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Determination of The Grafting Performance of Some Hybrid Chestnut Genotypes and Cultivars*

Bazı Hibrit Kestane Genotip ve Çeşitlerinin Aşı Performanslarının Belirlenmesi

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Abstract: This study has been carried out to determine the graft compatibility of newly registered some hybrid chestnut cultivars and genotype. The study was conducted between 2012-2018 at the Ondokuz Mayıs University and the Black Sea Agricultural Research Institute. In the study, 'Marigoule', Akyüz', 'Ali Nihat' and 'Macit 55' cultivars were tested as scion and rootstock, and A41 genotype was tested only as rootstock. Grafting studies were carried out on generative rootstocks in 2014 and mother plants in 2015 using the chip budding method. Graft success (%) was determined 30 days after grafting and survival ratio (%) at the end of the vegetation period. Also rootstock, graft area and scion diameter were measured at the end of the vegetation period by digital caliper. As a result of the study, graft success and survival ratio were low in combinations created with 'Ali Nihat' and A41 generative rootstocks. Among the plants, the best generative rootstock was the 'Macit 55' cultivar in terms of graft success and survival ratio. 'Macit 55' rootstock had good graft compatibility with both itself ('Macit 55'), 'Akyüz' and 'Ali Nihat' cultivars. 'Marigoule' cultivar has generally shown severe graft incompatibility with complex hybrids tested in the study. In the grafts made on clone plants, the 'Ali Nihat' rootstock was determined as a good rootstock for itself ('Ali Nihat'), 'Akyüz' and 'Macit 55' cultivars.

Keywords: *Castanea* spp., chip budding, mother plant, rootstock

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Öz: Bu çalışma, yeni tescil edilen bazı hibrit kestane çeşitlerinin ve genotipinin aşı uyuşma durumunu belirlemek amacıyla yapılmıştır. Çalışma 2012-2018 yılları arasında Ondokuz Mayıs Üniversitesi ve Karadeniz Tarımsal Araştırma Enstitüsü'nde yürütülmüştür. Çalışmada 'Marigoule', Akyüz', 'Ali Nihat' ve 'Macit 55' çeşitleri kalem ve anaç olarak, A41 genotipi ise sadece anaç olarak denenmiştir. 2014 yılında generatif anaçlarda, 2015 yılında da ana bitkilerde yongalı göz aşısı kullanılarak aşılamalar yapılmıştır. Aşı başarısı (%), aşılamadan 30 gün sonra ve yaşama oranı (%) vejetasyon sonunda belirlenmiştir. Ayrıca vejetasyon sonunda dijital kumpas yardımı ile anaç, aşı bölgesi ve kalem çapı ölçülmüştür. Çalışma sonucunda 'Ali Nihat' ve A41 generatif anaçlarıyla oluşturulan kombinasyonlarda aşı başarısı ve yaşama oranı düşük bulunmuştur. Bitkiler arasında aşı başarısı ve yaşama oranı açısından en iyi generatif anaç 'Macit 55' çeşidi olmuştur. "Macit 55" anacının hem kendisi ('Macit 55'), "Akyüz" hem de "Ali Nihat" çeşitleri ile aşı uyumunun iyi olduğu görülmüştür. 'Marigoule' çeşidi genellikle çalışmada denenen karmaşık melezlerle ciddi aşı uyuşmazlığı göstermiştir. Ana bitkilere yapılan aşılarda Ali Nihat anacının kendisi ('Ali Nihat'), 'Akyüz' ve 'Macit 55' çeşitleri için uyumlu bir anaç olduğu belirlenmiştir.

Anahtar Kelimeler: *Castanea* spp., yongalı göz aşı, ana bitki, anaç

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INTRODUCTION

There are 13 species of *Castanea* genus globally; 7 of them are divided into 3 sections (Johnson, 1988). Eucastanon section can be defined by having 3 fruits in the burr. This section includes European chestnut (*Castanea sativa* Mill.), Chinese chestnut (*C. mollissima* Blume), Japanese chestnut (*C. crenata* Sieb. & Zucc) and Seguinii chestnut (*C. seguinii* Dode) (Pereira-Lorenzo and Ramos-Cabrer, 2004). Chestnut cultivation is mostly done with species and hybrids of European chestnut (*C. sativa*), Chinese chestnut (*C. mollissima*) and Japanese chestnut (*C. crenata*).

Turkey is one of the origin centers of European chestnut (*C. sativa*). Turkey is in third place with 72.655 tones production (FAOSTAT, 2021). The fruit quality of European chestnut (*C. sativa*) is excellent, but it is not as resistant to pests and diseases as the Chinese chestnut (*C. mollissima*) and Japanese chestnut (*C. crenata*). Chestnut production in Turkey was higher in 1987, with 90.000 tones production (FAOSTAT, 2021). However, due to the epidemic of chestnut blight (*Cryphonectria parasitica*), Turkey's chestnut production was decreased nearly 50% in 13 years.

