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Effect of Grafting Method and Time on Grafting Success in Anatolian Chestnut (*Castanea sativa* Mill.)

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A R T I C L E I N F O

Research Article

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A B S T R A C T

Chestnut is an economically important tree because of wood and nut production, which is usually found in warm-temperate species, especially Mediterranean region. The fruit of chestnut is a good antioxidant source besides being rich in vitamin C. Anatolian chestnut (*Castanea sativa* Mill.) is a natural chestnut species in Türkiye. This study aimed to determine the most appropriate grafting time and grafting method for two chestnut cultivars (Marigoule and Erfelek) collected from the Western Black Sea region. Two different varieties (Marigoule and Erfelek) and three different grafting methods (chip budding, tongue, and cleft) were applied in different environments (in the hoop house and open field) for six months (December, January, February, March, April, and July). Analysis of variance was used for the grafting trials which was established and applied in the factorial trial design. Results showed that grafting success in the hoop house was twice as high in the open field, and the tongue was the most successful grafting method in all months except July. However, chip budding was observed as the most successful method in July. The highest grafting success was achieved in February, followed by July. According to the obtained results, Marigoule (*C. crenata* x *C. sativa*) cultivars were more successful than Erfelek cultivars. Marigoule cultivar's success rate was 86% for the tongue method in February at hoop house. In July, 83% grafting success was achieved.

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Introduction

Chestnut belongs to the Fagaceae family (Liu et al., 1988), which spreads in the Asian, European, and American continents of the Northern Hemisphere and partially in South America. The major chestnut-producing countries in Europe are Türkiye, Italy, Spain, Portugal, and France (Markovski et al., 2014). Chestnut stands spread throughout the Eastern Black Sea and in Western Anatolia along with the Marmara and are also locally found in the Mediterranean (Isparta-Center, Isparta-Sütçüler, Antalya-Serik, Antalya-Alanya) region in Türkiye (Kulaç et al., 2018). Therefore. Türkiye ranks fourth in the world's chestnut export with 11.39%, after China, Italy, and Portugal as of 2018. Türkiye earned more than $40 million in revenue from this export in 2018 (FAO, 2019).

Chestnut species are essential to fruit and wood production, as well as economic importance by producing high-quality wood and timber. Chestnut trees are durable because they decoratively easily penetrate varnish, and paint adheres well with nails or glue. The wood of chestnut is primarily preferred in pier construction and water vehicles such as furniture, boats, yachts, and ships (Conedera et al., 2004; Kakavas et al., 2018).

It has been suggested that the most suitable method of seedling production is vegetative production which is usually preferred in chestnut cultivation worldwide, especially in Türkiye (Ertan and Kılınç, 2005). Therefore, grafting, cutting, dipping, and micro-production are used for the vegetative propagation of chestnuts (Vieitez, 1981; Ferrini, 1993). Chestnut grafting methods are tongue, cleft, chip budding, split, etc. In addition, grafting has proven to be a more successful and feasible method than any other technique in the chestnut vegetative production industry (Keys, 1978; McKay and Jaynes, 1969). Grafted saplings are needed for producing chestnut saplings as demanded by the domestic and foreign markets and to spread the existing chestnut areas to larger areas (Ertan et al., 2015).

Grafting success depends on several factors: grafting time, rootstock age, size, developmental status, quality and quantity of cuttings, susceptibility to chestnut cancer, the experience of the practitioner, grafting cost, grafting environment, and technical possibilities (Ferrini and Pisani, 1994). Grafting time is the most critical factor for grafting success (Yetkin, 2010).

Plants' phenotypic characteristics are affected with both mutual interaction between genetic structure (Sevik, 2012; Yildiz et al., 2014; Güney et al., 2021; Tokatli et al., 2021; Aydın Uncumusaoglu and Mutlu, 2022; Ghoma et al., 2022) and environmental conditions such as climatic (Koç 2021a, b; Güney et al., 2022; Varol et al.,2022a, b; Key et al., 2022) and edaphic factors (Shults et al., 2020; Koç et al., 2021; Cesur et al., 2022; Kuzmina et al., 2022; Özel et al., 2022). It is known that many factors significantly influence plant metabolisms, such as stress level (Sevik et al., 2019; Savas et al., 2021; Isinkaralar et al., 2022; Key and Kulaç, 2022; Koç and Nzokou, 2022), genetic structure (Turna et al., 2006; Sevik et al., 2012; Isinkaralar et al., 2022a.b), hormone treatments (Turna et al., 2013; Sevik and Cetin, 2016; Yıldırım et al., 2020), cultivation practices such as pruning, irrigation, and shading (Wilson et al., 2013; Kulaç and Yıldız, 2016; Mutlu, 2019; Güney et al., 2020; Kutlu and Mutlu, 2021; Demir et al., 2021; Mutlu, 2021; Mutlu et.al., 2021). Therefore, the healing process of the grafted area of the tree is affected by the applied propagation method, rockstock-scion combination, insects and diseases, and environmental conditions (Hartmann et al., 1990).

