



## Determination of Grafting Adaptation of Some Chestnut (*Castanea sativa* Mill.) Genotypes with Marigoule (*C. Sativa* × *C. Crenata*) Cultivar

Şemsettin Kulaç<sup>1,a,\*</sup>, Hatice Nihan Nayır<sup>1,b</sup>

<sup>1</sup>Department of Forestry Engineering, Faculty of Forestry, Düzce University, 81620 Düzce, Turkey

\*Corresponding author

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### ABSTRACT

In this study, to reproduce natural chestnut genotypes, the fruit characteristics of the Düzce region determined by local people are good in a healthy way, compatible with Marigoule (*C. sativa* × *C. crenata*) hybrid varieties resistant to branch cancer and root rot was investigated. In this study, 24 different chestnut genotypes were used. As the grafting method, the most commonly used tongue grafting, split grafting, chip budding grafting, and side grafting method were used. All procedures related to grafting were carried out in the greenhouses of the Düzce University Faculty of Forestry. In this study, local genotypes that are compatible with Marigoule chestnut were determined. As a result, the native genotype, which showed the best adaptation to Marigoule seedlings, was 87.5% of Yalnızçam, and after that, 79.2% of the Ereğli Sefer genotype. The lowest fit showed Broken genotype with 15% and Akçakoca genotype with 17.5%. Besides, the compatibility of foreign varieties Maraval, Marigoule, and Betizac were also investigated. As a result, Betizac had the highest compatibility with 95%, while Maraval had 67.5%. The most successful in the grafting methods applied was the side graft (74.2%) followed by tongue grafting (59.9%), splitting grafting (51.4%), and chip budding grafting (29.7%).

<sup>a</sup> [semsettin61@msn.com](mailto:semsettin61@msn.com)

<sup>id</sup> <https://orcid.org/0000-0002-8398-3246>

<sup>b</sup> [nihanayir@gmail.com](mailto:nihanayir@gmail.com)

<sup>id</sup> <https://orcid.org/0000-0001-8365-2941>



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### Introduction

The chestnut is a member of the Fagaceae family, and *Castanea sativa* Mill. is the name given to the tree and its edible fruit. The chestnut is a notable temperate forest tree species, often found in mixed stands in Turkey, and has multi-purpose value because of its timber and nuts (Bozkurt et al., 1982; OGM, 2018). Chestnut forms enormous mixed stands with beech, hornbeam, oak, and other broad-leaved trees in the Marmara region and northern Anatolia. Although chestnut does not occur naturally in the Aegean and Mediterranean regions, its cultivation is carried out in those areas (Yaltrık, 1993; Kulaç et al., 2017; Okan et al., 2017).

Chestnut trees develop a sturdy, even structure with a spreading body, broad branches, and a dense crown. Chestnut grows up to 20-25 m in the semi-shade of timber forests and has a deep root system, while the increment is higher in coppice forests. Due to the fact that the height growth stops earlier in productive forests, trees are cut between the ages of 50 and 70 years (T.C. Tarım ve Orman Bakanlığı, 2010). If nut production is concerned, they are cut after the age of 80 (Mayer and Aksoy, 1998). Although various methods are used for chestnut propagation, the leading technique in Turkey and worldwide is via grafting

(Serdar, 1999). In this method, the upper part (the scion or budding) is combined with the lower part (the rootstock). Grafting is classified as scion or budding according to the part used, which is taken from the desired plant, and the grown plant shows all the characteristics of this scion or budding. Grafting methods are also divided into two types according to grafting time: during active shoot growth (early or late) or when scions are dormant (Serdar, 1999). In the production of a sapling, the first step of the grafting method is the selection of a compatible rootstock. The scions or buddings taken from the desired plant should be healthy, resistant to diseases, and have a high grafting retention rate (Ertan et al., 2014). Chestnut species are also used as the rootstock, and the best results in terms of rootstock-scion in chestnuts are from individuals of the same type (Şen et al., 1993; Soylu and Ufuk 1994; Serdar 1999). Several studies have been carried out to investigate the success of grafting methods (Tokar and Kovalovsky, 1971; Şen et al., 1993; Özçağırın et al., 2005; Serdar et al., 2010; Iliev et al., 2013; Kulaç et al., 2017). However, only a limited number of studies worldwide have focused on the effect of genotypes on grafting success.

The aim of this study was to determine the compatibility of 21 different local productive chestnut genotypes, two of which are resistant to chestnut gall wasp, and three foreign cultivars (Bouche de Bétizac, Marigoule, and Maraval) using four different grafting methods. In addition, Marigoule (*C. sativa* × *C. crenata*) which is resistant to branch cancer and root rot, was used as the rootstock. In this study, we tried to determine the most successful grafting methods and the chestnut genotypes that provide the best adaptation to the Marigoule hybrid chestnut.