Currently, the most important factors threatening chestnut cultivation in our country are chestnut blight (*Cryphonectria parasitica*), root rot (*Phytophthora* spp.) and gall wasp (*Dryocosmus kuriphilus*). There are several management methods against these pests and diseases. The most efficient method is to establish orchards with resistant cultivars. European chestnut (*C. sativa*) naturally grows in Turkey is favorable for its nuts; however it shows variable reactions to these pests and diseases (Pereira-Lorenzo et al., 2016). For this aim, a hybridization study was planned with Connecticut Agricultural Research Station in 2004. 'King Arthur' (*C. mollissima*/*C. seguinii*) and 'Lockwood' (*C. crenata*/*C. sativa*/*C. dentata*) cultivars were hybridized. Seeds of this hybridization were imported by Ondokuz Mayıs University (Macit et al., 2018). These genotypes were tested between 2006-2014 in terms of plant growth, yield, and some pomological features. For this purpose, juvenile infertility period, cumulative yield (for nine years after planting), earliness, number of fruits in burr, fruit size (pieces/kg), color, brightness and thickness of the shell and taste has been examined. As a result of selection studies, superior ones were determined (Macit et al., 2018; Pereira-Lorenzo et al., 2016). Selected genotypes were grafted onto 3-8 years old European chestnut (*C. sativa* Mill) plants. However, variable graft success and survival ratios were obtained (Serdar et al., 2014).

With the inclusion of interspecies hybrids in chestnut cultivation, the problem of graft incompatibility has begun to increase. Graft compatiblity in chestnut can be divided into two as early and delayed incompatibility. While early incompatibility can be seen in the first two years after grafting, delayed incompatibility can be seen even 5-7 years after grafting (Oraguzie et al., 1998). Numerous studies on interspecies grafting of chestnut had been done (Chapa et al., 1990; Craddock and Bassi, 1999; Huang et al., 1994; Oraguzie et al., 1998; Pereira-Lorenzo and Fernández-López, 1997; Serdar and Soylu, 2005; Serdar et al., 2010; Thomas et al., 2015; Tokar and Kovalovsky, 1971; Ufuk and Soylu, 1999; Viéitez and Viéitez, 1982). As a result of these studies, interspecies and intraspecies graft incompatibilities were observed. In this situation, it became an important subject to determine the suitable rootstock for newly registered superior chestnut cultivars.

This study aimed to determine the grafting performance of these hybrid chestnut cultivars and genotype important for Turkey and the World.

MATERIAL AND METHOD

This study was conducted between 2012 and 2018 at Ondokuz Mayıs University and Black Sea Agricultural Research Institute, Samsun, Turkey (41° 21' 55'' N, 36° 11' 14'' E; 190 m above sea level and 41° 13' 48'' N, 36° 29' 55'' E; 8 m above sea level respectively).

In the study, 'Akyüz', 'Ali Nihat', 'Macit 55', and 'Marigoule' cultivars and A41 genotype were used. 'Akyüz', 'Ali Nihat', 'Macit 55' cultivars and A41 genotype are hybrids of 'King Arthur' (*C. mollissima*/C. *seguinii*) and 'Lockwood' (*C. crenata*/*C. sativa*/*C. dentata*) cultivars (Macit et al., 2018). 'Ali Nihat' cultivar

was selected for its productivity and dwarf growing characteristics. 'Akyüz' cultivar has big nuts, and it has a spreading growth habit. Also, it is resistant to the Asian chestnut gall wasp (*Dryocosmus kuriphilus*) (Çil, 2018). 'Macit 55' cultivar has the highest cumulative yield amongst the cultivars tested (Macit et al., 2018). A41 genotype failed the selection studies for nut production; however, it remarked with its dwarf growing characteristics. It was only tested as a rootstock in the study.

'Marigoule' (MAR) cultivar was obtained from INRA research institute (France) by natural hybridization of *C. sativa* var. Migoule (female) and *C. crenata* (male) (Chapa and Verlhac, 1978). It is a popular and widely grown cultivar in Turkey for its tolerance to the chestnut blight (*Cryphonectria parasitica*). 'Marigoule' is a standard cultivar in Turkey, and it was used as a control in this study.

Graft compatibility between these genotypes and cultivars was tested on the one-year-old generative rootstocks and the one-year-old branches of the mother plants. Mother plants were planted in the Black Sea Agricultural Research Institute in 2005.

