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Generative rootstock potential of some hybrid chestnut genotypes

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ABSTRACT

In Turkey chestnut cultivation is threaten by chestnut blight, chestnut root rot and Asian chestnut gall wasp. In order to obtain tolerant cultivars to the pests and diseases hybridization studies were performed. As a result, A14, A25, A41 and A100 hybrid chestnut genotypes were selected as superior. Some of these genotypes were registered by 'Republic of Turkey Ministry of Agriculture and Forestry Variety Registration and Seed Certification Center'. A14 genotype named as 'Akyüz' and A100 named as 'Macit 55'. Grafting studies were performed with these genotypes and cultivars. However, due to graft incompatibility survival ratio was found low. To avoid graft incompatibility, own seedlings may be better candidate as rootstocks because of the degree of compatibility. In this study seedling rootstock potential of 'Akyüz', 'Macit 55' cultivars and A25, A41 genotypes were examined. 'Marigoule' cultivar used as control. The study was conducted between 2013 and 2015 at Ondokuz Mayıs University, Samsun, Turkey. In the study healthy seed ratio (%), seed emerging ratio (%), suitable for grafting seedling ratio (%), seedling diameter (cm), coefficient of variation (CV) of seedling diameter and seedling productivity (%) were investigated. As a result of the study, all genotype and cultivars were found superior in terms of their generative rootstock characteristics. However, a great variation was observed among the rootstocks over the years. Therefore, studies should be conducted to determine the effect of xenia on rootstock candidates in nut species where generative rootstock use is necessary. Also, to find out the graft compatibility of these genotype and cultivars grafting studies should be done and they should be evaluated for long-term.

Keywords:
Castanea spp.
Hybrid
Seedling potential
Seedling productivity

Bazı hibrit kestane genotiplerinin anaçlık potansiyelleri

ÖZET

Türkiye'de kestane yetiştiriciliği kestane kanseri ve kök çürüklüğü hastalıkları ile kestane gal arısı zararlısının tehdidi altındadır. Bu hastalık ve zararlılara karşı dayanıklı çeşitler geliştirmek amacıyla melezleme çalışmaları yapılmıştır. Çalışmalar sonucunda A14, A25, A41 ve A100 genotipleri ümitvar bulunmuştur. Bu çeşitlerden bazıları TTSM tarafından tescil edilmiştir. A14 genotipi 'Akyüz', A100 genotipi ise 'Macit 55' ismiyle tescil ettirilmiştir. Bu çeşit ve genotipler ile aşılama çalışmaları yapılmış ancak aşı uyuşmazlığı sonucunda yaşama oranları düşük olmuştur. Akrabalık derecelerinin daha yakın olması nedeniyle genotip ve çeşitlerin kendi çöğürleri ile aşı uyuşmasının daha iyi olabileceği düşünülmüştür. Bu çalışmada 'Akyüz' ve 'Macit 55' çeşitleri ile A25 ve A41 genotiplerinin anaçlık potansiyelleri incelenmiştir. 'Marigoule' ise kontrol çeşidi olarak kullanılmıştır. Çalışma 2013-2015 yılları arasında Ondokuz Mayıs Üniversitesi'nde yürütülmüştür. Çalışmada sağlam tohum oranı, çıkış oranı, aşı yapılabilir çöğür oranı, çöğür çapı, çöğür çapının CV değeri ve tohumdan elde edilen çöğür oranı incelenmiştir. Çalışma sonucunda tüm genotip ve çeşitler generatif anaçlık potansiyelleri bakımından üstün bulunmuştur. Ancak yıllara göre değişmekle beraber genotip ve çeşitlerin anaçlık değerleri arasında farklılıklar gözlenmiştir. Tohum anaçlarının kullanımının zorunlu olduğu türlerde, anaç adaylarında kseninin etkisinin de araştırılması gerekmektedir. Ayrıca bu kestane genotip ve çeşitleri ile ilgili aşı uyuşma çalışmaları da yürütülmeli ve arazideki uzun yıllar yaşama oranları belirlenmelidir.

