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Comparative Study of Two Bench Grafting Methods at Apple (*Malus domestica,* Borkh.) and Pear (*Pyrus communis, L.*)

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Abstract: The study of technological and histological aspects during early stages of graft union formation between apple varieties and apple rootstock respectively newly bred pear varieties and quince rootstock in order to assess the best bench mechanical grafting technique and to study the compatibility/incompatibility related growth characteristics in early stages of development is a critical step in the fruit nursery material production activity. Two bench grafting methods were adopted for apple and pear propagation at SCDP Bistrita, graft combinations were studied in greenhouse and open field conditions in the nursery. Scions of 'Golden delicious', 'Florina' were bench grafted on M26 apple rootstock using omega " Ω " and "V" top grafting techniques , as well as 'Argessis', 'Monica', 'Carpica' pear cultivars grafted on BN70 quince rootstock. Successful graft unions were observed in all combinations and samples; however sapling development was differentiated according the two methods. It was found that the overall percentage of graft survival was 80% at omega grafting and 85% at V grafting at apple respectively 65% of graft survival in omega grafting and 80% of graft survival at V top grafting at pear combinations. Results showed that sapling development was reduced at combination with omega grafting technique, plants were weak developed, without lateral shoots, while in V grafted saplings the development was more intense with numerous lateral shoots. Samples of graft union sections were examined under microscope and microphotographs were taken, results confirmed that after 10 month of sapling development no signs of incompatibility were observable, conductive tissue formation being adequate.

Keywords: apple trees, pear trees, grafting, callus, nursery material, sapling, cambial zone.

INTRODUCTION

In the fruit production development process, the establishment of new intensive and super-intensive orchards with new vegetative rootstocks grafted with acclimated scion cultivars represents the main factor. In the nursery material production system the bench grafting technique has a series of advantages like technological efficiency, production time reducing, with a greater economical efficiency than classical T budding in august using two years of development of new saplings (Bordeianu, 1964; Manughevici *et al*., 1968; Dolgun *et al.*, 2008; Soumelidou *et al.*, 1994) in order to assess the compatibility/incompatibility of grafted scion-rootstock combinations (Simmons *et al*., 1984, 1996). In several new scion-rootstock combination in order to assess the compatibility between the two graft members the classical two year development methodology is too time consuming therefore the only possibility to reduce the production period and the time for study is the adopting of a well developed bench grafting technique with a high productivity index. Studying transversal cut samples of the grafting zone gives us the opportunity to assess the development of callus tissue, position of necrotic layers, cambial differentiation, callus bridge formation, development of vascular tissues (Branza *et al*., 2008). Growing features of both scion and rootstock gives us information about the physiological and biochemical interactions between

graft members, histological evaluation of graft sections gives first information on compatibility and incompatibility in short time (Casavela *et al.*, 1970; Simmons, 1984, 1996; Zlati *et al.*, 2010). In several cases abnormal physiological features in graft union formation of apple trees from 1 to 2 year old age have been reported and associated with a different degree of dwarfism or different bud and rootstock interaction, poor vascular connection, phloem degeneration and cambial and vascular discontinuity in the union area especially at pear can cause mechanical weakness and subsequent breakdown of the union (Feucht *et al*.,1988, Hartman *et al*., 1977, Errea, 1994). Several multi-annual researches are needed to study the long term development process and the interaction between scion and rootstock with a direct influence on compatibility/incompatibility of both graft members.

MATERIALS AND METHODS

This study was carried out in 2013 at Fruit Research and Development Station Bistrita FRDS Bistrita) in a research plot at Ciceu Mihaiesti "Bata" Nursery in open field conditions and in greenhouse at the research station. In this trial 'Golden delicious' and 'Florina' apple cultivars were used as scion and medium vigor rootstock M26 as rootstock respectively 'Argessis', 'Monica', 'Carpica' pear cultivars as scion and BN70 as quince rootstock. This plant material was grafted in February 2013 using two bench mechanical grafting techniques: omega grafting (Ω) and top "V" grafting with German grafting machine Omega 1 and top "V" grafting tool Plesa 2. The plant material was calibrated according proper dimension classes (7-8 mm diameter scion and well rooted vegetative rootstock of 35-40 length with the same diameter) and bench grafted, subsequently on 15 cm of both sides grafted parts were applied with special paraffin wax having a temperature of 60˚, finally sealed with grafting band. Grafted nursery material was prepared for callusing by putting them into plastic boxes with deciduous, moist sawdust. Grafted saplings were maintained at 28˚C temperature with 70% relative humidity, sawdust was properly wetted, in 3 weeks new roots emerged along with new shoots. Apple combinations which are more drought and cold weather resistant were planted in open field conditions at Bata Nursery near Somes river in a sandy-clay soil with weak differentiated soil levels. No special chemical fertilization were made, trees utilized just the existent soil mineral content. Irrigations were effectuated when soil content dropped under field capacity. The planting of grafted roots has been effectuated in early spring at 85/35 cm distance. Pear combinations were potted and put in greenhouse conditions with proper automatic air humidification and a higher temperature condition. There have been effectuated transverse sections of 20-30 μm of the grafting zone with a rotary microtome, sections were fixated in ethanol and stained with red safranine. Sections were effectuated in august 2013 before the end of vegetation period. Histological observations were effectuated for different scion/rootstock combinations.

