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Araştırma Makalesi

Evaluation of Yields and Quality of Saplings Obtained by Grafting Combinations of Different American Grapevine Rootstocks and Kalecik Karası Grape Variety

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ABSTRACT

Cultivation on American grapevine rootstocks, which have been found to be an effective solution against phylloxera pest, which is the biggest threat to vineyard areas, has brought along the necessity of examining the affinity between rootstock and scion. In this regard, to increase the amount of current vineyard production by adding a new research to the studies examining the affinity situation in the literature, scions belonging to Kalecik Karası grape variety were grafted on 10 different rootstocks (41B, 5BB, 1103P, SO4, 99R, 1616C, 1613C, 5C, Ramsey ve Harmoney) by omega bench grafting method, and sapling yield and quality characteristics were examined in greenhouse conditions for two years in duplicate. After the measurements carried out in both years, it was determined that the average degree of callus formation of the combinations formed trough different rootstocks was 3.45, the length of the saplings was 10.90 cm, the number of leaves was 5.71 and the total sapling yield was 78%.

Key words: *Grafting, Omega, Kalecik karası, Grapevine rootstocks, Sapling*

Farklı Amerikan Asma Anaçları ve Kalecik Karası Üzüm Çeşidinin Aşılama Kombinasyonu ile Elde Edilen Fidanların Kalite Özellikleri ve Randımanlarının Saptanması

ÖZ

Bağ alanlarının en büyük tehditi olan filoksera zararlısına karşı etkili bir çözüm olduğu anlaşılan Amerikan asma anaçlarının kökleri üzerinde yetiştiricilik, anaç ve kalem arasındaki afinite durumunun incelenmesi gerekliliğini de beraberinde getirmiştir. Bu doğrultuda, literatürdeki afinite durumunun incelendiği çalışmalara yenisini katarak mevcut bağ üretim miktarını arttırmak amacıyla Kalecik Karası üzüm çeşidine ait kalemler 10 farklı anaç (41B, 5BB, 1103P, SO4, 99R, 1616C, 1613C, 5C, Ramsey ve Harmoney) üzerine omega masabaşı aşı yöntemiyle aşılanıp sera koşullarında iki yıl tekerrürlü olarak fidan randımanı ve kalite özellikleri incelenmiştir. Yapılan tek yönlü varyans analizi sonucunda her iki yılda da kallus skalası, sürgün uzunluğu ve yaprak sayısı açısından istatiksel farklar bulunmuştur. Her iki yılda yapılan ölçümler sonunda, farklı anaçlarla oluşturulan kombinasyonların ortalama kallus oluşum derecesi 3.45, fidanların sürgün uzunlukları 10.90 cm, yaprak sayısı 5.71 ve toplam fidan randımanı %78 olduğu saptanmıştır.

Anahtar kelimeler: *Aşılama, Omega, Kalecik Karası, Amerikan asma anacı, Fidan*

INTRODUCTION

Türkiye is located at the crossroads of grapevine gene centres and has a strong viticulture culture (Çelik et al., 1998). Türkiye's different regions and favourable conditions of these regions enable the cultivation of many varieties of winemaking grapes of both local and foreign origin (Çelik et al., 2010). However, when the production quantities in Türkiye are analysed, wine-growing ranks last compared to table and raisin cultivation On the other hand, there has been a decrease in both the area and production quantities of vineyards where

wine production takes place compared to the last 20 years, while fluctuations in yields are observed (Anonymous, 2023). The reason for the decrease in these amounts can be interpreted as the senescence of the vineyard areas and the lack of implementation of modern viticulture techniques. To bring the production grown for wine-growing to the desired position, especially considering that most of Türkiye's existing vineyard areas are contaminated with phylloxera pest, it becomes indispensable to establish vineyards with grafted vine saplings.

When the current production amounts of grafted grapevine saplings in Turkey are analysed, the production of saplings for wine production is in the second place with approximately 30%. Narince is the most common grape variety used in this production with approximately 60%, followed by Merlot and Cabernet Sauvignon with about 8%. Among the rootstocks, 1103 P, Kober 5 BB and 41 B are widely used (Anonymous, 2019; as cited in Söylemezoğlu et al., 2020). There are significant differences between the rootstocks used in viticulture and grape varieties in terms of adaptation to various ecological conditions and compatibility with each other. These variety-rootstock relationships can cause various difficulties in important factors such as plant growth, yield, quality, affinity and adaptation. Therefore, it is important to determine the compatibility of rootstocks used in viticulture with specific grape varieties, their adaptability to regional ecological conditions and their effects on the characteristics of the varieties during physiological growth periods (Çelik and Odabaşı, 1994; 1995; Türkben and Sivritepe, 2000; Çelik et al., 2010). In other words, in order to increase sapling production and to provide high yields with the obtained saplings, it is important to reproduce varieties adapted to various regions and favoured by consumers (Kaya and Karataş, 2023).

Kalecik Karası is one of the 3 most important red wine varieties of Turkey with its delicate, soft, lively, easy drinking and moderate aging characteristics with 23-24% total soluble solids and 0.7-0.8% acidity. However, viticulture in the Central Anatolia Region with the Kalecik Karası grape variety, which continued to exist until the 1960s, declined rapidly due to the phylloxera pest, neglect and marketing difficulties, and almost disappeared at the end of the 1980s. However, it was revitalised in the 1990s with the popularity of its wine, and intensive vineyard areas were set up throughout the country. Nowadays, clone selection projects based on wine quality are being carried out by various institutions and organisations in Turkey and infrastructure efforts for the production of certified saplings are continuing (Çelik et al., 2019).