Grafting on Generative Rootstock

In September 2012, seeds of the 'Akyüz', 'Ali Nihat', 'Macit 55', and 'Marigoule' cultivars and A41 genotype were harvested to obtain seedlings. They were placed in a cold room (2 °C) for stratification. Germinated seeds were potted (5.5 L, square-shaped (175*175*250 mm)) and placed in an open field near the greenhouse. Generative rootstock potentials of these genotypes and cultivars were also determined by Akyüz and Serdar (2020). In February 2014, seedlings were placed into the greenhouse and scion woods were collected from mother plants. On 10 April 2014, grafting was performed 15 cm above soil level by chip budding method. Graft combinations are stated in Table 1.

Table 1. Graft combinations performed on generative rootstocks in 2014 and on mother plants in 2015.
Çizelge 1. 2014 yılında generatif anaçlarda ve 2015 yılında ana ağaçlarda yapılan aşı kombinasyonları.

Combinations (Scion/Rootstock)				
Akyüz/Akyüz	Akyüz/Ali Nihat	Akyüz/A41	Akyüz/Macit 55	Akyüz/MAR
Ali Nihat/Akyüz	Ali Nihat/Ali Nihat	Ali Nihat/A41	Ali Nihat/Macit 55	Ali Nihat/MAR
Macit 55/Akyüz	Macit 55/Ali Nihat	Macit 55/A41	Macit 55/Macit 55	Macit 55/MAR
MAR/Akyüz	MAR/Ali Nihat	MAR/A41	MAR/Macit 55	MAR/MAR
		A41/A41		

After grafting, the graft area was wrapped with plastic grafting tape, and the whole graft area was covered by one layer of parafilm (Sigma P7543). Ten days after grafting, 15 cm above the graft area was cut. As the scion reached 15 cm in length, 1 cm above graft area was cut, and grafting wax was applied. At the beginning of June top of the greenhouse was covered with a 50% shading net. At the end of December in 2014, grafted plants were transferred into 45 L pots and were placed outside the greenhouse. This place was covered with a black agrotex ground cover (Abdioğulları plastic, Turkey) to prevent weed growth.

Grafting on Mother Plants

In 2015, one-year-old branches of the main plants were grafted by the combinations stated in Table 1. Scion woods were collected during the dormant period, and they were stored in the cold room (2±1°C and 85% relative humidity) until the grafting date. Grafting was performed with the chip budding method on 6 May 2015. The graft area was wrapped with plastic grafting tape, and the whole graft area was covered by one layer of parafilm (Sigma P7543). On 13 May 2015, 1 cm above the graft area was cut, and grafting wax was applied.

Examined Parameters

Graft success (%): It was calculated 30 days after grafting by counting formed shoots from the buds, and it was given as a ratio.

Survival ratio (%): It was calculated at the end of each vegetation period by counting living grafts, and it was given as a ratio.

Rootstock diameter (mm): At the end of each vegetative period, 5 cm below the graft area was measured by a digital caliper.

Graft area diameter (mm): Graft area was measured by a digital caliper at the end of each vegetative period.

Scion diameter (mm): At the end of each vegetative period, 5 cm above the graft area was measured by a digital caliper.

The study was designed with three repetitions, and a randomized plot design was used. The number of grafted generative rootstocks was changed according to the genotype or cultivar due to the number of seedlings obtained. 'Akyüz', 'Macit 55' and 'Marigoule' cultivars had 20 seedlings, 'Ali Nihat' cultivar and A41 genotype had 12 seedlings for each repetition. Arcsin transformation was applied to the graft success and survival ratios. Statistically significant differences between the studied genotype and cultivars were tested using analysis of variance (ANOVA). The Duncan Multiple Range Test determined the differences between the means. On the other hand, for grafting on mother plants, 9 grafts were done due to a lack of available one-year-old shoots. Graft success and survival ratio results on the mother plants were given as a ratio.

RESULTS

Results on Graft Success And Survival Ratio

Results of The Grafts Performed on Generative Rootstocks

The graft success ratio ranged from 2.60 to 93.9% (Table 2) according to the combinations grafted in 2014 on generative rootstocks.

Table 2. Graft success and survival rates of the grafts made on generative rootstocks in 2014.
Çizelge 2. 2014 yılında generatif anaçlarda yapılan aşılarda aşı başarısı ve yaşama oranları.