## Materials and Methods

### Genotype Selection for Scions and Rootstock

This study used natural chestnut genotypes having a thousand seed weight of between 13.5-15 kg (minimum) and 25-30 kg (maximum) from the area around Düzce, natural genotypes brought from other regions, and some foreign varieties. The scions from 21 different natural chestnut genotypes (Akçakoca-1, Akçakoca-2, Yığılca-2, Yığılca-4, Kırık, Alaplı-F1, Gümeli, Kadınca, Güven-1, Güven-2, Güven-3, Güven-4, Yalnızçam, Paşaoormanı-1, Paşaoormanı-2, Ereğli-Sefer, Gümeli-Sarısı, Dağlıca, Gümeli-Karası, Erfelek, and Kocaman) were used in this study and also three foreign chestnut cultivars (Bouche de Bétizac, Marigoule, and Maraval). The scions of these foreign genotypes were taken from Bouche de Bétizac, Marigoule, and Maraval chestnut trees grafted onto natural chestnut rootstock between 2006 and 2008 in the village of Dereköy in the Gümüşova District of Düzce Province.

### Seed Germination for Rootstock

Seeds collected from the Marigoule cultivars grafted onto natural chestnut in the village of Dereköy in the Gümüşova District of Düzce Province between 2006 and 2008 were washed with distilled water, and empty seeds remaining on the water surface were discarded. The selected healthy and good-quality seeds were placed in polyethylene bags and kept in the refrigerator (+4°C) until germination time (01 January 2016). For germination, first, the seeds were soaked in distilled water for about 48 h, and then washed with distilled water again and placed in the germination cabinet at +8°C. The seeds that had begun to germinate after the 28th day were planted in polyethylene bags with a potting mix composed of peat moss (30%), forest soil (30%), river soil (30%), and organic sheep manure (30%) which had been well-seasoned outside for over a year. The sowing of the germinated seeds was completed between 01 and 15 February 2016. Irrigation and weed control procedures were carried out regularly until the seedlings were one-year-old.

### Determination of Genotypes and Scion Preparation for Grafting

In order to determine the genotypes, first, we worked with the regional Forest Management Directorates under the General Directorate of Forestry from the provinces in the Western Black Sea Region. Genotypes in the natural forests in the region were determined through the Forest Enterprise managers, village headmen, and local villagers within the forest management boundaries. Also, we monitored the chestnut market of Düzce Province and identified the high-quality genotypes. The high-quality

genotypes were investigated for two years to determine the characteristics and quality of their nuts. Over January – February 2017, scions from selecting the best-quality chestnut genotypes were collected, wrapped in polyethylene bags, and taken directly to the laboratory. After sterilizing with 1-3% sodium hypochlorite (bleach), the scions, which were cut with 3-5 eyes in each, were placed in perlite in the refrigerator at +4°C for about a month until grafting.

### Selection of Grafting Methods

For this study, tongue grafting, cleft grafting, side grafting, and chip budding methods were chosen because they the local people commonly use them. The grafting procedures were begun on 10 February 2017 and were carried out in a temperature- and moisture-controlled greenhouse. The mean day and night ambient temperatures were 22-25°C and 12-18°C, respectively. All equipment was regularly sterilized. When the procedure was completed, grafting tape was used to add structural support and prevent water loss after the scion and rootstock were placed in contact with each other. Instead of grafting wax, beeswax with propolis was then melted and applied to the tips of the scions and the scion-rootstock grafting area.

### Grafted Sapling Maintenance and Control Procedures

Three to seven days after the chip budding process, the fast-growing shoots were cut, and 15-20 days after grafting, the shoot was cut 2-3 internodes above (approximately 5-10 cm) the grafting point. After 4-6 weeks, the shoots were cut 1 cm above the grafting point. In addition, irrigation, removal of unwanted shoots, and weed control were carried out regularly for all saplings throughout the experiment period. After six weeks of grafting, all grafting tape was removed, the grafted scions were checked, and then the wound surfaces were closed immediately with the beeswax-propolis. If the scion sapling shoots were more than 8-10 cm in length, the grafting was considered to have been successful.

### Data Analyses

The experimental setup consisted of a randomized complete block design with 24 genotypes with three replicates. Each grafting method (tongue, cleft, side graft, and chip budding) was considered as a block. Each genotype had 20 seedlings for each grafting method, totaling 1440 individual plants for each grafting method. In total, 4320 chestnut plants were grafted for this study. Analysis of variance (ANOVA) was performed using the SPSS 22.0 statistical program, with the level of statistical significance set as alpha = 0.05. When the model was significant, the Duncan test was used to compare the success rates of the grafting methods (Özdamar, 1999).

