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RESEARCH ON THE PROPAGATION OF SOME SWEET CHESTNUT CULTIVARS THROUGH DIFFERENT GRAFTING METHODS

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Abstract

Sweet chestnut was propagated till now in Romania mainly through seeds, and the trees obtained show a highly variable biological and economic behavior. Under current conditions of development and modernization of fruit growing, chestnut planting material for modern orchards should be obtained through clonal propagation. In the present study we aimed to propagate two Romanian sweet chestnut cultivars ('Casval' and 'Romval') and two French ones ('Bouche de Bétizac' and 'Marsol') using different grafting methods inside and in the field. As rootstock, local Castanea sativa seedlings were used. The bench grafting methods performed were: whip and tongue, cleft and side grafting followed by callus stimulation into a hot callusing device which provides temperatures of 26-27°C at the grafting point. In the nursery field same methods were used and also chip budding. Higher success rates were obtained for all cultivars when bench grafting was carried out inside (79.86%) compared with grafting in the field (69.94%). The highest success percentages were achieved inside when whip and tongue method was performed (85.33%), followed by cleft grafting (81.9%). The success rates obtained were: 'Bouche de Bétizac' (86.2%), 'Marsol' (85.27%), 'Casval' (75.0%) and 'Romval' (73.0%). In order to highlight the propagation capacity, the "Grafting Success Index" was determined, using three elements: callus formation, grafting success rate after callusing and grafting success rate at the end of the growing season. The scion/rootstock/grafting point ratio, as well as the scion growth in the first and second year after grafting, proved that the cultivars were compatible with the rootstock used. The results obtained in the given conditions allows us to recommend the adequate grafting methods in order to propagate the four sweet chestnut cultivars.

Keywords: *Castanea sativa*, European chestnut, grafting

1. INTRODUCTION

The sweet chestnut (*Castanea sativa* Mill.) is part of the *Castanea* genus which includes 12-13 species [1], the only one original from Europe being *C. sativa*.
This species was traditionally cultivated in Greece, Italy, Spain, France, Portugal, Switzerland, Turkey and other countries. In Romania, the sweet chestnut does not have a clear origin, but is certainly located in Oltenia region (Gorj and Vâlcea counties), near several old monasteries and also in Maramureș and Caraș Severin counties [2].
The expansion of sweet chestnut growing worldwide occurred in the last century through the new orchards plantings, as result of the nutritional quality of the nuts, the timber value and the role of chestnut trees in combating soil erosion.
The interest for this nut crop has also grown in Romania in recent decades, several local cultivars were obtained and used into culture along with other valuable foreign cultivars [3]. The interest for finding efficient and favorable methods for propagation sweet chestnut cultivars through layers, cuttings and grafting [4,5,6,7, 8] on various rootstocks accentuated [9].
Propagation through layering poses some inconveniences because not all cultivars are able to produce layers. Consequently, worldwide (USA, France, Italy, China, Korea, Japan) the propagation of edible chestnut cultivars by grafting has been adopted. Such rootstocks are usually derived from seeds and transmit different characters to the cultivars. That is why we have moved on to the selection and obtaining of rootstocks with vegetative propagation, after which they are grafted using various methods with the most valuable cultivars. The purpose of presenting the research paper is to contribute to the propagation of sweet chestnut cultivars as easily and efficiently as possible, using various grafting methods in order to favor the expansion of high-yield orchards.

2. MATERIALS AND METHODS

The study was setup at UCv-SCDP Vâlcea, a Fruit Growing Research Station which belongs to the University of Craiova. The station is located in the subCarpathian area of Oltenia, Romania, an area with mild temperate climate, where the average multi-annual temperature is 10.2° C and the annual rainfall 720 mm. The biological material used for grafting consisted of two types of cultivars:

- two Romanian cultivars obtained at UCv-SCDP Vâlcea: 'Casval' and 'Romval'. Both of them are belonging to *C. sativa* and are clonal selections from local populations of sweet chestnut.
- two French cultivars: 'Bouche de Bétizac' and 'Marsol', both are Euro-Japanese chestnut hybrid (*C. sativa x C. crenata*).