Scion/Rootstock Combination	Graft Success (%)	Survival Ratio (%)		
		1. Year	2. Year	3. Year
Akyüz/Akyüz	63.8 c-e*	100.0 a	77.2 b-d	70.5 a-d
Ali Nihat/Akyüz	56.5 de	91.7 a-d	45.4 f	37.5 f
Macit 55/Akyüz	73.3 b-d	100.0 a	86.9 a-c	73.2 a-c
MAR/Akyüz	91.7 a	81.9 cd	45.5 f	45.5 d-f
Akyüz/Ali Nihat	51.5 e	100.0 a	100.0 a	47.6 c-f
Ali Nihat/Ali Nihat	53.0 e	100.0 a	72.2 c-e	66.7 a-d
Macit 55/Ali Nihat	64.5 c-e	95.8 ab	95.8 ab	58.9 a-e
MAR /Ali Nihat	82.8 ab	92.6 a-c	77.8 b-d	40.7 ef
Akyüz/A41	51.0 e	79.6 d	74.9 b-e	41.4 ef
Ali Nihat/A41	58.3 de	100.0 a	86.1 a-d	77.2 ab
A41/A41	2.60 f	0.0 e	0.0 g	0.0 h
Macit 55/A41	64.1 c-e	95.8 ab	71.7 c-e	59.7 a-e
MAR /A41	93.9 a	93.3 a-c	66.1 de	56.1 a-f
Akyüz/Macit 55	65.0 c-e	90.0 a-d	85.0 a-d	50.5 b-f
Ali Nihat/Macit 55	57.7 de	90.5 a-d	90.5 a-c	61.6 a-e
Macit 55/Macit 55	72.6 b-d	92.8 a-c	86.9 a-c	82.4 a
MAR /Macit 55	90.0 ab	83.2 b-d	78.2 b-d	67.4 a-d
Akyüz/ MAR	63.3 c-e	96.9 a	72.3 c-e	55.8 a-f
Ali Nihat/ MAR	55.0 e	81.8 cd	57.6 ef	18.2 g
Macit 55/ MAR	76.7 a-c	100.0 a	82.8 a-d	69.7 a-d
MAR / MAR	78.4 a-c	88.8 a-d	73.3 c-e	73.3 a-c
P	≤0.01	≤0.05	≤0.01	≤0.01

* There is no difference between the means indicated by the same letter in the same column.

Statistically, MAR/A41 and MAR/Akyüz combinations obtained the best graft success (93.9 and 91.7%, respectively). A41/A41 combination got the worst graft success, 2.60 %. At the end of the 3rd year, Macit 55/Macit 55 combination with 82.4% obtained the best survival ratio, and A41/A41 received the lowest ratio with 0.0% (Table 2).

Results of The Grafts Performed on The Mother Plants

On the mother plants, the graft success varied between 18.2 and 100.0% (Table 3). Macit 55/Akyüz, Akyüz/Ali Nihat, Ali Nihat/Ali Nihat, Macit 55/Ali Nihat, Ali Nihat/A41, Macit 55/A41, MAR/A41, Macit 55/Macit 55, MAR/Macit 55, Akyüz/MAR, Macit 55/MAR and MAR/MAR combinations reached the best graft success ratio by 100.0%. On the other hand, A41/A41 combination had the worst graft success ratio (18.2%). At the end of the 4th year, Ali Nihat/Ali Nihat, Macit 55/Ali Nihat, A41/A41 and Macit 55/Macit 55 combinations gave the highest survival ratio by 100.0%. However, MAR/Akyüz, MAR/Ali Nihat, Akyüz/MAR, and Ali Nihat/MAR combinations had the worst score by 0.0% (Table 3).

Table 3. Graft success and survival rates made on mother plants in 2015.
Çizelge 3. 2015 yılında ana ağaçlarda yapılan aşılarda aşı başarısı ve yaşama oranları.

Scion/Rootstock Combination	Graft Success (%)	Survival Ratio (%)			
		1. Year	2. Year	3. Year	4. Year
Akyüz/Akyüz	83.3	100.0	100.0	83.3	83.3
Ali Nihat/Akyüz	93.3	100.0	86.7	78.3	71.7
Macit 55/Akyüz	100.0	93.3	60.0	60.0	53.3
MAR/Akyüz	80.0	88.9	38.9	38.9	0.0
Akyüz/Ali Nihat	100.0	100.0	100.0	100.0	88.9
Ali Nihat/Ali Nihat	100.0	100.0	100.0	100.0	100.0
Macit 55/Ali Nihat	100.0	100.0	100.0	100.0	100.0
MAR/Ali Nihat	33.3	66.7	33.3	33.3	0.0
Akyüz/A41	88.9	100.0	66.7	66.7	66.7
Ali Nihat/A41	100.0	100.0	94.4	94.4	82.2
A41/A41	18.2	100.0	100.0	100.0	100.0
Macit 55/A41	100.0	100.0	91.7	78.3	78.3
MAR/A41	100.0	100.0	93.3	85.0	85.0
Akyüz/Macit 55	92.9	100.0	93.3	93.3	93.3
Ali Nihat/Macit 55	95.0	84.1	79.4	73.8	73.8
Macit 55/Macit 55	100.0	100.0	100.0	100.0	100.0
MAR/Macit 55	100.0	100.0	93.3	93.3	93.3
Akyüz/ MAR	100.0	91.7	20.0	0.0	0.0
Ali Nihat/ MAR	95.7	82.1	31.5	0.0	0.0
Macit 55/ MAR	100.0	100.0	91.7	38.9	38.9
MAR / MAR	100.0	83.3	66.7	66.7	66.7