Anahtar Sözcükler:
Castanea spp.
Hibrit
Çöğür potansiyeli
Çöğür üretkenliği

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1. Introduction

Anatolia is considered one of the origin centers of European chestnut (*Castanea sativa* Mill.). According to FAO, Turkey's chestnut production in 2017 is 62.904 tones (FAOSTAT, 2019).

In terms of production, Turkey is the leader country in Europe and at second place in the world. On the other hand, in 1987 Turkey's chestnut production was approximately 90.000 tones.

In the following years, the chestnut blight (*Cryphonectria parasitica*) disease epidemic increased resulting in a decrease of nearly to 50.000 tones of chestnut production for Turkey in 2000 (FAOSTAT, 2019).

From 2000 to today, the impact of the chestnut blight is decreasing as Turkey's chestnut production continues to increase; however, chestnut blight still remains a threat to the chestnut production. Chestnut root rot, caused by oomycetes of various Phytophthora species, and Asian chestnut gall wasp, caused by *Dryocosmus kuriphilus*, have joined with chestnut blight by reducing nut production across Turkey.

There are multiple management methods against these pest and diseases. However, the most efficient method is to use resistant cultivars.

To obtain potentially resistant cultivars to these pests and diseases, complex hybrids of various chestnut species were imported from USA in 2005. From 2006 to 2014, adaptation studies were conducted and the performance of the genotypes were evaluated (Serdar et al., 2014; Pereira-Lorenzo et al., 2016).

As a result of the adaptation studies, A14, A25 and A100 genotypes were found to be superior for their nut quality and yield. Also, with their low growth vigor A25 and A41 genotypes were found superior as dwarf rootstock candidate. On 25.10.2019, A14 genotype registered as 'Akyüz' cultivar and A100 as 'Macit 55' cultivar by Republic of Turkey Ministry of Agriculture and Forestry Variety Registration and Seed Certification Center (TTSM) (TTSM, 2019).

Registration process is still going on with A25 genotype. In order to propagate these superior cultivars and genotypes, grafting studies were done.

They were grafted onto European seedling rootstocks, but in the following years various levels of graft incompatibilities were observed (Serdar et al., 2014). To avoid financial losses can be caused by graft incompatibility in the future it is important to determine suitable rootstocks for these genotype and cultivars. Their own seedlings may be better candidate as rootstocks because of the degree of compatibility.

For this aim, seedlings of these genotype and cultivars are tested for their rootstock potential.

The main purpose of this study was to find out the seedling rootstock potential of these hybrid chestnut genotypes.

2. Material and Methods

The experiment was conducted between 2013 and 2015 at Ondokuz Mayıs University, Agriculture Faculty, Horticulture Department, Samsun, Turkey (41º 21' 55'' N, 36º 11' 14'' E; 190 m above sea level).

2.1 Material

In the study A25 and A41 genotypes; 'Akyüz', 'Macit 55' and 'Marigoule' cultivar were used as generative rootstocks. 'Akyüz', 'Macit 55' cultivars and A25, A41 genotypes are hybrids of 'King Arthur' (*C. mollissima*/*C. seguine*) and 'Lockwood' (*C. crenata*/*C. sativa*/*C. dentata*) cultivars (Macit et al., 2018). 'Marigoule' cultivar is a natural hybrid of *C. sativa* and *C. crenata* (Chapa and Verlhac, 1978).

It was used as control to compare with genotypes and cultivars.

2.2 Methods

The study was carried out for three years (2013, 2014 and 2015). In all three years, after harvest chestnut seeds were brought to the laboratory immediately.

For surface sterilization, seeds were washed with 1 % NaOCl for 2 minutes.

After surface sterilization they were dried on blotting paper for 2 days.