This study aimed to determine the most appropriate grafting time and method for two chestnut cultivars, Marigoule and Erfelek, collected from the Düzce region, Türkiye. Two varieties and three different grafting methods (chip budding, tongue, and cleft) were used in two environments: hoop house and open field for six months (December, January, February, March, April, and July). In the study, scions were taken from the gardens of the citizens of the Sinop Erfelek and the Gümüşova District-Dereköy village. Saplings obtained from the seeds collected from the natural chestnuts of Düzce province Chestnut Bayırı were used as a rootstock.

Materials and Methods

Study Area

Seeds were collected from natural chestnut forests in Kestane Bayırı, Akçakoca district of Düzce province. Firstly, the seeds were stored at +2°C for two weeks for cold and moist stratification. Then, seeds were put into petri dishes and moved into the germination chamber (+8°C) in the silviculture laboratory at Düzce University. The seeds, which were kept in the room for 12h once a week were washed with distilled water and then put into the germination chamber at +8°C.

Figure 1. The location where scions and seeds were taken, yellow row shows that Akçakoca where supply of seeds, red rows indicate the location where the scions were collected.

The seeds that started to germinate after the 28th day were transferred to polyethylene bags (11×25 cm). A mixture of peat forest soil, stream sand and organic burnt sheep manure at a ratio of 1:1:1:1 was used as the growing medium. Regular maintenance procedures were carried out until the age of 1 year. Grafting procedures were carried out on seedlings that were 1 year old. The study was conducted in Düzce University Forestry Faculty hoop house and silviculture laboratory in June 2013 to December 2016.

Supply of Scions

Scions were obtained from grafted chestnut trees in the Dereköy village of Erfelek district in Sinop and Gümüşova, Düzce district in Düzce. The scions were placed in polyethylene bags and stored in a cold storage room. Scions were cut which had 3-4 eyes in each scion, and were kept in perlite at +4°C for about 3 weeks (excluding April and July grafts) until grafting.

Grafting Methods

Three different grafting methods (chip-budding, tongue, and cleft) were used in this study. For each grafting method, 10 seedlings were grafted with 3 replicates. Grafts were applied to two different genotypes (Marigoule and Erfelek). The hoop house and open field were applied as two environmental conditions. Grafting was done in the last week of every month. It was repeated in 6 different months (December, January, February, March, April, and July), and three different grafting methods were subjected on the same day. A total of 2160 saplings were grafted in this study.

Determination of Grafting Success

After grafting, the lower shoots on the rootstocks were cut. Grafting was applied to the cut surface. The rate of grafting was determined by proportioning the grafted seedlings to all seedlings, and also success rates were determined by counting the grafts that were shed 45 days after the grafting, which had a shoot size of 5 cm. The daily average, maximum and minimum temperature differences were provided by the Düzce Meteorology Regional Directorate for the climate data of the times (Table1).

Statistical Analysis

In the study, analysis of variance (ANOVA) was performed with the SPSS 22.0 statistical package program to determine the significant difference in terms of the effects of chestnut cultivars and grafting methods and times on the success of grafting, and the Duncan test was used to determine homogeneous groups.

Results

Success of Grafting Methods in the Open Field

The chip budding method had the lowest success rate, which was an average of 20.19% for Marigoule varieties, while the cleft method had an average of 5.19% for Erfelek varieties in the open field. On the other hand, the highest success was observed in tongue graft, with an average of 34.54% for Marigoule varieties, while the tongue method with an average of 18.15% for Erfelek varieties in the open field (Figure 2).

