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THE EFFECTS OF DIFFERENT METHODS AND ROOTSTOCKS PHENOLOGYCAL STAGES ON SWEET CHESTNUT (*CASTANEA SATIVA* MILL.) SPRING GRAFTING

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Abstract

In this study Sweet chestnut spring grafting onto same rootstocks has been made. Two-year-old seedlings in the open field were used as rootstocks. Two phenological stages of the rootstocks have been tested – dormancy condition (end of February) and the beginning of growing season (end of March). The grafting methods were chip budding, cleft graft, side veneer graft and splice graft. Criteria for assessment of the results were the percentage of grafting components union, main shoot growth, lateral shoots number and growth. Significant differences in grafting success and plant development were found in different phenological stages and in grafting methods. It was mainly recommended to perform splice grafting during the spring using “dormant” and “active” rootstocks. By splice grafting both graft success and growth of the scion (86.7 to 93.3 % graft success and 23.7 to 26.2 cm graft shoot height) can be realized.

Key words: Sweet chesnut, spring grafting, chip budding, cleft graft, side veneer graft, splice graft.

Introduction

Sweet chestnut in Bulgaria is found in limited and fragmented isolated fields. It has entered the customs and traditions of people, in the places where it can be found; therefore it has high social and economic importance, especially for fruit production (Glushkova 2006).

Castanea sativa Mill. belongs to the so-called “multipurpose” species due to its various beneficial properties and wide range of valuable products obtained from it (Milev and Iliev 2007).

The problems in chestnut breeding are mainly due to ubiquitous disease known as cancer caused by the fungus *Cryphonectria parasitica* Murrill. (Avramov 2002). Recent studies showed that everywhere in the world where this species is cultivated, plantations were in disquieting phytosanitary state. In Bulgaria, almoust all plantations are affected. The disease raises the question about the biological existence of the species (Iliev and Mirchev 1992, Milev and Sotirovski 2007).

Currently a number of researchers and international research groups are exploring diverse ways for overcoming the problem (Robin and Heiniger 2001, Milev et al. 2005, Milgroom et al. 2008, Risteski et al. 2013). A possibility in this direction is to explore and to produce resistant clones with additional requirements of the harvest qualities, the wood and the growth rate (Iliev and Mirchev 1992, Craddock and Bassi 1999, Petrov 2005, Milev and Sotirovski 2007).

However, genotypes with high resistance to cancer can't be selected through seed propagation. These problems with Sweet chestnut breeding outline the importance of vegetative propagation for production of seedling. In addition, vegetative propagation allows the achievement of early, frequent, abundant and benign harvests in the garden type crops.

In vegetative propagation, cloning of mature genotypes is always preferred because they have the phenotype show of their genetic potential (Franclet 1980, Zobel 1981). It is known, however, that the rooting of Sweet chestnut cuttings is difficult, depends on many factors, including the stages of the donor plant and does not give practically significant results (Dirr and Heuser 1987).

When propagation by cuttings is very difficult or impossible, the method of grafting is applied for reproduction of valuable genotypes (Broshtilov 2000).

This study aimed to determine suitable phenological stages of chestnut rootstocks and successfull methods for spring grafting and budding.

Material and Methods

The vegetative propagation experiment was carried out in forest nursery "Ivanik", Forest Enterprise Petrich. A source of scion wood was the existing clonal colection in the nursery. The analysis of embedded cultivars on their resistance to cancer showed that the only variety with no visible damage from the disease is the French variety Marigoule (cv. '*Marigoule*'), wherefore it was used for the extraction of shoots. The scions were taken in the beginning of February and were stored at 4°C until they were used for grafting.

Two-year-old seedlings were used as rootstocks in the open field.

To study the influence of the phenological stage of the rootstock, the grafting was carried out in two periods:

End of February (26–27.02.2011) – dormant stage ("dormant" rootstocks);

End of March (20.03.2011) – beginning of growing period ("active" rootstocks).

To determine the optimal methods of grafting were tested:

1. Budding: chip budding;

2. Grafting of three bud scions: cleft graft, side veneer graft, splice graft.

For each method 3 repetitions with 25 graftings were used. The scion and the rootstock were fasten by special grafting rubber strips. The rootstock shoots were remorved on 03 May 2011.

The grafted saplings were not shaded during the growing period.

The percentage of graft success, length of main and lateral shoots and the number of scion shoots were the criteria for the evaluation of the results.

The results were analysed by One-Way ANOVA followed by a post hoc LSD test at p<0.05 (statistical package SPSS11).

Results and Discussion

T-budding trials showed that the rootstocks' bark could not be separated from the wood in both phenological periods of grafting work Therefore, this method has not been included in the experiment and the chip budding had been selected as a primary grafting method.

The results of the grafting success are presented in Table 1.