For stratification, seeds were counted and then put in plastic cases with wet perlite medium and transferred into a cold room (2-4°C) (Table 1).

To avoid loss in moisture top of the cases were covered with plastic wrap.

Every two weeks moisture level of the perlite was checked by hand and if necessary, water was added. Stratification was ended as the radicle of seeds reached about 2 cm long in 50 % of the seeds (2.5-3 months).

After stratification healthy seed ratio was calculated by counting the unmolded or undecayed (healthy) nuts. Healthy seeds were planted into the 5.5 L pots. For each year the same soil mixture was used (5:1:1, Soil, Perlite, Peat). Soil mixture properties are indicated in Table 2 for each year.

At the end of the vegetation period, seed emerging ratio was calculated by counting the number of seedlings. Planted seed numbers for each genotypes and cultivars were given in Table 3.

Çizelge 1. Çeşit ve genotiplere ait katlamaya alınan tohum sayıları
Table 1. Seed numbers of the cultivars and genotypes placed in to the stratification

Cultivars/ Genotypes	Year		
	2013	2014	2015
Akyüz	420	360	420
A25	255	90	300
A41	255	210	60
Macit 55	520	420	420
Marigoule	520	270	420

Çizelge 2. Yıllara göre kullanılan toprak karışımının özellikleri
Table 2. Soil mixture properties for each year

	2013	2014	2015
Structure	Sandy Loam	Sandy Loam	Sandy Loam
pH 1:1	7.64	7.64	7.66
EC dS/m	0.35	0.35	0.32
$CaCO_3$ %	2.57	2.57	2.77
Organic Matter %	3.10	3.10	4.03
Total N %	0.13	0.13	0.25
P ppm	31.66	31.66	22.15
K ppm	16.47	16.47	20.67
Na me/100g	1.72	1.72	1.63

Çizelge 3. Çeşit ve genotiplere ait dikilen tohum sayıları
Table 3. Planted seed numbers for each cultivars and genotypes according to the years

Cultivars/ Genotypes	Year		
	2013	2014	2015
Akyüz	375	300	360
A25	210	90	270
A41	210	180	60
Macit 55	375	375	360
Marigoule	375	210	360

Also, seedling diameter was measured 5 cm above soil level with a digital caliper at the end of the vegetation period. 6 mm or thicker seedlings were counted as suitable for grafting (Soylu and Serdar, 2000). Coefficient of variation (CV) of seedling diameter (%) was calculated as Soylu (1986) stated (standard deviation of seedling diameter / mean of seedling diameter). Seedling productivity (%) is an important term for the nurseries. It was calculated as the ratio between seedlings which are thicker than 6 mm at 5 cm above soil level and seeds put into stratification.

The study was designed with three repetitions and randomized plot design was used. The total seed or seedling number was varied according to the genotype and cultivar and the year. Healthy seed ratio, seed emerging ratio, seedling diameter and suitable for grafting seedling ratio were analyzed statistically in SPSS 17.0 package program. Angle (arcsin$\sqrt{x}$) transformation was applied to values calculated as a percentage in applications (Tosun, 1991). The significance level of the differences between the means was determined by Duncan Multiple Range Test.

To make a general evaluation about the rootstock potentials of these cultivar and genotypes, a comparison was made by using the weighted-rankit method as modified by Ertan (1999) in Table 4.

Çizelge 4. Çeşit ve genotiplerin anaçlık değerlerini belirlemede kullanılan tartılı derecelendirme puan cetveli (Ertan, 1999'dan modifiye edilmiştir)
Table 4. Weighted-rankit method table for evaluation of the rootstock potentials of the variety and genotypes (Modified from Ertan, 1999)