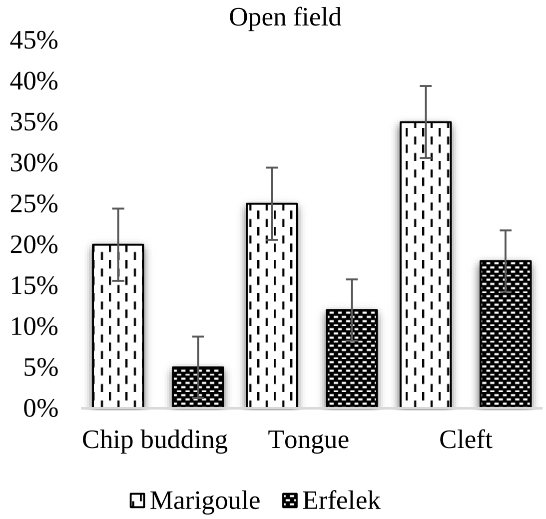

Figure 2. Success rates of grafting methods in open field

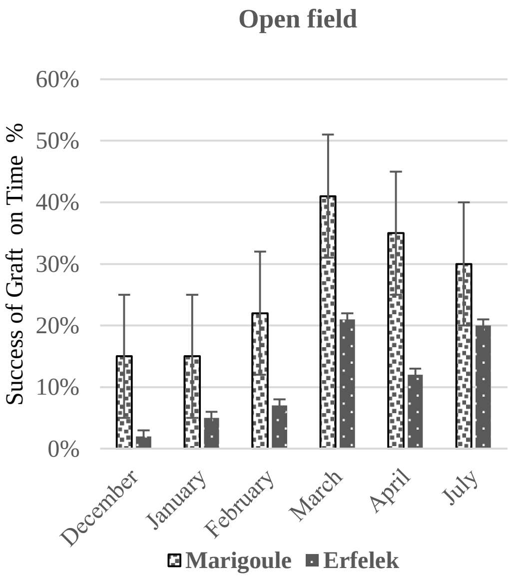

Figure 3. Success rate of grafting times in open field

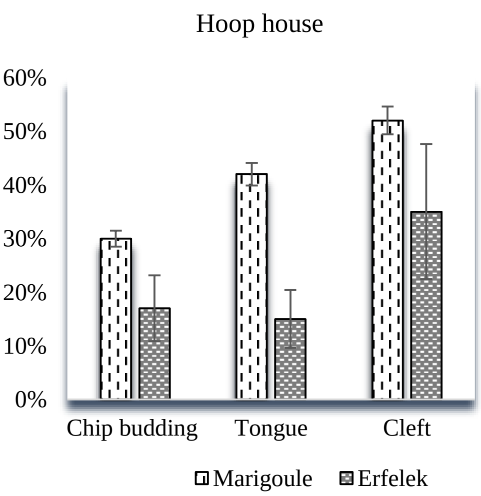

Figure 4. Success rates of grafting methods in the hoop house

As a result of Duncan's analysis, the highest grafting success rate of the Marigoule variety was observed in March in the open field, with an average of 42.22%, while the lowest success rate was in December and January, with an average of 15.56%. On the other hand, the highest grafting success rate for the Erfelek variety was observed in March in the open field, with an average of 21.48%, while the lowest success rate was in December, with an average of 2.86% (Figure 3). Regarding grafting success, the effect of grafting methods, environments, and grafting time showed significant differences ($P<0.01$). The interaction of grafting types x months was significant for the Erfelek variety.

As a result of analysis for the Marigoule cultivar, the lowest success was seen in the chip budding with an average of 20.19%, while the highest success was observed in the tongue with an average of 34.54%. On the other hand, for the Erfelek cultivar, the highest grafting success rate was seen in Dilcikli graft in March, while the lowest success in cleft was observed in December.

Success of Grafting Methods in the Hoop House

As a result of Duncan's analysis, the highest grafting success rate was found to be 54.08% in the grafting varieties applied to the Marigoule variety in the hoop house environment. The lowest rate was observed in the chip budding (30.56%). The highest grafting success rate was observed in the tongue (35.56%), while the lowest rate was observed in the cleft (15.19%) for Erfelek cultivars (Figure 4).

On the other hand, the highest grafting success rate was observed in February with an average of 54.08% in grafting varieties made in different months in the hoop house in the Marigoule variety. The lowest success rate was observed in December, with an average of 32.59%. The highest grafting success rate in the Erfelek cultivar was observed in February and March with an average of 33.33% with an average of 7.41%. The lowest success rate was observed in December (Figure 5). The effect of grafting methods environments and grafting time observed statistically ($P<0.01$) significant differences in terms of grafting success as a result of ANOVA analysis. The interaction of the methods of months x grafting in Marigoule and Erfelek varieties graft cultivars is statistically significant in the hoop house (Table 4).

While the highest grafting success rate was observed in Marigoule with tongue in February, the lowest success rate was observed in December for the chip budding method. The highest grafting success rate was observed with an average of 35.56% in the graft varieties applied in the Erfelek variety in the hoop house, while the lowest rate was observed in the cleft with an average of 15.19% as a result of Duncan analysis (Table 5).