The highest percentage of grafting success occurs in splice graft (average 90.0 %), and the worst/lowest result – in chip budding (average 37.3 %). This result is different from the recommendations of Nedev et al. (1966), Petrov (1976), Milev et al. (2007), Celik et al. (2009), Bratels (2012), Dirr and Heuser (1987) to use chip budding when the bark separation is not possible.

Table 1. Grafting success of the components, depending on the phenological stage of the rootstocks and the method of grafting (%).

Grafting methods	Phenological stage of the rootstock		Average per method
	Dormant	Active	
Chip budding	21.33±8.74a	53.33±8.74bc	37.33±4.40a
Cleft graft	40.00±2.00b	81.33±8.11de	60.67±4.40b
Side veneer graft	69.33±5.81cd	77.33±3.53de	73.33±4.40b
Splice graft	86.67±5.33de	93.33±3.53e	90.00±4.40c
Average of phenological stage	54.33±3.11	76.33±3.11	

Note: Means with the same letter are not significantly different.

As a whole, the various types of grafting showed better possibilities for scion and rootstock union for both phenological periods. On average, for both periods of work, lower grafting results were obtained by using dormant rootstocks – 54.3 %. Grafting on "active" rootstocks leaded to an average percentage of components' union – 76.3 %.

The phenologycal periodicity of grafting has been studied by many authors. The majority of them agree with the recommendation for a summer (July to August) T-budding (Nedev et al. 1966, Petrov 1976, Grbić 2004, Dirr and Heuser 1987). According to other authors, the suitable phenological periods for grafting are winter (Grbić 2004, Bartels 2012) and spring before the beginning of growing (Petrov 1976, Celik et al. 2009, Ozturka et al. 2009). The results of the present study however showed lower percentage of components' union during the dormancy period regardless of used grafting metods.

The success of grafting is higher in spring, after the begining of the growing season. In support of these results are the studies made by Serdar and Soylu 2005. However, their results show that splice grafting can be equally successful in both phenological periods.

In addition, during spring, it is possible to obtain inverted radicle graft (Ozturk and Serdar 2011) and inverted T-budding (Serdar and Soylu 2005).

The statistical indicators of significance of various studied factors (rootstocks phenological state and method of grafting) are presented in Table 2.

As it can be seen from Table 2, grafting success depends on the phenological state of the rootstock (Significance level 0.000), used method of grafting (Significance level 0.000) and the combined effect of the two factors (Significance level 0.029).

From a production point of view, a matter of interest is not only the successful union of the grafted seedlings, but also the growth of the top shoot of the grafting (Table 3). The growth rate of the top shoot

Table 2. Significance of stock phenological stage and grafting method on the results.

Indicator	Grafting success		Top shoot length		Average nomber of shoots		Average length of the lateral shoots	
Factors	F	SL	F	SL	F	SL	F	SL
Phenological stage	24.99	0.000	0.42	0.519	0.06	0.941	1.94	0.165
Method	25.45	0.000	68.91	0.000	57.72	0.000	95.54	0.000
Phenological stage x Method	3.89	0.029	1.08	0.360	0.47	0.703	0.81	0.489

Note: *F* – Fisher's criterium; *SL* – Significance level.

is not only criterion for the duration of the production cycle, but also an indicator for the physiological status of the scion as a result of more or less successful graft union.

The longest top shoot growth was observed at splice grafting while the shortest one was observed at chip budding and side veneer grafting method in both phenological stages of the rootstock.

The most delayed growth of the top shoot was reported for chip budding and side veneer grafting (from 3.8 to 9.5 cm), while the most intensive growth was observed in splice grafting (from 23.7 to 26.2 cm). The results showed that the phenological stage of the rootstock does not affect the growth of the vegetative sapling. This was the reason why the obtained results from the different methods of work in both periods were synonymous from a statistical point of view.

In general, the reason for the small size of the scion shoots could be caused by the comparatively unsuitable conditions in the open field. Ozturk and Serdar

Table 3. Effect of the phenological stages of the rootstock and the method of grafting on the growth of the top shoot (cm), average number of scion shoots and average length of the lateral shoots (cm).

Grafting method	Growth of the top shoot		Average number of scion shoots		Average length of the lateral shoots	
	Phenological stage of the rootstock					
	dormant	active	dormant	active	dormant	active
Chip budding	3.77±0.87a	5.68±0.85a	1.00±0.00a	1.10±0.07a	0.00±0.00a	0.11±0.08a
Cleft graft	17.35±1.68b	15.03±1.35b	2.43±0.11c	2.27±0.10c	9.97±1.56b	10.31±1.21b
Side veneer graft	8.74±1.26a	9.48±1.23a	1.60±0.09b	1.69±0.07b	1.81±0.73a	3.21±0.67a
Splice graft	23.68±1.70c	26.21±1.36c	2.49±0.14c	2.46±0.08c	17.14±1.43c	20.77±1.39d

Note: Means with the same letter are not significantly different.

(2011) indicated that the most intensive growth of scion shoots could be obtained by using an overshadow of the saplings.