	Characters	Relative Scores	Value Ranges
1.	Healthy seed ratio (%)	20	≤ 50 %: 1, 51-60 %: 3, 61-70 %: 5, 71-80 %: 7, 81-90 %: 9, ≥ 91 %: 10
2.	Seed emerging ratio (%)	20	≤ 50 %: 1, 51-60 %: 3, 61-70 %: 5, 71-80 %: 7, 81-90 %: 9, ≥ 91 %: 10
3.	Seedling diameter (mm)	20	≤ 5 mm: 1, 5.1-5.5 mm: 3, 5.6-6.0 mm: 5, 6.1-6.5 mm: 7, 6.6-7.0 mm: 9, ≥ 7.1 mm: 10
4.	Coefficient of variation (CV) of seedling diameter	20	≥ 29: 1, 25-28: 3, 21-24: 5, 18-20: 7, 15-17: 9, ≤ 14: 10
5.	Suitable for grafting seedling ratio (%)	20	≤ 60 %: 1, 61-68 %: 3, 69-76 %: 5, 77-84 %: 7, 85-92 %: 9, ≥ 93 %: 10
	Total	100	

3. Results and Discussion

In the study, healthy seed ratio was ranged between 89.0-100.0 % in 2013; 86.5-100.0 % in 2014 and 90.0-100.0 % in 2015. In all three years, the lowest healthy seed ratio was obtained from 'Marigoule' cultivar. In 2013 the highest health seed ratio was obtained from A25 and A41 genotypes. In 2014 'Akyüz' cultivar and in 2015 A41 genotype gave the best results (Table 5).

Çizelge 5. Healthy seed ratio after stratification (%)
Table 5. Healthy seed ratio after stratification (%)

Cultivars/ Genotypes	Healthy seed ratio (%)		
	2013	2014	2015
Akyüz	97.1 b*	100.0 a	96.9 c
A25	98.9 a	96.8 b	96.5 c
A41	100.0 a	93.8 c	100.0 a
Macit 55	91.7 c	91.1 d	98.1 b
Marigoule	89.0 d	86.5 e	90.0 d
P	≤0.01	≤0.01	≤0.01

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

Seed emerging ratio was ranged between 79.6-95.4 % in 2013; 80.1-96.8 % in 2014 and 81.1-93.9 % in 2015. In all three years, the lowest seedling emerging ratio was obtained from 'Marigoule' cultivar, probably because of the low healthy seed ratio. This could be related with the shell thickness of the nuts. Shell thickness can affect the penetration of the water into the nuts. In 'Marigoule' cultivar shell thickness of the seeds can be thinner than others. So, this can lead to increase mold and decay. In 2013 and 2014, the highest seed emerging ratio was obtained from A25 genotype 95.4 and 96.8 % respectively. In 2015 'Akyüz' cultivar gave the best results with 93.9 % (Table 6).

Çizelge 6. Tohum çıkış oranı (%)
Table 6. Seed emerging ratio (%)

Cultivars/ Genotypes	Seed emerging ratio (%)		
	2013	2014	2015
Akyüz	93.1 b*	93.6 b	93.9 a
A25	95.4 a	96.8 a	81.8 d
A41	92.6 b	84.6 c	88.6 c
Macit 55	88.0 c	82.9 d	92.3 b
Marigoule	79.6 d	80.1 e	81.1 e
P	≤0.01	≤0.01	≤0.01

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

In our study the seed emerging ratio was ranged between 79.6-96.8 %. In a study about chestnut rootstock selection in Marmara region, seed emerging ratio was ranged between 32.5-86.6 % (Soylu et al., 1999). Another study was conducted in Black sea region with the same purpose and the seed emerging ratio was ranged between 51.6-97.3 % (Soylu and Serdar, 2000). Our results are in accordance with these studies.