RESULTS AND DISCUSSIONS

There have been effectuated observations and calculations regarding successful callus formation, bud take, shoot fresh weight, shoot dry weight, grafting survival, average trunk cross section area, average planting height, diameter, vigor index, sapling height below and above 1m, dynamics of shoot development (Fig.1). Separately development characteristics were also examined like length of roots, insertion angle of roots, root diameter. Results regarding successful callus formation, bud take and grafting survival are presented in Tab. 1. As callus formation at graft union level is a prerequisite for successful graft/bud take, rootstock might play an important role in this regard as well as the grafting technique. Several

researchers (Sadowski *et al.*, 2003) reported that bud take was higher in bench grafted seedlings with formerly produced callus than those without preformed callus, thus the callusing phenomena is a key element in the nursery production system. In our experiment at 'Golden delicious'/M 26 combination in the top "V" grafted saplings a higher percent of callus formation was observed (90%) when compared with omega grafting - "Ω" (85%), similar results were found in bud take (84% versus 80%) and grafting survival (84% vs.79%). Slight higher callus formation was observed in the 'Florina'/M26 graft combination (91% in "V" grafting, 83% in Ω grafting), bud take (86% in "V" grafting, 81% in "Ω" grafting), overall grafting survival (86% in "V" grafting, 81% in "Ω" grafting) (Tab. 1).