Results on Rootstock Diameter, Graft Area Diameter and Scion Diameter

Results of the Grafts Performed on Generative Rootstocks

In the study, the rootstock diameter at the end of the first year varied between 7.90-10.9 mm, the diameter of the graft area was between 9.59-12.9 mm, and the scion diameter was between 4.29-6.84 mm. Akyüz/MAR combination gave the thickest rootstock diameter, while the Ali Nihat/Ali Nihat gave the thinnest. Akyüz/A41 combination had the thickest graft area. On the other hand, Ali Nihat/Ali Nihat and Macit 55/MAR combinations owned the thinnest graft area. The thickest scion diameter was measured from MAR/Macit 55 and Akyüz/A41 combination. Macit 55/MAR and Ali Nihat/Ali Nihat combination had the thinnest scion diameter (Table 4,5,6).

At the end of the 3rd year, the thickest rootstock was measured from MAR/Ali Nihat, Akyüz/Macit 55 and MAR/Ali Nihat combinations (19.6, 19.3 and 19.0 mm respectively) and the thinnest rootstock was measured from Akyüz/Akyüz combination with 14.4 mm. In scion diameter, the thickest scions were obtained from the Ali Nihat/Macit 55 combination, while the thinnest scions were obtained from the Ali Nihat/MAR combination (Tables 4-6).

Table 4. Rootstock diameter of the grafts made on generative rootstocks in 2014.
Çizelge 4. 2014 yılında generatif anaçlara yapılan aşıların anaç çapları.

Scion/ Rootstock Combination	Rootstock Diameter		
	1.Year	2. Year	3.Year
Akyüz/Akyüz	8.79 fg*	13.6 a-e	14.4 f
Ali Nihat/Akyüz	9.44 de	14.9 ab	16.6 c-e
Macit 55/Akyüz	8.72 fg	13.2 b-e	15.7 d-f
MAR/Akyüz	9.01 ef	15.2 a	17.2 b-d
Akyüz/Ali Nihat	8.20 gh	12.7 de	17.2 b-d
Ali Nihat/Ali Nihat	7.90 ı	12.5 e	15.2 ef
Macit 55/Ali Nihat	8.40 f-h	14.5 a-c	17.1 b-e
MAR/Ali Nihat	8.13 gh	13.8 a-e	19.6 a
Akyüz/A41	9.83 cd	14.7 a-c	18.5 ab
Ali Nihat/A41	9.02 ef	14.9 ab	16.1 d-f
Macit 55/A41	8.37 f-h	15.3 a	17.0 b-e
MAR/A41	8.48 f-h	13.7 a-e	18.1 a-c
Akyüz/Macit 55	8.59 fg	13.7 a-e	19.3 a
Ali Nihat/Macit 55	8.77 fg	13.9 a-e	18.5 ab
Macit 55/Macit 55	8.28 gh	13.0 c-e	15.6 d-f
MAR/Macit 55	8.77 fg	14.7 a-c	19.0 a
Akyüz/MAR	10.90 a	14.2 a-e	15.5 d-f
Ali Nihat/MAR	10.40 ab	14.1 a-e	15.5 d-f
Macit 55/MAR	9.52 de	15.0 ab	15.4 d-f
MAR/MAR	10.10 b	14.3 a-d	15.7 d-f
P	<0.01	<0.05	<0.01

* There is no difference between the means indicated by the same letter in the same column.

The thickest graft area was measured from Ali Nihat/MAR and MAR/Ali Nihat combination (23.9 and 23.7 mm respectively). Besides, the thinnest graft area was measured from Akyüz/Ali Nihat, Macit 55/Macit 55, Akyüz/Akyüz and Macit 55/Akyüz combinations.

Table 5. Graft area diameter of the grafts made on generative rootstocks in 2014.
Çizelge 5. 2014 yılında generatif anaçlara yapılan aşıların aşı bölgesi çapları.