While the phenological stage of the rootstock have not proven its influence on the growth of vegetative saplings (Significance level 0.519), the grafting method has proven its significant effect (Significance level 0.000) from a statistical point of view. The combination of these two factors, however, also showed a statistically significant impact on the length of the top shoot (Significance level 0.360) (Table 2).

The average number of shoots formed from scion wood (Table 3) can also be used as an indicator for the physiological stages of the graft. From a production point of view, this criterion correlates with the capacity of the crown of the vegetative saplings, respectively their quality.

The lowest average number of lateral shoots is developed after chip budding (1 to 1.1 pc.). It is a logical result because only one bud is used in the budding methods. The highest number of shoots was observed in splice graft and cleft graft (2.3 to 2.5 pc.).

According to this indicator the results showed (Table 2) that the phenological stage of the rootstock itself is not significant (Significance level 0.941) as in combination with the method of grafting (Significance level 0.703). Statistically significant impact on the average number of shoots has only the method of grafting (Significance level 0.000).

As an additional indicator of the graft growth potential, the average length of lateral shoots can also be considered as a criterion of its successful union. From a production standpoint, it can be relied on the lateral shoots to form part of the stem when the top shoot is damaged by a biotic or abiotic factor. In the production of cultivated saplings, most often the cause of such damage is its breaking off as a consequence of the rapid growth and the influence of wind.

Statistically reliable differences between the two different phenological stages of the rootstocks were not observed in chip budding, cleft grafting and side veneer grafting (Table 3).

The average length of the lateral shoots of the scion is lowest (0 to 3.2 cm), after chip budding and side veneer grafting. The growth of the lateral shoot was most intensive after splice grafting (from 17.1 to 20.8 cm).

The results showed (Table 2) that the growth of the scions, with regard to the three selected criteria, is influenced only by the grafting method but not by the phenological stage of the rootstock.

The present study outlines the splice grafting as a method that ensures both the highest percentage of components union and the best scion's growth, which is in line with the recommendations of (Bratels 2012, Grbić 2004).

Despite of the recommendations for cleft grafting (Serdar et al. 2005, Duman and Serdar 2006, Bratels 2012) and side veneer grafting (Huang et al. 1994, Milev et al. 2007), which methods can reach components' union up to 100 %, the present study showed that these methods are more suitable for work with rootstocks in “active” condition and do not lead to sufficient results before the growing season. Other authors recommend whip grafting (Celik et al. 2009, Serdar and Soylu 2005) and whip and tongue grafting (Craddock and Bassi 1993).

In correspondence with the opinions of many authors (Nedev et al. 1966, Petrov 1976, Grbić 2004, Milev et al. 2007, Bartels 2012, Dirr and Heuser 1987), Sweet chestnut seedlings, including newly germinated seeds (Ozturk and Serdar 2011) are suitable rootstocks for grafting of the same species, though (Grbić 2004) shows the possibility of using *Quercus cerris* L. seedlings as well.

As it is known, in heterovegetative propagation, the scion transfers its ontogenetic stages of vegetative development to the vegetative sapling, thanks to which, early harvests could be expected. During the implemented studies, reproductive organs were formed even during the first growing season after grafting – in splice grafting during the first and second phenological period, respectively 2.67 % and 1.33 % of the saplings and in side veneer grafting during the dormant period – 2.67 %.

As a technological element of Sweet chestnut grafting, it can be noted that after the transplantation of the scions, the rootstock reacts actively by wakening of numerous dormant buds and by intensive development of shoots on the rootstock below the point of grafting. As it is known from practice, shoots hold up the growth of the scions, which requires their regular removal during the entire growing period.

Conclusion

Based on the obtained results, the following conclusions and recommendations can be made:

In the vegetative propagation of Sweet chestnut both rootstocks in active or dormant stage can be used. This finding allows for significant expansion of work periods and increase of the production volumes.

In spring grafting the T-budding is not applicable, and the side veneer grafting does not provide reliable union of the components.

In cleft grafting and in chip budding “active” rootstocks shoud be used and for splice and side veneer grafting both “active” and “dormant” rootstocks can be used.

The phenological stage of the rootstock affects the components union in chip budding and in cleft grafing. In splice grafting and side veneer grafting, results in both work periods are identical.

The highest rate of union and growth of grafts are reached in splice grafting, which determine it as the general and the most appropriate method of grafting.

The highest percentage of components union is obtained in splice, side veneer and cleft grafting on “active” rootstocks.

Based on the complex criteria it can be recommended in spring grafting of Sweet chestnut, with relatively equal width of the components the method of splice grafting to be used, and when the scions are collected from significantly thinner shoots in comparison to the rootstocks, the side veneer grafting is recommended.

The results, conclusions and recommendations offer the possibility to go beyond the established narrow frames in the practice for work with summer T-budding in the production of cultivated saplings of Sweet chestnut. They enable the utilization of the spring period of work, by using rootstocks in different phenological stages and methods of grafting.

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