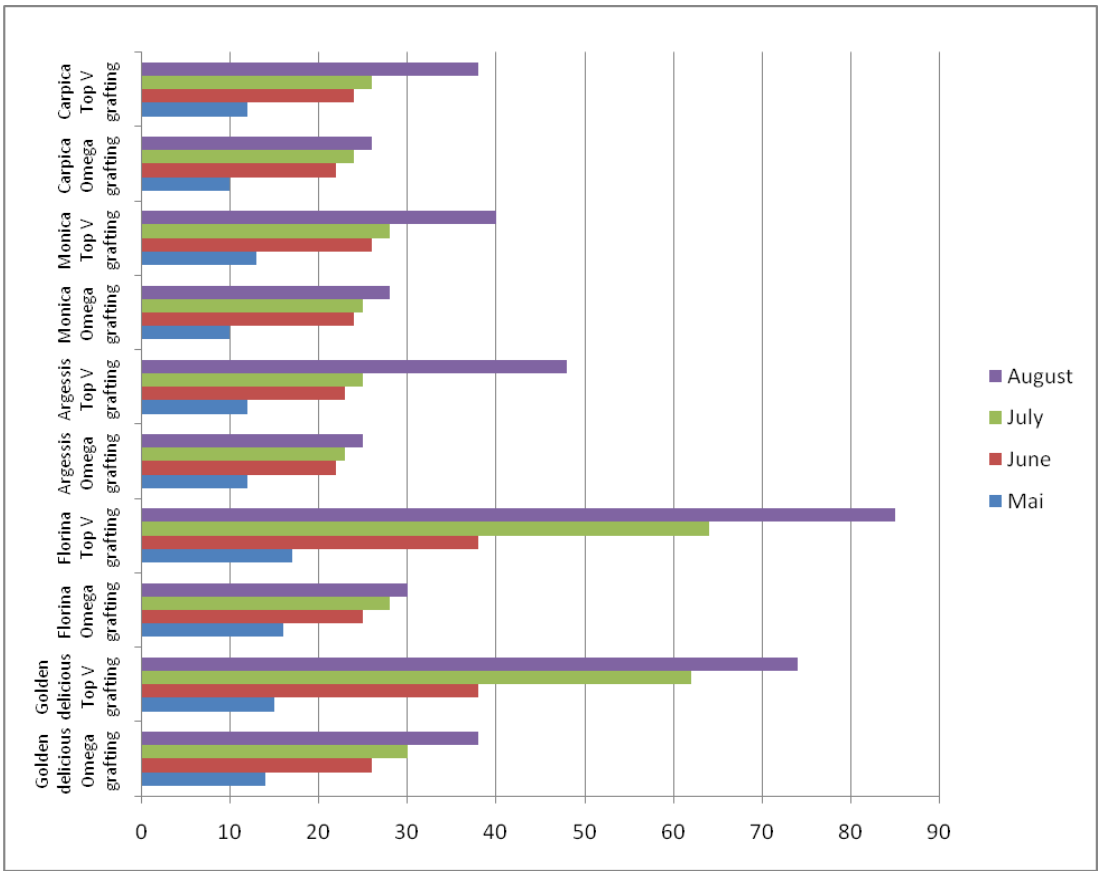

Fig. 1. Shoot development in the vegetation period may-august 2013 at the studied apple and pear graft combinations

In the pear graft combinations the callus formation realized lower levels when compared with apple graft combinations. At pear combination 'Argessis'/BN 70 callus formation was 84% in "V" grafting and only 70% in "Ω" grafting, bud take presented also slight lower values (80% in "V" grafting versus 65% in Ω grafting). In 'Monica'/BN70 cultivar-rootstock combination a similar effect was observed with higher callus formation in "V" variants (87%) versus omega grafting (72%). Lower callus formation was observed at 'Carpica'/BN70 cultivar (85% in "V" grafting, 68% in "Ω" grafting). Grafting survival ratio after 10 months showed slight reduced values of 79% in "V" grafting versus 64% in "Ω" grafting in 'Argessis'/BN70 combination, 80% in "V" grafting when compared with 67% in "Ω" grafting at 'Monica'/BN 70 combination and finally at 'Carpica' 81% in "V" grafting while 64% in "Ω" grafting.

Tab. 1

The effect of rootstock / scion interaction on successful callus production percentage, shoot dry and fresh weight, bud take, grafting survival

Species	Cultivar	Rootstock	Grafting type	Succesfull callus formation	Bud take	Shoot fresh weight	Shoot dry weight	Grafting survival [%]	Average trunk cross section area [mm2]	Average sapling height [m]	Diameter [mm]	Vigurosity index	Sapling heigh <1 m [%]	Sapling heigh >1 m [%]
Apple	Golden delicious	M26	Omega	85	80	18.5	16.2	79	63.6	38	9	1.67	2	98
Apple	Golden delicious	M26	Top grafting "V"	90	84	21.3	18.5	84	132.7	74	13	1.79	25	75
Apple	Florina	M26	Omega	83	81	19.5	17.4	81	38.5	30	7	1.28	3	97
Apple	Florina	M26	Top grafting "V"	91	86	24.3	22.3	86	113.0	85	12	1.33	30	70
Pear	Argessis	BN70	Omega	70	65	18.4	16.8	64	28.3	25	6	1.13	2	98
Pear	Argessis	BN70	Top grafting "V"	84	80	24.5	22.4	79	38.5	48	7	0.80	15	85
Pear	Monica	BN70	Omega	72	68	19.3	17.1	67	38.5	28	7	1.37	1	99
Pear	Monica	BN70	Top grafting "V"	87	82	25.6	23.4	80	50.2	40	8	1.26	12	88
Pear	Carpica	BN70	Omega	68	68	18.8	16.4	64	19.6	26	5	0.75	0	100
Pear	Carpica	BN70	Top grafting "V"	85	81	24.6	21.2	81	38.5	38	7	1.01	10	90

First results indicate that graft survival was reduced in "Ω" grafting than in "V" grafting in both apple and pear cultivars probable due to histological cambial connectivity reasons, weak development of transport elements of xylem and phloem cultivars, thus further results are needed to elucidate these hypothesis.

Overall development dynamics showed that the pear cultivars grafted on BN 70 rootstock by the top mechanical grafting technique developed more intensively in the second physiological development stage, transport elements were more developed whereas the "Ω" grafted trees developed weakly with close values in the second physiological growth period.