Scion/ Rootstock Combination	Graft Area Diameter		
	1.Year	2. Year	3.Year
Akyüz/Akyüz	10.50 e-ı*	18.9 a-c	19.9 d
Ali Nihat/Akyüz	10.90 c-g	18.3 a-d	21.5 a-d
Macit 55/Akyüz	10.30 f-ı	18.1 a-d	20.0 d
MAR/Akyüz	10.70 d-h	19.6 a	21.8 a-d
Akyüz/Ali Nihat	10.20 f-ı	14.8 h	19.6 d
Ali Nihat/Ali Nihat	9.67 ı	15.6 f-h	20.4 cd
Macit 55/Ali Nihat	9.85 hı	16.4 d-h	22.0 a-d
MAR/Ali Nihat	9.94 g-ı	16.9 d-g	23.7 a
Akyüz/A41	12.90 a	18.0 a-d	21.1 b-d
Ali Nihat/A41	11.10 c-f	18.0 a-d	20.6 cd
Macit 55/A41	9.94 g-ı	17.5 c-e	21.8 a-d
MAR/A41	11.30 c-e	19.5 ab	23.4 ab
Akyüz/Macit 55	10.90 c-f	16.7 d-g	21.8 a-d
Ali Nihat/Macit 55	11.00 c-f	16.6 d-g	23.5 ab
Macit 55/Macit 55	9.90 hı	15.1 gh	19.8 d
MAR/Macit 55	11.00 c-f	19.4 ab	22.8 a-c
Akyüz/MAR	11.60 b-d	19.8 a	21.5 a-d
Ali Nihat/MAR	12.20 ab	17.7 c-e	23.9 a
Macit 55/MAR	9.59 ı	17.2 c-f	20.8 cd
MAR/MAR	11.70 bc	15.8 e-g	20.8 cd
P	<0.01	<0.01	<0.01

* There is no difference between the means indicated by the same letter in the same column.

Table 6. Scion diameter of the grafts made on generative rootstocks in 2014.
Çizelge 6. 2014 yılında generatif anaçlara yapılan aşıların kalem çapları.

Scion/ Rootstock Combination	Scion Diameter		
	1. Year	2. Year	3. Year
Akyüz/Akyüz	5.58 cd*	10.8 d-h	17.0 ef
Ali Nihat/Akyüz	5.78 b-d	11.9 a-d	19.2 a-e
Macit 55/Akyüz	5.67 b-d	10.0 f-ı	18.1 c-e
MAR/Akyüz	6.26 a-c	12.6 ab	18.5 b-e
Akyüz/Ali Nihat	6.04 a-d	10.2 e-ı	14.9 fg
Ali Nihat/Ali Nihat	4.29 e	8.56 j	16.8 e-g
Macit 55/Ali Nihat	5.34 d	11.6 b-e	20.9 ab
MAR/Ali Nihat	5.94 b-d	10.2 e-ı	20.1 a-d
Akyüz/A41	6.83 a	10.6 d-ı	18.8 a-e
Ali Nihat/A41	5.50 cd	10.6 d-ı	17.9 de
Macit 55/A41	5.60 cd	10.5 d-ı	20.4 a-c
MAR/A41	6.34 a-c	12.4 a-c	19.6 a-d
Akyüz/Macit 55	5.82 b-d	11.0 c-h	16.8 e-g
Ali Nihat/Macit 55	6.10 a-d	11.4 b-f	21.2 a
Macit 55/Macit 55	5.58 cd	10.8 d-h	18.1 c-e
MAR/Macit 55	6.84 a	13.1 a	19.3 a-e
Akyüz/MAR	5.62 cd	9.71 h-j	19.1 a-e
Ali Nihat/MAR	5.93 b-d	9.22 ij	14.5 g
Macit 55/MAR	4.45 e	9.82 g-j	20.8 ab
MAR/MAR	6.56 ab	11.2 b-g	19.0 a-e
P	<0.01	<0.01	<0.01

* There is no difference between the means indicated by the same letter in the same column.

Results of the Grafts Performed on the Mother Plants

At the end of the first year, the rootstock diameter of the mother plants varied between 8.77 and 12.0 mm. The diameter of the grafting area was 9.77-16.7 mm, and the diameter of the scion ranged between 2.18-8.24 mm in terms of combinations.

Table 7. Rootstock, graft area and scion diameters of the grafts made on mother plants in 2015.
Çizelge 7. 2015 yılında ana anaçlara yapılan aşıların anaç, aşı bölgesi ve kalem çapları.