According to the success of grafting results, environment and grafting time showed statistically significant differences ($P<0.01$). While the interaction of chestnut cultivar and grafting methods is significant, the interaction of area x chestnut cultivar and medium x graft type is not significant (Table 6).

The highest grafting success was for Marigoule (34%) when considering the average of all media and grafting methods. This rate was 17% for Erfelek cultivar. The highest grafting success was observed in Marigoule (33%).

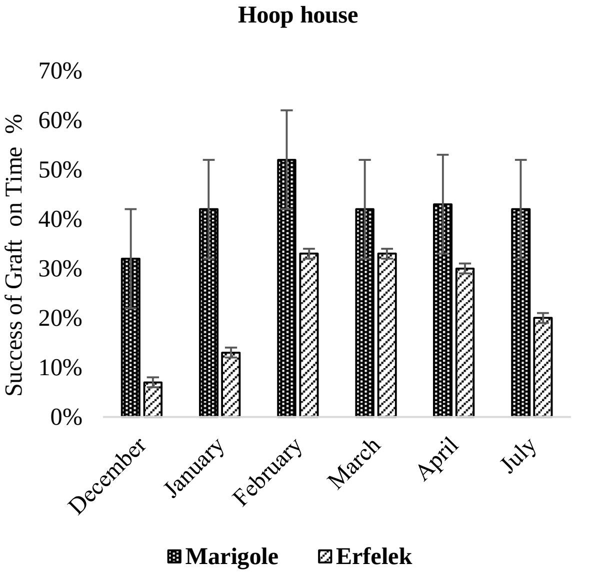

Figure 5. Success rate of grafting times in the hoop house

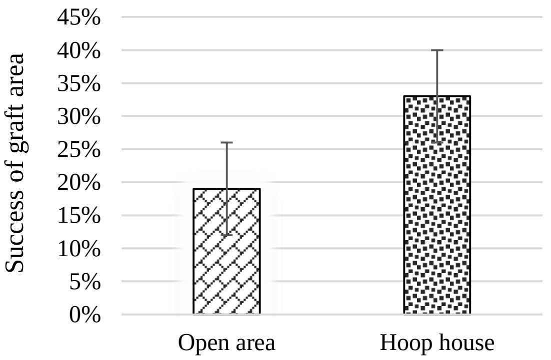

Figure 6a) Grafting success depend on environment.

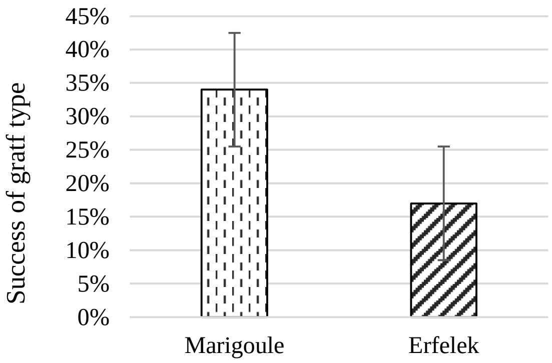

Figure 6b) Success of grafting according to the Margoule and Erfelek varieties.

The cleft method showed the highest success in all months except for July, while the lowest success was in December. The chip budding method was determined to have the highest success in January and February while the lowest in March. However, the highest success was detected in the chip budding grafting in July (Table 7).

The highest grafting success was observed in the cleft (35%), while the lowest success was observed in chip budding (20%), depending on the averages of environment conditions and grafting times (Figure 7b). On the other hand, the highest grafting success was observed in grafting in March (35%), while the lowest success was observed in December (15%), depending on chestnut cultivar's average and all of the grafting types (Figure 7a).

Discussion

The planet has faced many casualties in the last two centuries, such as climate change, air pollution, flooding, and earthquakes. Especially the results of climate change cause many disorders on climate local and global scale (Canturk and Kulaç, 2021; Koç, 2021a, b; Koç, 2022a). First, changing climate causes global warming worldwide and results in drought stress in conifer (Koç, 2021c, d; Koç et al., 2021; Koç and Nzokou, 2022) and broad leaf (Koç, 2022b) plant species. Second, air pollution is another factor that has many side effects on plants, such as heavy metal contamination (Koç, 2021e; Isinkaralar et al., 2022; Key et al., 2022; Koç et al., 2022). All these environmental factors adversely affect plant growth, development, and physiology (Koç, 2019). Chestnut, a sensitive plant species, is most affected by these unfavorable factors. While plant propagation can be done both vegetatively and generatively the cultivation of chestnut seedlings is done by the grafting method in terms of fruit quality and productivity.