After 150 days of culture in open field conditions apple combinations realized an average sapling height of 79.5 cm in "V" grafted saplings and 34 cm at "Ω" grafted trees with a corresponding 122.85 mm^2 average trunk cross section area in "V" combinations and 51.05mm^2 in Ω grafted trees. At pear combinations cultivars grafted in "V" technique realized a sapling height of 42 cm with a corresponding value of 42.4 mm^2 TCSA and an average value of 28.8 mm^2 $_{TCSA}$ in "Ω" combinations with a sapling height of 26.33 cm. At both cultivars no special chemical fertilization was undertaken.

Development of lateral shoot formation was differentiated between the two grafting methods. At the apple species in "Ω" grafting method an average value of 1.4 lateral shoots formed (1.2 at 'Golden delicious' cultivar and 1,6 at 'Florina' cultivar) (Tab. 2) whereas at "V" top grafting a higher number of 4,4 lateral shoots were counted (4.2 at 'Golden delicious' and 4.6 at 'Florina' cultivar). In the case of pear cultivars at "Ω" grafting there were counted an average number of 1.4 shoots (1.5 at 'Argessis', 1.5 at 'Monica', 1.2 at 'Carpica') whereas in "V" grafting there were found 3.6 shoots (3.5 at 'Argessis', 3.7 at 'Monica', 3.8 at 'Carpica'). Regarding the height of insertion of lateral shoots there was found that in "Ω" grafting (10.8 at 'Golden delicious', 11 at 'Florina') shoots were inserted at 10.9 cm, while in "V" grafted variants insertion height was observed at 14.1 cm (12.2 at 'Golden delicious' and 16 at 'Florina'). In pear cultivars at omega Ω grafted variants average insertion hight was 8.6cm (10.5 cm in 'Argessis', 7.5 cm at 'Monica', 7.8 cm at 'Carpica') whereas at "V" grafted combination average insertion height was higher 12.26 cm (14 cm 'Argessis', 'Monica' 10.5cm, 12.3 cm at 'Carpica').

Tab. 2

Development of lateral shoot formation

Scion cultivar	Grafting type	Rootstock	Average length of sapling August 2013	Average number of lateral shoots	Height of insertion of lateral shoots	Length of sapling with lateral shoots
Golden delicious	Omega – "Ω"	M26	38	1.2	10.8	10
	Top grafting "V"	M26	74	4.2	12.2	15
Florina	Omega – "Ω"	M26	30	1.6	11.0	8
	Top grafting "V"	M26	85	4.6	16.0	17
Argessis	Omega – "Ω"	M26	25	1.5	10.5	10
	Top grafting "V"	M26	48	3.5	14.0	18
Monica	Omega – "Ω"	M26	28	1.5	7.5	7
	Top grafting "V"	M26	40	3.7	10.5	15
Carpica	Omega – "Ω"	M26	26	1.2	7.8	8
	Top grafting "V"	M26	38	3.8	12.3	17

Vigor index shows the length and diameter ratio expressed by the Trunk Cross Sectional Area (TCSA) per length of scion/rootstock combination and it is a good indicator in the appreciation of the development ratio of the two graft members. Results indicate that there

is a notable difference between the two adopted techniques since in both apple cultivars this index varied between 1.33-1.79 at “V” grafted saplings and 1.28-1.67 in omega grafting. This index generally was significantly lower at the pear scion-rootstock combinations being in the range of 0.80-1.25 in V grafting and 0.75-1.37 in omega grafting combinations.

Fig. 2 - Open field measurements of apple scion-rootstock graft combinations

Analyzing sapling height above 1 m in percent of total saplings, results showed that generally just 25-30% achieved the dimension above 1m in both grafting variants, however the “V” top grafted variants realized a higher percentage (25-30%) when compared with omega grafted trees (2-3%) at apple. In the pear cultivars the “V” grafted combinations exceeded in percentage (10-15%) the omega grafted variants. Results indicate that higher metabolic and development process were registered at “V” grafted variants.

Fig. 3 Apple scion-rootstock variant

There were rootstock development measurements (Fig.3) carried out in the frame of researches, results show that at M26 rootstock the root system is of fasciculate nature, there were counted 6 groups of roots inserted directly on the bark , the majority being of the same order. The first group (Tab. 3) had an insertion angle of -45˚ (0˚ being ground level), length of roots ranged from 3-13 cm with an average of 5.82 cm with a root dimension of 0.5-1 mm this root zone explores the 0-5 cm of the upper part of soil.