Seedling diameters were ranged between 6.21-7.76 mm in 2013, 4.35-6.34 mm in 2014 and 6.20-7.89 mm in 2015 (Table 7). In all three years, the difference between the seedling diameters were statistically significant and the thickest diameter was measured from 'Marigoule' cultivar. As predicted, the seedling diameter was thinner in A25 genotype, which was thought to have weak growth character. After 'Marigoule' the thicker seedlings were obtained from 'Akyüz' cultivar in 2013 and 2014 and from A41 genotype in 2015. In 2014, strong pruning was performed to obtain more scions from A41 genotype. That led us to harvest less nuts compared to other years. This situation affected positively the size of the seeds in 2015. Macit et al. (2018) stated seed weight of A41 genotypes as 8.3 g. However, in 2015 seed weights were measured as 12.3 g. According to some studies, there is a positive relationship between seed size and seedling development (Soylu, 1986; Cicek and Tilki, 2007). Therefore, it can be stated that the thicker diameter of the A41 genotype in 2015 is due to the larger seed size of the seeds planted this year.

Çizelge 7. Çeşit ve genotiplerde ölçülen çap (mm), çöğür çapı üniformitesi (CV) ve aşı yapılabilir çöğür oranı (%) değerleri

Table 7. Seedling diameter (mm), Seedling diameter uniformity (CV) and suitable for grafting seedling ratio (%) of the genoypes and cultivars

Cultivars/ Genotypes	2013			2014			2015		
	Seedling diameter (mm)	Diameter CV (%)	Suitable for grafting seedling ratio (%)	Seedling diameter (mm)	Diameter CV (%)	Suitable for grafting seedling ratio (%)	Seedling diameter (mm)	Diameter CV (%)	Suitable for grafting seedling ratio (%)
Akyüz	6.89 b*	13.8	93.7	5.48 b	12.1	93.9	6.65 c	17.5	95.1
A25	6.21 d	12.7	92.8	4.35 d	15.7	96.6	6.45 c	20.2	95.7
A41	6.75 bc	12.7	93.2	4.81 cd	21.8	97.2	7.28 b	18.1	91.6
Macit 55	6.46 cd	12.8	94.6	5.13 bc	14.8	93.9	6.20 c	15.3	95.1
Marigoule	7.76 a	14.1	94.1	6.34 a	20.4	96.5	7.89 a	16.2	92.9
P	≤0.01		NS	≤0.01		NS	≤0.01		NS

* There is no difference between the means indicated by the same letter in the same column.
NS: Not significant

The coefficient of variation (CV) of seedling diameter indicates the uniformity. Lower value means the seedling diameter is more uniform. The CV values of the seedling diameter varied from year to year and varied between 12.7-14.1 % in 2013, 12.1-21.8 % in 2014 and 15.3-20.2 % in 2015 (Table 7).

Suitable for grafting seedling ratio was ranged between 92.8-94.6 % in 2013, 93.9-97.2 % in 2014 and 91.6-95.7 % in 2015 (Table 7). There was no statistical difference between the rates of suitable for grafting seedling ratio in all three years.

In our study, the seedling diameter was 4.35-7.89 mm, the CV of the diameter was 12.1-21.8 % and the suitable for grafting seedling ratio was ranged between 91.6-97.2 % according to years. Bilgener and Serdar (1995), found that seedling diameter was ranged between 3.13-7.21 mm according to genotype and stratification medium and year. On the other hand, Ertan (1999) found the seedling diameter between 6.56-10.74 mm, coefficient of variation (CV) of seedling diameter between 4.15-35.75 % and the suitable for grafting seedling ratio between 0.0-24.28 %. Soylu et al (1999), in the first year of their study they measured seedling diameter between 4.3-6.5 mm and in the second year between 3.6-6.8 mm and CV of seedling diameter between 18.60-36.65 %. Soylu and Serdar (2000), measured the diameter of the seedlings between 4.87-7.83 mm and the seedling diameter uniformity (CV) calculated between 12.5-32.1 %. Besides, in the same study, the suitable for grafting seed ratio was varied between 20.6-88.9 %. It is thought that the differences seen in our study may be due to the genetic structure of the genotype, ecology and genotype x ecology interaction.