There are various studies investigating the effect of different grafting methods on the success of grafting for all that the limited number of studies on chestnut grafting time in the world and especially in our country (Ertan et al., 2015). Soylu (1982) observed that the highest grafting success rate was obtained from the T and inverted T budding methods in May. Beshir et al. (2019) proposed that whip graft for mango in open-field grafted tree production in parallel to Hardy (1960), and Craddock and Bassi (1993) after the start of the growth activity of the rootstocks. Woodroof (1967) and Craddock and Bassi (1993) reported that low temperatures may delay the healing of the grafting and that the grafts lead to damage by late spring frosts. On the contrary, the study of Soylu and Serdar (2005), in parallel with our study, showed that the highest chestnut grafting success in a hoop house was obtained from inverted Tbudding after leaf appearance on rootstocks in spring. Our study obtained successful grafting in the chip budding graft in March. In another study, five grafting methods, nine Spanish hybrid rootstocks and French CA15 Marigoule, different grafting dates, growth of grafted plants and short diameter of up to four years, and compatibility between scion and rootstocks were examined, but the results were not successful in all methods. However, grafting was most successful in late August and early September (Pereira and Fernandez, 1997). In our study, while the chip budding was the most successful in July, the tongue gave the most successful results in other months, especially in March. Grafting time and types are different between the two studies.

In a study conducted in Sinop Bektaşağa Forest Nursery, the success of grafting was investigated by applying different grafting methods at different times and three different grafting methods (T budding, inverted T, and tongue) were used in two chestnut types (SA 5-1. SE21-9) and 8 grafting periods between spring and autumn (Serdar, 2000).

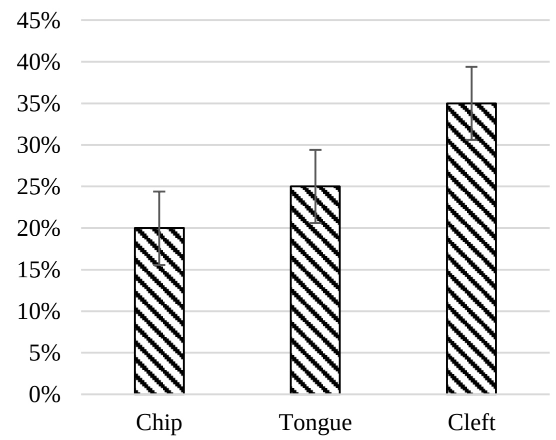

Figure 7a) Success of grafting depend on grafting types

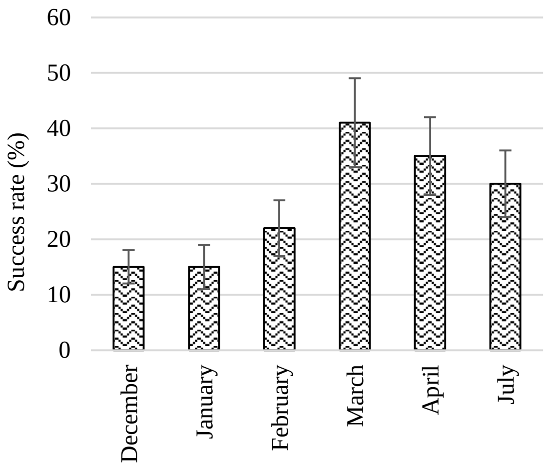

Figure 7b) Success of grafting depend on time.

Table 1. Climate data for the study period.