## Results

The grafting methods, genotypes, and their interactions significantly ( $P < 0.05$ ) affected the grafting success rate (Table 1) at ambient temperature. The chestnut genotypes and grafting methods yielded different success rates. The side graft method had the highest success rate (74,2%), while chip budding had the lowest (29,7%), as shown in Figure 1.

Table 1. Analysis of variance (ANOVA) results for grafting methods, genotypes, and their interactions.

| Source of Variance   | Degrees of Freedom (df) | Sum of Squares | Mean Square | F-value | P-value |
|----------------------|-------------------------|----------------|-------------|---------|---------|
| Grafting methods (M) | 3                       | 78691.2        | 26230.4     | 515.7   | 0.000   |
| Genotypes (G)        | 23                      | 139243.2       | 6054.1      | 119.0   | 0.000   |
| M × G                | 68                      | 51096.3        | 751.4       | 14.8    | 0.000   |
| Error                | 193                     | 9816.7         | 50.9        |         |         |
| Total                | 288                     | 1095800.0      |             |         |         |

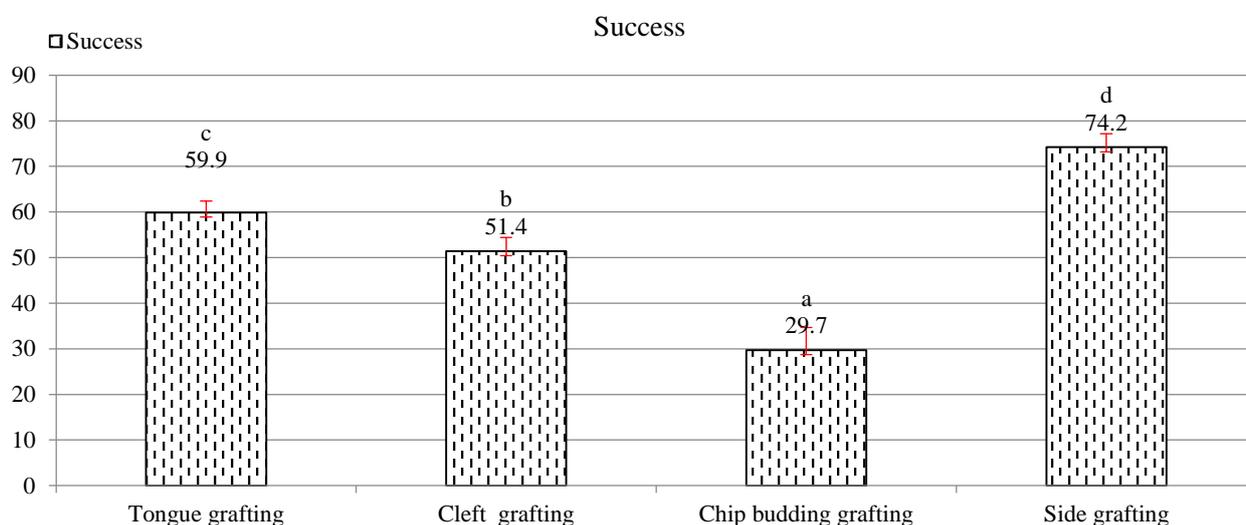


Figure 1. Success rates of the grafting methods.