C. sativa seedlings, obtained from seed mother plants of UCv-SCDP Vâlcea, were used as rootstocks. The grafting referred to:

a. Bench grafting methods carried out inside rooms during March:
   - whip and tongue;
   - cleft;
   - side grafting.

b. Field grafting methods performed during June:
   - whip and tongue;
   - cleft;
   - side grafting;
   - chip budding.

The trial presents 2 factors (A – cultivars and B – grafting methods) and was organized in 5 repetitions.
Bench grafting was followed by callus stimulation for 28-30 days, in a hot callusing installation with constant temperature of 26-27°C at the grafting union and atmospheric humidity of 70-80%. Bench grafting was done in the second half of March, with dormant scion wood and after 7 days forcing of the rootstocks in heated room. The successful grafted plants were transferred to the nursery field 30-35 days after grafting. Grafting in the field was carried out in the first half of June, using scion wood kept in the refrigerator. Data was collected regarding the growth of scions, rootstocks and the grafting point. In order to highlight the propagation capacity, the "Grafting Success Index" (GSI) was determined, where GSI = (callused plants + graft shoots in vegetation + successful unions)/100. The following levels for GSI were taken into account: 0.5 – 1.0 (low), 1.0 – 1.5 (medium), 1.5 – 2.0 (good), >2.0 (very good). At the end of the vegetation period (1st and 2nd year after grafting), the growth in length of the shoots/twigs were determined.

3. RESULTS AND DISCUSSIONS

The Romanian ('Casval' and 'Romval') and French ('Bouche de Bétizac' and 'Marsol') chestnut cultivars were grafted onto seedlings of *Castanea sativa*. The results obtained in the two years of study are presented in Table 1 and show that the most advantageous grafting method was whip and tongue (85.33% mean success rate), followed by cleft (81.9%) and side grafting (72.35%). The cultivars grafted through whip and tongue achieved the highest grafting percentages: 'Bouche de Bétizac' (90.4%), 'Marsol' (87%), 'Casval' (85%) and 'Romval' (79%). The Romanian chestnut cultivars, although they achieved high grafting rates, were still 2-8% below the French ones. When grafting was performed directly in the field, the whip and tongue method achieved the highest mean success percentages (78.25%), but 7.0% lower than bench grafting by the same method. The average bench grafting success rate (with 3 methods) was 79.86%, while directly in the field, it was 60.94% (Table 1). If we compare the average of the cultivars, in both grafting cases, we find that 'Marsol' achieved 81.89% grafting success, followed by 'Casval' (69.87%), 'Romval' (65.5%) and 'Bouche de Bétizac' (64.35%).
After the first period of graft shoots vegetation, we find that the percentages of survival grafts are lower compared to the initial percentages of callused grafts (Table 2). In bench grafting, the highest graft percentages at the end of first vegetation ranged between 77.12% (whip and tongue) and 60.75% (side grafting). Compared to the initially callused grafts, the percentages at the end of first vegetation period were lower with 8.2% (whip and tongue) up to 11.6% (side grafting). The same situation was also observed in case of field grafts, in which case the strongest decrease occurred in in chip budding (8.25%) and side grafting (9.5%), followed by whip and tongue (6.75%) and cleft (6.5%). In case of bench grafting, between 63% ('Romval') and 72% ('Marsol') grafted trees are obtained in the fall, while for field grafting between 34% ('Bouche de Bétizac') and 72% ('Marsol') trees are resulting. In order to precisely determine the results obtained in the final grafting of chestnut cultivars, callus formation at the grafting point and the percentage of grafts resulting at the end of the vegetation period were monitored. Based on these elements, the "Grafting Success Index" (GSI) was determined (Table 3). In the case of whip and tongue grafting method, all cultivars achieve an index of over 2.0 (very good). 'Casval', 'Bouche de Bétizac' and 'Romval' cultivars achieve for side grafting in the field GSI of 1.0-1.5% (average value) as does 'Bouche de Bétizac' (cleft and chip budding).
The average value of GSI is 2.399 (very good) for bench grafting and 1.915 (good) for grafting in the field. At the end of the vegetation period (1st year, in autumn) the cross-section areas of the scion, rootstock and grafting union were determined. With the help of these elements, the cultivar/rootstock/grafting union ratios were calculated (Tables 5 and 6). The value of this ratio above 0.330 shows us a very good graft-rootstock combination, between 0.300-0.330 it shows good compatibility, and below 0.300, the compatibility is poor. In the case of bench grafted chestnut trees, the ratio is over 0.300, thus showing that there is a very good cultivar - rootstock compatibility.