Day/Month/Year	2015 December		2016 January		2016 February		2016 March		2016 April		July 2016	
Temprature °C	ATD (°C)	MTD (°C)	ATD (°C)	MTD (°C)	ATD (°C)	MTD (°C)	ATD (°C)	MTD (°C)	ATD (°C)	MTD (°C)	ATD (°C)	MTD (°C)
1	6.7	11.6	-2.5	3.9	2.2	7.0	22.0	18.3	15.8	19.3	23.6	11.1
2	7.1	9.3	-5.7	11.9	5.7	7.2	13.3	10.1	13.0	11.7	23.5	12.7
3	3.5	4.1	-3.8	4.6	4.8	14.8	11.1	4.4	11.3	9.8	24.1	8.3
4	2.7	6.1	2.6	9.1	8.6	15.8	11.4	10.9	9.9	17.9	24.6	9.7
5	2.2	8.6	2.5	3.0	7.5	7.5	9.3	4.2	12.3	19.6	23.6	9.6
6	1.4	13.0	3.2	4.3	2.9	5.9	7.9	18.4	14.4	22.1	22.2	7.1
7	3.2	14.0	12.2	16.1	1.4	6.5	12.7	18.7	16.4	22.0	23.0	8.3
8	3.8	10.7	6.2	4.0	1.3	13.7	13.0	17.6	16.7	17.3	22.3	10.1
9	6.9	10.2	5.8	12.3	3.0	15.3	14.3	21.6	19.0	17.4	20.3	14.3
10	4.3	7.3	5.8	17.0	5.1	19.6	14.2	11.6	15.7	7.9	21.4	14.7
11	6.7	1.8	11.6	11.4	11.3	5.9	11.4	11.9	12.9	10.6	22.1	10.4
12	6.8	4.6	13.2	16.0	10.3	11.7	10.9	14.0	11.7	2.6	23.0	14.3
13	7.5	4.6	10.7	10.5	11.8	7.9	10.3	8.2	14.6	12.0	24.6	15.7
14	3.7	11.7	6.2	4.8	15.5	11.3	8.6	4.1	16.6	19.0	26.1	10.4
15	3.7	11.8	2.1	10.2	16.3	13.9	6.3	3.4	16.3	10.2	24.7	13.8
16	7.4	6.2	9.6	12.0	16.3	18.1	6.0	3.9	15.3	18.7	26.2	17.8
17	4.5	9.2	15.8	9.1	14.8	15.9	4.9	6.5	17.0	22.1	25.7	16.4
18	2.9	11.8	3.9	9.5	12.6	14.2	5.2	17.2	19.2	20.7	23.9	5.9
19	1.8	12.6	0.2	2.7	9.8	3.7	6.9	5.4	20.7	21.5	20.8	5.4
20	1.8	11.5	-4.0	8.8	7.6	2.1	6.7	4.4	18.1	13.8	20.4	8.7
21	0.2	6.6	-0.4	5.4	6.7	2.6	8.4	15.5	10.8	6.7	21.3	7.7
22	0.7	11.6	1.2	2.9	5.7	14.6	12.0	14.1	10.4	20.1	21.0	14.4
23	2.5	15.2	-0.5	3.5	8.7	20.6	15.5	15.8	13.5	15.8	21.7	14.5
24	3.4	15.3	-2.3	6.9	9.9	9.5	19.8	10.5	15.3	15.8	22.1	16.6
25	4.3	12.8	-1.5	4.2	9.9	3.3	9.7	5.8	16.0	11.5	22.7	13.1
26	4.6	5.7	-4.7	6.1	10.4	12.8	5.8	3.0	13.9	15.5	23.8	12.4
27	2.9	4.5	-1.3	6.8	12.4	6.0	3.2	7.3	12.4	9.6	23.3	12.8
28	1.1	7.9	0.7	5.5	10.6	13.6	5.2	5.4	12.4	15.6	24.4	10.5
29	1.9	12.7	0.3	6.4	10.8	23.0	5.0	16.7	12.0	16.4	24.7	12.1
30	2.2	8.2	2.0	1.7	0.0	0.0	11.7	13.3	14.3	10.5	24.9	10.2
31	-1.8	2.8	2.3	5.6	0.0	0.0	13.0	18.7	0.0	0.0	24.9	12.9
Avg.	3.57	9.16	2.95	7.62	8.19	10.45	10.18	11.00	14.13	14.64	23.25	11.67

ATD: Avg Temp of Day, MTD: Max. min. temp. Difference of Day

The results showed that the highest success was tongue grafts (85.2%) in 3rd period (2-13 May), but our study differently determined that the highest grafting success rate was obtained tongue grafting with the Marigoula variety in February. On the other hand, Öztürk et al. (2009) studied that Marigoule grafting was applied to seedlings with SE 3-12 genotypes and 554-14 genotypes between 10 May 2006 and 29 April 2007 using different grafting methods in different plantation conditions (shaded and unshaded hoop house) and researchers showed that the highest success was achieved in the shaded hoop house in May 2006. Therefore, our study showed that the highest success (86%) was achieved in February 2016 in the Marigoule variety in the hoop house.

Table 2a. The results of ANOVA variance analysis in open field for Margoule varieties

Source of variance	Sum of squares	Degrees of freedom	Mean of squares	F value	Importance level (p)
Corrected Model	10900.320	17	641.20	10.35	0.00
Intercept	32667.66	1	3266.66	624.29	0.00
Months	5226.40	5	1057.22	17.07	0.00
Types of Graft	1292.52	2	946.29	15.22	0.00
Months x Types of Graft	3721.35	10	372.13	6.01	0.00
Error	2229.79	36	61.94		
Total	51797.72	54			
Corrected Total	13130.11	53			

Table 2b. The results of ANOVA variance analysis in open field for Erfelek varieties.