Soylu and Serdar (2000) stated that 6 mm and thicker seedlings were counted to determine the suitable for grafting seedling rate. However, Ertan (1999) determined this value as 10 mm and thicker were suitable for grafting. This explains the differences about the suitable for grafting seedling ratio between two studies.

The seedling productivity ratio indicates that how many seedlings were obtained from the seeds which were put into stratification. The highest ratio for

seedling productivity was obtained from A25 genotype in 2013 and 2014 by 88.5 % and 93.6 %, respectively. On the other hand, the lowest ratio was obtained from 'Marigoule' cultivar in 2013 and 2014 by 74.9 % and 77.2 %, respectively. In 2015, the highest ratio was obtained from 'Akyüz' cultivar by 89.2 % and the lowest ratio from A41 genotype by 71.4 % (Table 8). According to variety/genotypes' all three years average, the highest ratio of seedling productivity was obtained from 'Akyüz' cultivar with 88.0 % and the lowest was from 'Marigoule' cultivar with 76.2 %.

Çizelge 8. Aşı yapılabilir tohum oranı (%)
Table 8. Seedling productivity ratio (%)

Cultivars/ Genotypes	2013	2014	2015	Mean
Akyüz	87.3	87.9	89.2	88.0
A25	88.5	93.6	78.3	84.6
A41	86.3	82.2	71.4	83.0
Macit 55	81.8	77.9	87.7	80.3
Marigoule	74.9	77.2	75.3	76.2
Mean	82.2	79.7	79.2	

General evaluation was done with all the data obtained from the study by using the weighted-rankit method. In 2013, 'Akyüz' cultivar and A41 genotype had the highest score with 980. These two genotypes were followed by A25 genotype with 940 points (Table 9). In 2014, the highest score was obtained from 'Akyüz' cultivar with 860. This cultivar was followed by 'Macit 55' cultivar with 820 points. In 2015, 'Akyüz' and 'Marigoule' cultivars had the highest score with 960 points.

Table 9. Çeşit ve genotiplerin tartılı derecelendirme puanları
Table 9. Weighted-rankit scores of the cultivars and genotypes

Year	Cultivars/ Genotypes				
	Akyüz	A25	A41	Macit 55	Marigoule
2013	980	940	980	920	920
2014	860	800	700	820	800
2015	960	860	900	920	960
Mean	933	867	860	887	893

According to the average weighted-rankit scores, 'Akyüz' cultivar had the highest score with 933. 'Akyüz' cultivar was followed by 'Marigoule' cultivar with 893 points. 'Akyüz' (Macit et al., 2018) and 'Marigoule' (Serdar et al., 2011) cultivars are thought to have bigger seeds compared to other genotypes, resulting in a thicker seedling diameter than others.

For rootstock potential, rootstock diameter and suitable for grafting seedling ratio criteria are very important. In Turkey, 'Marigoule's popularity as seedling is getting increase every day. Serdar et al (2011) determined that the seed size of 'Marigoule' is 61 nuts per kilogram. Although this value is approximately the same as 'Akyüz' cultivar. However, in 'Marigoule' cultivar, healthy seed ratio and suitable for grafting seedling ratio were found lower than others.

'Marigoule' which was examined as control cultivar in our research was registered as cultivar and also rootstock by Ondokuz Mayıs University Faculty of Agriculture on 06.04.2010 (TTSM, 2019). Among the hybrid chestnut genotypes used in the study, 'Akyüz' cultivar received a better weighted-rankit score than 'Marigoule' cultivar, while the other genotypes scored close to it.

4. Conclusion

The cultivars and genotypes examined in our study were evaluated in terms of their generative rootstock characteristics and all were found as superior. However, a great variation was observed among the rootstocks over the years. Therefore, studies should be conducted to determine the presence of xenia effect of rootstock candidates on rootstock selection in nut species where generative rootstock use is necessary. Also, graft compatibility studies should be done with these superior genotypes.

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