A few exceptions were obtained that deviate from the rule: 'Marsol' cleft grafted (0.257), as well as 'Bouche de Bétizac' (0.265) and 'Marsol' (0.139) side grafted, proved to have poor compatibility, although in the second year after grafting they developed normally. In the case of cultivars grafted in the field, those by whip and tongue present a ratio of over 0.300 (very good compatibility).

In the other types of grafting, there are some cases with ratios below 0.300 ('Bouche de Bétizac' and 'Romval', when cleft grafted; 'Bouche de Bétizac', 'Marsol' and 'Romval' side grafted and 'Bouche de Bétizac' and 'Romval' when chip budded). The graft length after 1st and 2nd year is presented in Table 7.

If in the first year the grafts length growth was 35.8 cm on average (in the field) and 39.5 cm (for bench grafting), in the second year the average length, regardless of the grafting methods, was 168 cm (in the field) and 174 cm (for bench grafting). Growth differences between cultivars are reduced in both study years.

In the first year, a growth ratio was made between the grafting cross section area and the growth in length. The ratios between cultivars and grafting methods were very close, ranging between 0.01 and 0.021.

The average ratio for bench grafts was 0.018, and that of grafts in the field was 0.017. According to the study conducted over 2 years with these grafting methods, we find that this type of propagation is advantageous and suitable for chestnut cultivars.

4. CONCLUSIONS

- The development of the sweet chestnut culture is an objective necessity for its food and ecological value, and for this, modern propagation is required through methods that preserve the characteristics of the most valuable cultivars.
- The propagation of sweet chestnut cultivars can be done through various methods, among which grafting responds to some desires for the modernization of plantations (high and early yielding, superior nut quality, etc.).
- Bench grafting and using the heating at the grafting point proved superior in mean grafting success rates (79.86%), compared to grafting in the field (60.94%).
- The highest bench grafting success rates were recorded in the case of whip and tongue (85.33%), followed by cleft grafting (81.9%) and whip and tongue grafting in the field (78.25%).
- Under bench grafting conditions, the grafted cultivars achieved average success rates of 86.2% ('Bouche de Bétizac'), 85.27% ('Marsol'), 75% ('Casval') and 73% ('Romval').
- Grafting Success Index was determined depending on the cultivar and the grafting method. All chestnut cultivars bench grafted have GSI of over 2, i.e. "very good". For field grafting, the index is "very good" for all cultivars grafted by whip and tongue method.
- The ratio between the scion/rootstock/grafting union sections (the end of 1st year) and the growth in length of the scion (end of 1st and 2nd years) shows a uniform growth without any particular problems regarding the grafting compatibility of the studied sweet chestnut cultivars.