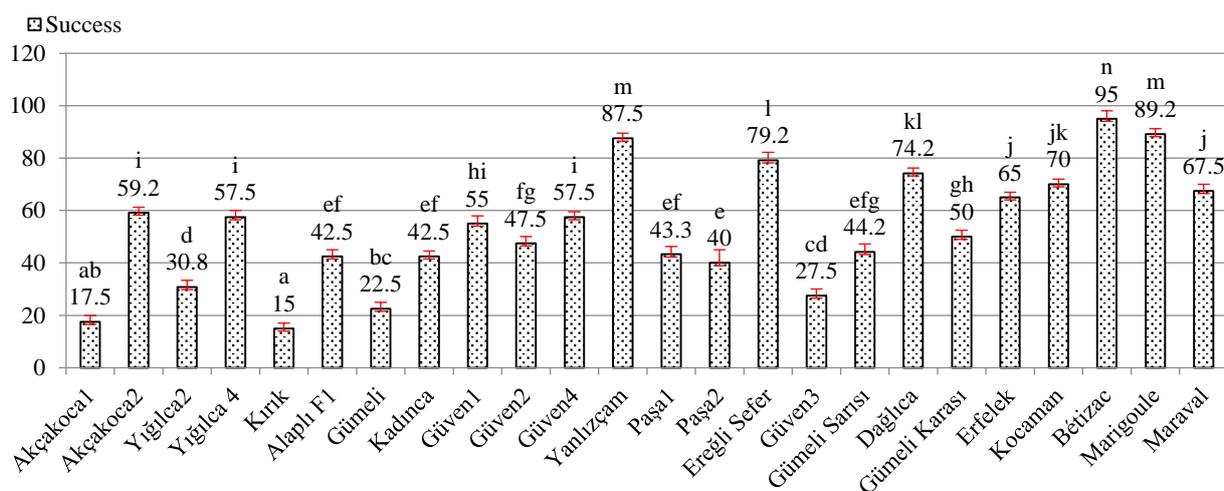


Figure 2. Grafting success rates of genotypes.

The sapling Bouche de Bétizac genotype had the highest grafting success rate (95%), followed by Marigoule (89,2%) and Yalnızçam (87,5%) genotypes, whereas Kırık had the lowest rate (15%), followed by Akçakoca-1 (17,5%), as seen in Figure 2.

All M × G interactions are shown in Table 2. The interactions of the foreign genotype Bouche de Bétizac and Marigoule saplings with the tongue, cleft, and side grafting methods yielded 100% grafting success rates (Table 2). As for the local genotypes, the interactions of the Yalnızçam genotype saplings with the tongue, cleft, and side grafting methods showed 100% grafting success. In addition, Paşormanı-1, Paşormanı-2, Dağlıca and Gümeli-Karası genotype saplings yielded 100% success with side grafting. Ereğli-Sefer genotype saplings also had 100% success with

tongue and cleft grafting. The lowest grafting success rate (10%) was found with the interactions of Akçakoca-1 and Kırık chestnut genotypes with the chip budding, cleft, and tongue grafting methods (Table 2). Furthermore, interactions of Paşormanı-2, Güven-3, Akçakoca-1, Kırık, Yığılca-2, Gümeli, and Kadınca genotypes with chip budding showed the lowest (10%) grafting success (Table 2).

## Discussion

The first step for success in the production of saplings using grafting is to choose the appropriate rootstock. Healthy, desirable rootstocks must be selected on the basis of resistance to disease and high grafting success rates

(Ertan and Seferoğlu, 1998). Therefore, the Marigoule cultivar, which is resistant to many diseases, was chosen as the rootstock in the study. For example, Spanish chestnut genotypes were grafted onto Marigoule rootstocks by Pereira-Lorenzo and Fernandez-Lopez (1997) using the cleft, tongue, T-budding, and chip budding methods. In that study, most grafting methods had more than 70% grafting success, while in our study, the tongue, cleft, and chip budding had 59%, 51%, and 29% success rates, respectively. This result indicates that chestnut genotypes in our region are generally less compatible with Marigoule chestnut cultivars than Spanish chestnut genotypes. Another study conducted in the spring on Anatolian chestnut rootstocks focused on the phenological stage effect of grafting success on different grafting methods such as cleft, chip budding, side graft, and others (Iliev et al., 2013). In that study, the success rates of the chip budding, cleft, and side grafting methods were 37%, 60%, and 73%, respectively. These results were in line with our study regarding similar success values for grafting methods and success ranking within methods. A field study was carried out by the Eğirdir Horticultural Research Institute (Isparta, Turkey) using three grafting methods (chip budding, splice grafting, and tongue grafting) on chestnut clone rootstocks (M9, MM106, and MM1111). The grafting success rates of the tongue, splice, and chip budding methods were 80,60%, 75,91%, and 33,58%, respectively. Considering all interactions, tongue and splice grafting were the methods recommended for application, while chip budding was not found to be economical (Özongun et al., 2004). In parallel with their

study, chip budding had a low success rate (29%) in the chestnut saplings compared to the other grafting methods and was seen as uneconomical in the present study.