Tab. 3

Measurement results of rootstock development, insertion angle, length of roots and diameter of roots

No crt	Insertion angle of roots [°]	Length of roots [cm]	Diameter of roots [mm]	Insertion angle of roots [°]	Length of roots [cm]	Diameter of roots [mm]	Insertion angle of roots [°]	Length of roots [cm]	Diameter of roots [mm]	Insertion angle of roots [°]	Length of roots [cm]	Diameter of roots [mm]	Insertion angle of roots [°]	Length of roots [cm]]	Diameter of roots [mm]	Insertion angle of roots [°]	Length of roots [cm]	Diameter of roots [mm]
1	-45	13	1	-20	10	1	-50	12	1	-15	6	0.7	0	13	1	5	12	1.2
2	-45	8	1	-20	10	1	-50	13	1	-15	6	0.8	0	11.5	0.8	5	13	1.2
3	-45	6	1	-20	11	1	-50	13	1	-15	7.5	0.5	-10	10.5	1	5	11.5	1.1
4	-45	5.5	0.5	-20	10.5	1	-50	14	1.1	-15	7.5	0.5	-5	10	1	0	10.5	1.2
5	-45	6	0.5	-20	11.5	1	-50	13	1.1	-15	6	1	0	9	0.5	0	9	1.2
6	-45	5.5	0.5	-20	11.5	1	-30	10	1	-10	5.5	0.8	0	8	0.5	0	9	1
7	-45	5.2	0.5	-20	10.5	1	-30	11	1	-10	9.5	1	5	8	0.5	5	8.5	1
8	-45	4.8	0.4	-35	7	0.7	-30	12	1	-35	9.5	1	5	8.5	0.8	5	8.5	1
9	-45	3	0.3	-35	8.5	0.7	-30	13	1	-35	8	0.8	5	9.5	0.5	5	7.5	0.8
10	-45	4	0.5	-35	8.5	0.8	-30	10	1.1	-10	8	0.8				5	7.5	0.8
11	-45	5	1	-45	7.5	0.7	-30	8	0.9	-10	9	1				5	8.5	1.3
12	-45	6	1	-45	7	0.5	-30	8	0.9	-30	12	0.9				5	7.5	0.8
13	-45	5.2	0.5	-45	8	0.6	-30	9	0.8	-10	7	0.7				5	8.5	0.7
14	-45	5.4	1	-45	6.5	0.6	-30	7	0.7	-10	5.5	0.6				5	9	0.7
15	-45	4.9	1	-45	5	0.5	-30	6	0.6							5	7.5	0.5
16	-45	5.7	0.5	-45	7	0.6										5	7.5	0.5
17				-45	9	0.8										5	8.5	0.6
18				-45	10	0.9										5	8.5	0.5
19				-45	11	1										5	4.5	0.7
20				-45	5.2	0.5										5	4.5	0.8
21				-45	6.5	0.5										5	4.5	0.9
22				-45	5.5	0.5										45	3.7	0.7
23				-45	6.7	0.4										45	3.8	0.5
24				-45	7.5	0.5										45	3.2	0.5
25				-45	7	0.7										45	4.5	0.4
26				-45	8	0.7										45	4.5	0.4
27																45	4.8	0.4
28																		

The second group had lower insertion angles -20˚-35˚-40˚ exploring the 0-10 cm depth of soil with an average length of 8.32 cm ranging 5-11.5 cm and 0.73 in diameter. In the 0-15 cm soil depth roots were longer ranging 6-14 cm (with an average of 10.6 cm) and a diameter of 0.94 mm with more vertical inclination (-30˚-50˚) showing a deeper exploring of soil. Group 4 roots had a smaller inclination, close to 0˚ (10˚-15˚) exploring more horizontally the soil (average length of 7.64 cm, diameter 0.79 mm).Group 5 roots presented 0˚ inclination angle exploring entirely the 0-20 cm soil depth with roots ranging 8-13 cm in length (average value of 9.7 cm and 0.73 mm in diameter.

The greatest amount of roots were in group 6 with a total number 27 roots with several secondary and tertiary hairy roots and medium sized first order roots ranging 4.5-11.5cm in average 7.42 cm and 0.79 mm in diameter. This group of roots are working in the lowest zone of soil (0-20 cm) having a difficult task, to anchor the whole tree and to uptake minerals and water from the same humus complex. The highest number of roots were found by group 2 (5-10 cm zone with 26 roots) and group 6 (15-20 cm with 28 roots), these regions having the highest mineral nutrient content and water availability.