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Table 1. Average grafting percentages when grafting sweet chestnut cultivars using different methods

Cultivar	Bench grafting				Grafting in the field					Overall mean (%)
	Whip and tongue (%)	Cleft (%)	Side (%)	*Mean (%)*	Whip and tongue (%)	Cleft (%)	Side (%)	Chip budding (%)	*Mean (%)*	
'Casval'	85%	80.0	60.0	***75.00***	86.0	65.0	28.0	80.0	***64.75***	69.87
'Bouche de Bétizac'	90.4	86.8	82.0	***86.20***	68.0	36.0	26.0	40.0	***42.50***	64.35
'Marsol'	87.0	88.4	80.4	***85.27***	87.0	75.0	73.0	79.0	***78.50***	81.89
'Romval'	79.0	73.0	67.0	***73.00***	72.0	61.0	51.0	48.0	***58.00***	65.50
Mean (%)	***85.33***	***81.9***	***72.35***	***79.86***	***78.25***	***59.25***	***44.5***	***61.75***	***60.94***	***70.40***

Table 2. Percentages of sweet chestnut grafts resulting at the end of the first year of vegetation after bench and field grafting

Cultivar	Bench grafting				Grafting in the field					Overall mean (%)
	Whip and tongue (%)	Cleft (%)	Side (%)	*Mean (%)*	Whip and tongue (%)	Cleft (%)	Side (%)	Chip budding (%)	*Mean (%)*	
'Casval'	74.0	61.0	59.0	***64.67***	78.0	60.0	20.0	75.0	***58.25***	61.46
'Bouche de Bétizac'	84.5	71.0	60.0	***71.80***	60.0	30.0	18.0	28.0	***34.00***	52.90
'Marsol'	76.0	70.0	70.0	***72.00***	80.0	69.0	68.0	72.0	***72.25***	72.12
'Romval'	74.0	61.0	63.0	***63.00***	68.0	52.0	64.0	49.0	***50.75***	56.88
Mean (%)	***77.12***	***65.75***	***67.87***	***67.87***	***71.5***	***52.75***	***53.5***	***53.5***	***53.81***	***60.78***

Table 3. Grafting Success Index depending on cultivar and bench grafting methods

No. Crt.	Grafting methods	Post grafting results	Cultivar				Mean
			'Casval'	'Bouche de Bétizac'	'Marsol'	'Romval'	
1	Whip and tongue	Callus formation (%)	95.0	100	94.0	88.0	***94.75***
		Success rate after callusing (%)	85.0	90.4	87.0	79.0	***85.35***
		Success rate after first year of vegetation (%)	74.0	84.5	7.0	74.0	***77.12***
		Grafting Success Index (GSI)	2,540	2,749	2,570	2,410	***2,572***
2	Cleft	Callus formation (%)	92.0	96.0	100	82.0	***93.50***
		Success rate after callusing (%)	80.0	86.2	88.4	73.0	***81.90***
		Success rate after first year of vegetation (%)	61.0	71.0	70.0	61.0	***65.75***
		Grafting Success Index (GSI)	2,330	2,532	2,584	2,160	***2,412***
3	Side	Callus formation (%)	91.0	96.0	90.0	82.0	***89.75***
		Success rate after callusing (%)	60.0	82.0	80.4	67.0	***72.35***
		Success rate after first year of vegetation (%)	59.0	60.0	70.0	54.0	***60.75***
		Grafting Success Index (GSI)	2,100	2,380	2,404	2,030	***2,229***
	Mean	***Callus formation (%)***	***92.67***	***97.30***	***94.67***	***84.00***	***92.16***
		Grafting success rate after callusing (%)	***75.00***	***86.30***	***85.27***	***73.00***	***79.86***
		Grafting success rate after first year of vegetation (%)	***64.67***	***71.80***	***72.00***	***63.00***	***67.87***
		Grafting Success Index (GSI)	***2,323***	***2,554***	***2,519***	***2,200***	***2,399***

Table 4. Grafting Success Index depending on cultivar and grafting methods under field conditions