Some studies have focused on the success of grafting methods on mango, black mulberry, shea tree, and tallow tree. In a study of mango grafting methods and the success rate depending on grafting time in the Amhara region of the Kalu district in northeastern Ethiopia, the highest grafting success (100%) was achieved in both June and March with the cleft grafting method, while a grafting success rate of 20% was obtained in January and October using the tongue grafting method (Wubeshet et al., 2019). It was suggested that the reason for achieving high rates of success with cleft grafting was due to the different mango types and grafting in different months (Wubeshet et al., 2019). A study conducted on black mulberry found success rates for tongue grafting, chip budding, and side grafting as 75%, 70%, and 58%, respectively (Zenginbal and Eşitken, 2016). Another study conducted on the shea tree (*Vitellaria paradoxa*) found that cleft, tongue, and side grafting methods had 86%, 80%, and 58% success rates, respectively (Sanou et al., 2004). In a study investigating the suitability of the tallow tree (*Allanblackia floribunda*), a new oil tree for Africa, for grafting, the highest success was achieved with side grafting (80%), followed by side-veneer grafting (52%), and cleft grafting (50%), with the lowest grafting success found for the chip budding method (13%) (Asaah et al., 2008). In the present study, the chip budding method had the lowest grafting success, whereas side grafting had the highest. These results were attributed to species differences.

Table 2. Interactions of chestnut genotypes and grafting methods.

| Chestnut Genotype | TG   |    | CG   |    | CBG  |     | SG   |    |
|-------------------|------|----|------|----|------|-----|------|----|
|                   | Ort. | DG | Ort. | DG | Ort. | DG  | Ort. | DG |
| Akçakoca-1        | 10%  | a  | 10%  | a  | 10%  | a   | 40%  | cd |
| Akçakoca-2        | 80%  | g  | 80%  | g  | 20%  | ab  | 56%  | Ef |
| Yığılca-2         | 40%  | cd | 23%  | ab | 10%  | a   | 50%  | de |
| Yığılca-4         | 70%  | fg | 50%  | de | 55%  | def | 60%  | fg |
| Kırık             | 10%  | a  | 10%  | a  | 10%  | a   | 30%  | bc |
| Alaplı-F1         | 60%  | ef | 20%  | ab | 30%  | bc  | 60%  | ef |
| Gümeli            | 20%  | ab | 20%  | ab | 10%  | a   | 40%  | cd |
| Kadınca           | 50%  | de | 50%  | de | 10%  | a   | 60%  | ef |
| Güven-1           | 60%  | ef | 60%  | ef | 30%  | bc  | 70%  | fg |
| Güven-2           | 60%  | ef | 30%  | bc | 30%  | bc  | 70%  | fg |
| Güven-4           | 60%  | ef | 60%  | ef | 40%  | cd  | 70%  | fg |
| Yanlızçam         | 100% | h  | 100% | h  | 50%  | de  | 100% | h  |
| Paşaormanı-1      | 20%  | ab | 40%  | cd | 13%  | a   | 100% | h  |
| Paşaormanı-2      | 30%  | bc | 20%  | ab | 10%  | a   | 100% | h  |
| Ereğli-Sefer      | 100% | h  | 100% | h  | 46%  | d   | 70%  | fg |
| Güven-3           | 30%  | bc | 20%  | ab | 10%  | a   | 50%  | de |
| Gümeli-Sarısı     | 80%  | g  | 40%  | cd | 16%  | a   | 40%  | cd |
| Dağlıca           | 96%  | h  | 80%  | g  | 20%  | ab  | 100% | h  |
| Gümeli- Karası    | 40%  | cd | 40%  | cd | 20%  | ab  | 100% | h  |
| Erfelek           | 60%  | e  | 60%  | e  | 40%  | c   | 100% | h  |
| Kocaman           | 80%  | g  | 60%  | e  | 40%  | c   | 100% | h  |
| Bétizac           | 100% | h  | 100% | h  | 80%  | g   | 100% | h  |
| Marigoule         | 100% | h  | 100% | h  | 56%  | e   | 100% | h  |
| Maraval           | 80%  | g  | 60%  | e  | 30%  | B   | 100% | h  |

\* TG: Tongue Grafting, CG: Cleft Grafting, CBG: Chip Budding Grafting, SG: Side Grafting, DG:Duncan Group.

## Conclusion

This study affirms the importance of chestnut genotypes on the grafting success of the plant propagation system. The local chestnut genotypes most compatible with the Marigoule rootstock were the Yalnızçam and Ereğli-Sefer cultivars, while the Kırık and Akçakoca-1 genotypes had the lowest success rates. The propagation of these genotypes (Yalnızçam and Ereğli-Sefer) can prove economical when mass chestnut production is considered. Among the foreign genotypes, Bouche de Bétizac had the highest grafting compatibility with the rootstock. As a grafting method, the side graft had a high compatibility rate, while chip budding had the lowest rates compared to the other methods. As a result, side grafting and tongue grafting methods can be recommended to produce grafted chestnut saplings in Turkey and around the world. Additional genotypes that have resistance to both gall wasp and branch cancer should be detected, tested, and propagated across Turkey.

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