Scion/Rootstock Combination	Rootstock Diameter				Graft Area Diameter				Scion Diameter			
	1. Year	2. Year	3. Year	4. Year	1. Year	2. Year	3. Year	4. Year	1. Year	2. Year	3. Year	4. Year
Akyüz/Akyüz	10.10	12.70	14.5	16.1	11.8	14.3	16.5	18.7	6.02	8.80	12.3	13.1
Ali Nihat/Akyüz	10.80	14.60	16.6	17.1	12.8	16.9	18.6	19.4	6.82	10.6	14.1	15.1
Macit 55/Akyüz	10.90	15.50	17.8	18.2	12.7	17.2	19.2	19.4	6.72	12.9	15.0	16.1
MAR/Akyüz	11.60	15.80	22.0	-	16.7	22.0	29.8	-	7.39	14.2	22.9	-
Akyüz/Ali Nihat	12.00	16.30	17.9	18.9	13.6	17.8	20.2	21.4	7.58	12.4	14.0	16.1
Ali Nihat/Ali Nihat	10.30	11.60	12.9	15.4	11.3	13.3	14.6	17.9	5.46	8.47	10.2	12.6
Macit 55/Ali Nihat	11.90	15.60	19.0	21.2	13.6	17.3	20.2	22.2	7.41	11.6	15.3	17.3
MAR/Ali Nihat	8.77	15.40	15.5	-	10.5	19.6	25.0	-	3.62	7.61	12.8	-
Akyüz/A41	11.70	13.80	18.4	22.9	13.2	16.1	21.2	24.8	7.05	10.2	15.2	20.1
Ali Nihat/A41	10.30	11.90	14.3	16.0	12.0	13.5	15.6	17.7	6.07	8.41	10.6	13.0
A41/A41	9.77	12.40	16.1	17.3	12.2	15.2	17.5	20.0	6.03	10.8	12.7	15.1
Macit 55/A41	10.60	13.10	15.2	18.3	11.9	14.6	17.3	20.1	6.14	9.68	11.1	14.6
MAR/A41	11.80	16.70	19.8	25.2	14.3	19.7	23.0	28.7	8.24	14.3	18.4	23.1
Akyüz/Macit 55	10.20	12.80	14.4	16.2	11.4	14.4	16.3	18.1	5.93	9.42	11.8	13.1
Ali Nihat/Macit 55	11.30	13.60	15.7	17.4	12.4	15.3	18.0	19.1	6.33	9.48	12.4	14.2
Macit 55/Macit 55	11.40	13.90	16.8	17.5	12.6	15.5	18.0	19.2	6.63	10.6	13.8	15.2
MAR/Macit55	11.00	13.80	16.8	18.9	12.5	16.6	19.9	22.3	6.61	11.6	15.2	17.6
Akyüz/MAR	10.40	10.30	-	-	10.6	10.8	-	-	3.58	4.04	-	-
Ali Nihat/MAR	9.10	9.82	-	-	9.77	9.82	-	-	2.18	2.71	-	-
Macit 55/MAR	9.51	11.30	14.9	15.8	10.3	12.3	16.1	16.6	4.32	6.86	10.9	12.1
MAR/MAR	10.00	12.30	15.7	19.5	11.1	13.6	17.0	20.7	4.66	7.55	11.8	15.4

The thickest rootstock diameter was measured from the Akyüz/Ali Nihat combination, and the thinnest rootstock diameter was obtained from the MAR/Ali Nihat combination. The thickest graft area diameter was measured from the MAR/Akyüz combination, and the thinnest graft area diameter was measured from the Ali Nihat/MAR combination. The thickest scion diameter was obtained from the MAR/A41 combination, while the thinnest scion diameter was obtained from the Ali Nihat/MAR combination (Table 7).

At the end of the 4th year, The thickest rootstock, graft area and scion diameter were obtained from the MAR/A41 combination and the thinnest rootstock diameter was measured from the Ali Nihat/Ali Nihat combination Also, the thinnest grafting area and scion diameter were obtained from the Macit 55/MAR combination (Table 7).

DISCUSSION

In other studies, related to chestnut grafting, Huang et al. (1994) had 6-100%, Oraguzie et al. (1998) had 0-100%, Ertan (1999) had 0-100%, Serdar (2000) had 10-100%, Bueno et al. (2009) had 25-100% and Serdar et al. (2010) had 32-90% graft success. The results of our study are similar to the literature results. Variation in graft success was relatively high both in our research and in studies mentioned in the literature.

Oraguzie et al. (1998) divided graft incompatibility into two as early (first 15 months after grafting) and late (starting 32 months after grafting). In the generative rootstocks grafted in 2014, in the first and the second year Ali Nihat/Akyüz and MAR/Akyüz combinations; and in the second and the third year Akyüz/Ali Nihat, MAR/Ali Nihat, Akyüz/A41 and Ali Nihat/MAR combinations survival rates suddenly decreased. Those combinations could be affected by graft incompatibility. According to Oraguzie et al. (1998), Ali Nihat/Akyüz and MAR/Akyüz combinations showed early graft incompatibility and MAR/Ali Nihat and Ali Nihat/MAR showed late graft incompatibility. Also, ambrosia beetle damage was seen in Akyüz/Ali Nihat and Akyüz/A41 combinations. Mainly ambrosia beetle attacks damaged or weak plants (Mendel et al., 2021). Graft incompatibility could weaken those plants and attract the ambrosia beetle (Hulcr and Stelinski, 2017).