No. Crt.	Grafting methods	Post grafting results	Cultivar				Mean
			'Casval'	'Bouche de Bétizac'	'Marsol'	'Romval'	
1	Whip and tongue	Callus formation (%)	92.0	81.0	96.0	90.0	89.75
		Success rate after callusing (%)	86.0	68.0	87.0	72.0	78.25
		Success rate after first year of vegetation (%)	78.0	60.0	80.0	68.0	71.50
		Grafting Success Index (GSI)	2,560	2,090	2,630	2,300	2,395
2	Cleft	Callus formation (%)	74.0	55.0	89.0	84.0	75.50
		Success rate after callusing (%)	65.0	36.0	75.0	61.0	59.25
		Success rate after first year of vegetation (%)	60.0	30.0	69.0	52.0	52.75
		Grafting Success Index (GSI)	1,990	1,210	2,330	1,970	1,875
3	Side	Callus formation (%)	52.0	60.0	87.0	64.0	65.75
		Success rate after callusing (%)	28.0	26.0	73.0	51.0	44.50
		Success rate after first year of vegetation (%)	20.0	18.0	68.0	34.0	35.00
		Grafting Success Index (GSI)	1,080	1,040	2,280	1,490	1,453
4	Chip budding	Callus formation (%)	94.0	56.0	93.0	71.0	78.50
		Success rate after callusing (%)	80.0	40.0	79.0	58.0	64.25
		Success rate after first year of vegetation (%)	75.0	28.0	72.0	49.0	56.00
		Grafting Success Index (GSI)	2,490	1,240	2,440	1,780	1,989
	Mean	***Callus formation (%)***	***75.50***	***63.00***	***91.25***	***77.25***	***76.75***
		Success rate after callusing (%)	***64.75***	***42.50***	***78.50***	***58.00***	***60.94***
		Success rate after first year of vegetation (%)	***58.25***	***34.00***	***72.25***	***50.75***	***53.81***
		Grafting Success Index (GSI)	***1,985***	***1,395***	***2,420***	***1,860***	***1,915***

Table 5. The growth pattern of chestnut cultivars in the first year of vegetation depending on bench grafting methods

Cultivar	Whip and tongue grafting				Cleft grafting				Side grafting				Mean			
	Cross sectional area (cm^2)			Cultivar/Rootstock/Grafting union Ratio (%)	Cross sectional area (cm^2)			Cultivar/Rootstock/Grafting union Ratio (%)	Cross sectional area (cm^2)			Cultivar/Rootstock/Grafting union Ratio (%)	Cross sectional area (cm^2)			Cultivar/Rootstock/Grafting union Ratio (%)
	Rootstock	Grafting union	Shoot graft in autumn		Rootstock	Grafting union	Shoot graft in autumn		Rootstock	Grafting union	Shoot graft in autumn		Rootstock	Grafting union	Shoot graft in autumn	
'Casval'	1.34	1.65	0.98	0.443	1.04	1.19	0.77	0.622	1.20	2.20	0.69	0.261	1.19	1.68	0.81	0.432
'Bouche de Bétizac'	1.28	1.57	0.83	0.413	0.01	1.34	0.48	0.355	1.34	1.97	0.70	0.265	1.21	1.63	0.64	0.329
'Marsol'	1.39	1.54	0.76	0.355	1.47	1.72	0.65	0.257	1.93	2.77	0.74	0.139	1.60	2.01	0.72	0.250
'Romval'	1.15	1,312	0.59	0.392	0.92	1.09	0.37	0.369	1.24	1.40	0.68	0.392	1.10	1.27	0.54	0.381
Mean	***1.29***	***1.52***	***0.77***	***0.400***	***1.04***	***1.34***	***0.57***	***0.400***	***1.43***	***2.08***	***0.70***	***0.264***	***1.27***	***1.65***	***0.68***	***0.340***

Table 6. The growth pattern of chestnut cultivars in the first year of vegetation depending on the grafting method in the field