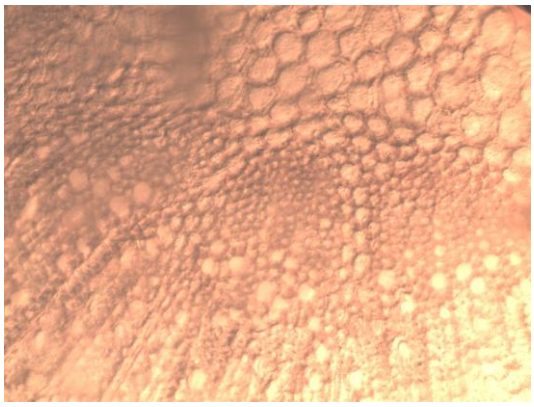

Fig. 4 Detail of apple scion xylem tissues

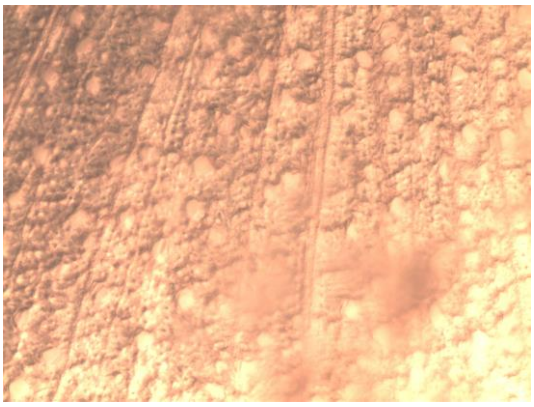

Fig. 5 Conductive tissues in grafting union at pear

Microphotographs of transversal cross sections showed no necrotic layer accumulation at apple combinations (Fig. 4, Fig. 5), callus bridges derived from parenchyma cells, callus started to spread into gaps, callus proliferation was observed more at 'Golden delicious'/M26 combination and slightly less at 'Florina' cultivar. High ratio of vigor index of scion/rootstock combination influenced positively the graft union development and implicitly the development of new saplings. At the final period of 150 days of development good cambial continuity was observed, necrotic layers were eliminated, the cortex region had no point-form residues of necrotic layer resorption. In apple all graft combinations have a good graft bridging, xylem and phloem elements; it was not observed an abnormal cellular or histological development related to different growing features of scion-rootstock combination.

In the pear combinations weaker xylem and phloem continuity was observed, callus proliferation was slightly reduced, but further development was negatively influenced in omega grafting. Small necrotic zones were observed in omega grafting. These results indicate that in omega grafting ("Ω") the cambial zones of both graft members are not in a direct connection in such an extent as in "V" top grafting thus the latter "V" grafting technique is superior to the omega grafting. The top "V" grafting has the feature that xylem and phloem elements have a greater surface contact, influencing positively the graft formation and intercellular communication.

A good union was observed in "V" graft combinations with good cambial connectivity and continuity, callusing occurred successfully, outer necrotic layers were transformed to bark, outer restoration of tissues occurred well, no visible discontinuity was observed in the bark after eight months of graft union survival, thus no evidence for tissue incompatibility were found in this study.

CONCLUSION

Mechanical bench technique is a cost effective innovative, efficient propagation method, results indicate that a proper choosing of grafting method can provide a high success rate of propagation. Biometrical nursery parameters showed the superiority of "V" grafting technique, achieving higher success rate in callus formation, bud take, grafting survival, along with sapling development parameters, TCSA sapling height, diameter, vigor index, when compared with omega grafting technique. In both fruit tree species the second propagation method showed a higher success rate, data regarding the development of young saplings showed an increased metabolic activity resulting higher sapling heights and diameter, increased vigor index, whereas saplings of omega grafted trees stagnated in development achieving the half of the "V" grafted saplings. Also the lateral shoot formation indicated that the "V" grafted trees are well developed when compared with the before mentioned omega Ω trees. The highest number of lateral shoots gave the 'Florina' in "V" grafted combination.

In both apple and pear variants in "V" grafting successful grafting occurred which included the formation of necrotic layer, callus production, first cohesion of rootstock and scion by the callus junction. Subsequent reduction or elimination of necrotic layer in callus, differentiation of some cells to the cambial cells, bridging the cambium tissues of stock and scion, finally formation and strong connection of vascular tissues (Atkinson, 2003; Sitarek, 2006).

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