Source of variance	Sum of squares	Degreesof freedom	Mean of squares	F value	Importance level (p)
Corrected Model	6750.661	17	397.10	25.95	0.00
Intercept	6269.56	1	6259.56	409.77	0.00
Months	2578.25	5	515.65	33.70	0.00
Types of Graft	15211.45	2	760.22	49.69	0.00
Months x Types of Graft	2588.77	10	258.88	16.92	0.00
Error	520.21	34	15.30		
Total	14504.98	52			

Table 3. Variance analysis results of grafting varieties according to months in Marigoule and Erfelek cultivars in open field.

Months		Marigoule			Erfelek		
		Mean	Std. Deviation	N	Mean	Std.Deviation	N
December	Chip budding	0.00	0.00	3	0.00	0.00	1
	Tongue	20.00	6.67	3	0.00	0.00	3
	Cleft	26.67	6.67	3	6.67	6.67	3
	Total	15.56	12.91	9	2.86	5.24	7
January	Chip budding	0.00	0.00	3	0.00	0.00	3
	Tongue	20.00	13.33	3	21.22	3.85	3
	Cleft	26.67	17.64	3	13.33	6.67	3
	Total	15.56	16.33	9	5.19	7.29	9
February	Chip budding	8.89	3.85	3	0.00	0.00	3
	Tongue	20.00	0.00	3	4.45	3.85	3
	Cleft	37.72	3.85	3	15.55	3.85	3
	Total	22.22	12.91	9	6.67	7.45	9
March	Chip budding	40.00	6.67	3	28.89	3.85	3
	Tongue	33.33	6.67	3	4.45	3.85	3
	Cleft	53.33	13.34	3	31.11	3.85	3
	Total	42.22	12.02	9	2148	13.24	9
April	Chip budding	26.67	0.00	3	2.22	3.85	3
	Tongue	40.00	6.67	3	6.67	6.67	3
	Cleft	37.72	13.22	3	28.89	3.85	3
	Total	34.22	9.27	9	12.59	13.10	9
July	Chip budding	45.56	1.93	3	33.33	2.89	3
	Tongue	20.00	0.00	3	13.33	0.00	3
	Clef	25.00	0.00	3	13.33	0.00	3
	Total	30.19	11.77	9	20.00	10.11	9
Total	Chip budding	20.19	19.02	12	12.08	15.47	16
	Tongue	25.56	10.29	12	5.19	5.39	18
	Cleft	34.54	13.22	12	18.15	9.92	18
	Total	26.76	15.74	54	1179	11.94	52

Table 4a. The results of ANOVA variance analysis in the hoop house for Margoule varieties

Source of variance	Sum of squares	Degrees of freedom	Mean of squares	F value	Importance level (p)
Corrected Model	32861.022	17	1933.00	102.16	0.00
Intercep	102270.26	1	102270.26	5405.24	0.00
Months	3088.86	5	417.77	22.08	0.00
Types of Graft	5135.41	2	2567.70	135.71	0.00
Months xTypes of Graft	25636.75	10	2563.68	135.50	0.00
Error	68.14	36	18.92		
Total	135812.42	54			
Corrected Total	33542.16	53			

Table 4b. The results of ANOVA variance analysis in the hoop house for Erfelek varieties.

Source of variance	Sum of squares	Degrees of freedom	Mean of squares	F value	Importance level (p)
Corrected Model	15644.830	17	920.23	43.02	0.02
Intercep	28474.00	1	28474.07	1331.13	0.03
Months	5521.28	5	1104.26	51.62	0.03
Types of Graft	4360.05	2	2180.02	101.91	0.03
Months xTypes of Graft	5763.50	10	576.35	26.94	0.03
Error	770.07	36	21.39		
Total	44888.98	54			
Corrected Total	16414.90	53			

Table 5. Variance analysis results of grafting varieties according to months in Marigoule and Erfelek cultivars in the hoop house.