Cultivar	Whip and tongue grafting				Cleft grafting				Side grafting				Chip budding				Mean		
	Cross sectional area (cm^2)			Cultivar/Rootstock/Grafting union Ratio (%)	Cross sectional area (cm^2)			Cultivar/Rootstock/Grafting union Ratio (%)	Cross sectional area (cm^2)			Cultivar/Rootstock/Grafting union	Cross sectional area (cm^2)		Cultivar/Rootstock/Grafting union		Cross sectional area (cm^2)		Cultivar/Rootstock/Grafting union
	Rootstock	Grafting union	Shoot graft in autumn		Rootstock	Grafting union	Shoot graft in autumn		Rootstock	Grafting union	Shoot graft in autumn	Rootstock	Grafting union	Shoot graft in autumn	Rootstock		Grafting union	Shoot graft in autumn	
'Casval'	0.90	1.07	0.43	0.447	1.10	1.32	0.62	0.427	0.66	1.28	0.42	0.497	1.21	1.97	0.73	0.470	0.98	1.26	0.41	0.332
'Bouche de Bétizac'	0.95	1.88	0.46	0.378	2.16	2.67	1.27	0.220	1.26	1.54	0.52	0.268	1.30	1.72	0.61	0.272	1.42	1.90	0.72	0.284
'Marsol'	1.09	1.29	0.45	0.320	0.75	0.98	0.40	0.558	1.56	1.96	0.50	0.163	0.97	1.21	0.61	0.519	1.09	1.36	0.49	0.330
'Romval'	1.16	1.36	0.61	0.326	1.17	1.49	0.35	0.200	1.10	1.42	0.38	0.243	1.86	1.94	0.84	0.233	1.17	1.55	0.55	0.303
Mean	***1.04***	***1.40***	***0.49***	***0.383***	***1.30***	***1.61***	***0.66***	***0.344***	***1.15***	***1.55***	***0.46***	***0.293***	***1.19***	***1.71***	***0.71***	***0.349***	***1.17***	***1.57***	***0.58***	***0.312***

Table 7. Characteristics of sweet chestnut scions in the second year after grafting

No. crt.	Grafting methods	Cultivar	Growth length of scion (cm)					
			1st year after grafting	2nd year after bench grafting	Graft cross-section /length Ratio		Graft length growth in 2nd year	
					Field	Bench grafting	Field	Bench grafting
1	Whip and tongue	'Casval'	31.0	37.5	0.014	0.026	167	177
		'Bouche de Bétizac'	26.7	43.6	0.017	0.019	160	172
		'Marsol'	43.0	48.8	0.011	0.016	178	186
		'Romval'	30.5	34.3	0.020	0.017	160	173
		Mean	***32.8***	***41.1***	***0.015***	***0.019***	***166***	***177***
2	Cleft	'Casval'	36.7	39.0	0.017	0.020	172	170
		'Bouche de Bétizac'	60.0	47.7	0.021	0.017	185	181
		'Marsol'	42.3	34.7	0.011	0.019	176	175
		'Romval'	25.0	39.2	0.014	0.012	162	172
		Mean	***41.0***	***40.2***	***0.016***	***0.017***	***174***	***175***
3	Side	''Casval'	34.4	35.7	0.012	0.019	162	165
		'Bouche de Bétizac'	29.2	38.0	0.018	0.019	159	171
		'Marsol'	38.0	47.3	0.013	0.016	167	182
		'Romval'	31.1	34.6	0.012	0.020	161	166
		Mean	***33.2***	***38.9***	***0.014***	***0.018***	***162***	***171***
4	Chip budding	'Casval'	32.3	-	0.024	-	164	-
		'Bouche de Bétizac'	38.3	-	0.016	-	173	-
		'Marsol'	34.4	-	0.018	-	171	-
		'Romval'	39.6	-	0.021	-	177	-
		Mean	***36.2***	***-***	***0.019***	***-***	***171***	***-***
	Overall mean	***'Casval'***	***33.6***	***35.4***	***0.017***	***0.022***	***166***	***171***
		'Bouche de Bétizac'	***38.6***	***43.1***	***0.019***	***0.018***	***169***	***175***
		'Marsol'	***39.4***	***43.6***	***0.016***	***0.017***	***173***	***181***
		'Romval'	***31.6***	***36.0***	***0.017***	***0.016***	***165***	***170***
		Mean	***35.8***	***39.5***	***0.017***	***0.018***	***168***	***174***