On the mother plants, more stable results were obtained. At the end of the 1st and the 2nd year, a remarkable decrease in the survival ratio was observed on MAR/Akyüz, Akyüz/A41, Akyüz/MAR and Ali Nihat/MAR combinations. Also, graft success was low in MAR/Ali Nihat combination. This decrease could result from early graft incompatibility in MAR/Akyüz, MAR/Ali Nihat, Akyüz/A41, Akyüz/MAR and Ali Nihat/MAR combinations.

'Akyüz' and 'Ali Nihat' cultivars and A41 genotype are the hybrid of 'King Arthur' (*C. mollissima*/*C. seguine*) and 'Lockwood' (*C. crenata*/*C. sativa*/*C. dentata*). However, growing characters showed that they are more like Chinese chestnut (*C. mollissima*). Huang et al. (1994) found that the survival rate in intraspecific grafting of Chinese chestnut was 80%. Zhang et al. (1987) found that Chinese chestnuts in China have 0.5% genetic incompatibility. Santamour (1988) stated that there is intraspecies incompatibility in Chinese chestnut and this incompatibility may be associated with cambial peroxidase isoenzyme band groups. Huang et al. (1994) and Warmund et al. (2012) stated that phloem bundles concentrated in Chinese chestnuts, may negatively affect graft success. This situation may be the cause of the graft incompatibilities in Ali Nihat/Akyüz, Akyüz/Ali Nihat and Akyüz/A41 combinations in generative plants and Akyüz/A41 combination on mother plants.

'Marigoule' cultivar is a European x Japanese (*C. sativa* x *C. crenata*) hybrid, and it was used as a control in the study. As stated before, 'Akyüz' and 'Ali Nihat' cultivars and A41 genotype mostly reflect the Chinese chestnut characteristics. It has been reported that there is a low graft success and survival ratio between European and Chinese chestnut grafting (Tokar and Kovalovsky, 1971). Also, graft incompatibility was reported between Chinese and Japanese chestnuts (Dayong et al., 2004; Huang et al., 1994). Severe graft incompatibility between 'Marigoule' and 'Akyüz' and 'Ali Nihat' cultivars could be caused by this problem. On the other hand, some graft incompatibility symptoms were observed in

MAR/A41 combination. However, satisfactory results were obtained in grafts made on mother plants. A41 could be more affected by the xenia effect.

Our study observed overgrowth at the grafting area in combinations of MAR/Akyüz, MAR/Ali Nihat, MAR/A41, MAR/Macit 55, Akyüz/MAR, Ali Nihat/MAR and Macit 55/MAR. Serdar et al. (2010) stated that overgrowth might occur in the grafting areas in some combinations where incompatibility is seen intensely. However, overgrowth in the graft area is only one of the symptoms of graft incompatibility. In addition, not every overgrowth may be a sign of the graft incompatibility (Hartmann et al., 2014). Different incompatibility signs were observed in some combinations, or the scion/rootstock was dried without any external symptoms. According to Oraguzie et al. (1998), grafted chestnuts can dry without showing any external symptoms. When these grafts were examined, a physical separation layer was found in the graft areas. Also, in the same study, differences in the thickness of the rootstock and the scion were observed in incompatible grafts (Oraguzie et al., 1998). Ishibashi et al. (1983) stated that the diameter of the grafting area was very different from the diameter of rootstock and scion in incompatible combinations, while Huang et al. (1994) stated that there were differences between the growth habit of rootstock and scion in the incompatible Kiansheng/AU-17 combination. Similarly, our study found

CONCLUSION

In grafts made on generative rootstocks, graft success and survival ratio have shown a wide variation over the years. This variation has shown that these genotypes or cultivars could be affected by ecology or xenia. Therefore, the fruit species that must use generative rootstock, xenia degree should be determined.

Among the analyzed genotypes or cultivars, 'Macit 55' cultivar was the best candidate for graft success and survival ratio. This cultivar has good graft compatibility with both itself and the 'Akyüz' and 'Ali Nihat' cultivars. Orchards should be established with these combinations and should be examined for yield and other characteristics.

In grafts made on mother plants, it has been determined that the 'Ali Nihat' cultivar is a good clonal rootstock candidate for itself, 'Akyüz' and 'Macit 55' cultivars, while the A41 genotype is a good clonal rootstock candidate for the 'Marigoule' cultivar. Studies should also be carried out to propagate these plants by using different vegetative methods. Although, graft success was high in some combinations. However, the survival ratio was found to be low in the following years. In this context, survival ratio could be a more reliable parameter than graft success to determine graft incompatibility.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

DECLARATION OF AUTHOR CONTRIBUTION

Burak Akyüz and Ümit Serdar designed the study, participated in experiment, and drafted the manuscript.

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