Months		Marigoule			Erfelek		
		Mean	Std. Deviation	N	Mean	Std. Deviation	N
December	Chip budding	0.00	0.00	3	0.00	0.00	3
	Tongue	46.67	6.67	3	8.89	3.85	3
	Cleft	51.11	3.85	3	13.33	0.00	3
	Total	32.59	24.82	9	7.41	6.18	9
January	Chip budding	0.00	0.00	3	0.00	0.00	3
	Tongue	64.44	7.70	3	6.67	0.00	3
	Cleft	66.67	6.67	3	33.33	6.67	3
	Total	43.70	33 .18	9	13.33	15.63	9
February	Chip budding	8.89	3.85	3	11.11	3.85	3
	Tongue	66.67	0.00	3	24.45	3.85	3
	Cleft	86 .67	0.00	3	64.45	3.85	3
	Total	54.08	35.03	9	33.33	2427	9
March	Chip budding	44.44	7.70	3	24.45	3.85	3
	Tongue	35 .55	3.85	3	26.67	6.67	3
	Cleft	48.89	3.85	3	48.89	3.85	3
	Total	42.96	7.54	9	33.33	12.47	9
April	Chip budding	46.67	6.67	3	42.22	10.18	3
	Tongue	40.00	0.00	3	11.11	3.85	3
	Cleft	46.67	0.00	3	37.78	3.85	3
	Total	44.45	4.71	9	30.37	15.67	9
July	Chip budding	83.33	0.00	3	31.11	3.85	3
	Tongue	22.22	3.85	3	13.33	6.67	3
	Cleft	24.45	3.85	3	15.55	3.85	3
	Total	43.33	30.14	9	20.00	9.43	9
Total	Chip budding	30 .56	31.59	18	18.15	16.77	18
	Tongue	45.93	16 .63	18	15.19	8.82	18
	Cleft	54.08	19. 92	18	35.56	18.72	18
	Total	43 .52	25.16	54	22.96	17.60	54

Table 6. The results of variance analysis for success of grafting.

Source of variance	Sum of squares	Degrees of freedom	Mean of squares	F value	Importance level (p)
Place	10183.79	1	10183.79	36.52	0.00
Chestnut variable	16555.68	1	16555.68	59.37	0.00
Graft variable	9355.12	2	4677.56	16.78	0.00
Place x Chestnut variable	374.36	1	374.36	1.34	0.25
Chestnut x Graft variable	2109.12	2	1054.56	3.78	0.02
Place x Chestnut x Graft variable	56324.51	2	95.27	0.34	0.71
Error	56324.51	202	278.83		
Total	244236.89	214			

Table 7. Variation of the success of grafting according to the time of grafting of the grafting varieties and Duncan results.

Types of Graft	December	January	February	March	Aprıl	July
Chip budding	0.00^{a}	0.00^{a}	7.22^{a}	34.44^{b}	29.45^{a}	48.06^{b}
Tongue	18.89^{b}	23.33^{b}	28.89^{b}	25.00^{a}	24.44^{a}	17.22^{a}
Cleft	24.44^{c}	35.00^{c}	50.00^{c}	45.55^{c}	37.78^{b}	19.58^{a}

Accordingly, it can be said that the hoop house environment has a positive effect on the success of the grafting.

In another study conducted on chestnuts, different temperatures (10, 15, 20, 25, 15/5, and 20/10°C) were determined by cleft, tongue, chip budding grafting methods. The results, which were not parallel to our study, showed that the highest success was achieved with chip budding graft at a temperature of 15/5°C (Aslan, 2019). When the meteorological data is examined, considering the averages of February and the temperature differences, it can be concluded that higher grafting success was observed in February, as temperatures similar to the environments with a different temperature of 15/5 degrees occurred. According to the results, it can be proposed that the tongue method for chestnut grafting was more successful in hoop house conditions in March, and chipped budding type grafting was more successful in July for the Düzce region. The variety of Marigoule is more successful than Erfelek because it is more compatible with local seedlings and more resistant to cancer. Düzce province has optimum growing conditions for chestnut trees.

It is crucial for the future of chestnuts and the development of the country to do different studies on the identification and reproduction of naturally grown chestnut trees. In addition, similar studies with different grafting methods (patch, T budding, and inverted T., etc.) that were not used in this study should be done and testing in different regions that may be beneficial for the future of chestnut.

According to this study, it is advisable to use chip budding and tongue methods for chestnut grafting or propagation studies in Düzce region. February and July may be recommended in the greenhouse environment for the most appropriate grafting studies. Especially in winter or spring periods, the grafting success is better in the greenhouse environment.

According to the results of this study, it can be recommended greenhouse conditions more successful than open field in Düzce and it could be recommended that tongue grafting method preferred in March and also chip budding method could be preffered in July. Düzce province has optimum growing conditions for chestnut trees. It is important for the future of chestnut and the development of our country by doing different studies on the identification and reproduction of varieties in naturally grown chestnut trees. In addition, it may be beneficial for the future of chestnut to be carried out again with different grafting methods.

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