Until now, it is seen that different rooting media, plant growth regulators and different grafting combinations have been applied in studies on the propagation of Kalecik Karası grape variety (Çelik, 1982; Kıraç and Çelik, 1996; Çakır and Yücel, 2016; 2017). On the other hand, the affinity status of Kalecik Karası on different grapevine rootstocks has been investigated by electrophoretic method, but the quality characteristics of the saplings and affinity of some rootstocks namely 1616C, Ramsey and Harmoney have not been determined and It has been emphasised that because of the difficulty in interpreting the data, using enzymes to predict graft incompatibility is daunting (Gökbayrak et al. 2007). In this respect, grape variety of Kalecik Karası should be grafted on the widely used grapevine rootstocks and then the yield and quality characteristics of the saplings should be examined.

The grafted grapevine saplings are obtained by hand or machine grafting of one year old scions of *Vitis vinifera L.* grape varieties onto American grapevine rootstocks and rooting them under nursery or controlled conditions (Çelik et al., 1998). Grapevine saplings obtained in greenhouses and similar controlled conditions have advantages such as a high sapling yields, being ready for planting in about 3 months and early maturity compared to bare-rooted ones (Akman and Ilgın, 1991).

In this study, it was aimed to investigate the affinity and quality characteristics of Kalecik Karası, a high performing red wine grape variety in Central Anatolia Region, by grafting on different American vine rootstocks with Omega grafting method under controlled conditions in order to eliminate the negative effects of environmental factors and to obtain saplings in a shorter time.

MATERIAL and METHOD

This study was carried out in the viticulture research and application area of Dicle University, Faculty of Agriculture, Department of Horticulture.

Plant material

The plant materials used in this study consisted of cuttings of different American grapevine rootstocks (41B, 5BB, 1103P, SO4, 99R, 1616C, 1613C, 5C, Ramsey and Harmoney) and scions of Kalecik Karası, a winemaking grape variety.

Method

The cuttings were collected from healthy and strongly growing one-year-old branches of the plant during the pruning period and kept in cold storage at 4^{o}C ± 1 and 85-90% humidity level until the graft period. Before grafting, the cuttings were kept in a water pool for two days to regain the water lost in the structure during cold storage and to soften the wood tissue (Değirmenci Karataş et al., 2023). The cuttings removed from the water pool were treated with fungicide, and the omega bench grafting method was used for the grafting process. The grafting was carried out in the second week of March (15 March) and a total of 60 grafts were made for each combinations. To control the loss of moisture at the grafting point, the cuttings were quickly dipped in paraffin melted at 70-75^{o}C containing fusion-promoting ingredients (Akman and Ilgın, 1993; Çelik et al., 1998). The paraffinised cuttings were stacked in plastic boxes in the order of moistened sawdust and cuttings. The plastic boxes, which were transported to the callusing room, whose temperature and humidity were controlled by the automation system and set 25˚C ±1 - %85-90 humidity, were kept in this room for around 3 weeks (Çoban and Kara, 2003) to ensure the formation of callus at the grafting point of the cuttings. In addition, to prevent the increase in moisture concentration in the callusing room, 30 minutes of ventilation was carried out every day. The callused cuttings were removed from the callusing room and rinsed in plastic containers containing water to remove the sawdust adhering to the cuttings. Grafted cuttings were again treated with paraffin and planted in 12×24 cm polyethylene tubes containing peat, perlite and sand (2:2:1) and transferred to the greenhouse at the beginning of the second week of April for rooting. In case the air temperature in the greenhouse exceeded 30 ˚C, fans were activated to reduce the environment temperature, and the greenhouse floor was wetted in case of low humidity to maintain the humidity level.

Investigated parameters

After rinsing the grafted cuttings taken out in the callusing room, the callus scale was used to observe the level of callus development, and the parameters of shoot length and number of leaves were examined in the greenhouse saplings. On the other hand, the viability ratio, which represents the percentage of fused cuttings planted in the greenhouse, was determined. The examination of all parameters was carried out on 10 randomly selected plants and the methods followed during the examination are presented below:

Callus scale (0-4): Grafted cuttings removed from callusing room were classified as per callus development. For this classification, numbers from 0 to 4 were used, and meanings of numbers are presented below (Çelik, 1982; Doğan, 1996):

0 : No callus formation
1 : One-way callus
2 : Callus in two directions
3 : Callus in three directions
4 : Callus in four (fully) directions

Shoot length (cm): The main shoot length of the saplings were measured with the help of a tape measure from the shoot exit point to the shoot tip.

Number of leaves (n): Leaves that have completed their development on the main shoot were counted (Cangi and Etker, 2019).

Viability ratio (%): It expresses how many of the grafted cuttings left in the greenhouse to be rooted have turned into saplings. For this purpose, $\frac{Number\ of\ saplings}{Number\ of\ grafted\ cuttings\ planted} \times 100$ formula was used (Cangi and Etker, 2019).

Statistical analysis

The data obtained in the experiment, which was designed according to random plots experimental design with four replications and 10 plants in each replicate, were subjected to analysis of variance by using Jump Pro 16.0 statistical software. After the analysis of variance, TUKEY HSD analysis was performed to compare the means.

FINDINGS AND DISCUSSION

Callus scale (0-4)

According to the analysis of variance for the callus scale parameter of the Kalecik Karası grape variety grafted on different rootstocks, there were significant differences (5%) only between the rootstocks in the second year, while differences at 5% level of significance were observed between the replicates and rootstocks in the first year (Table 1).

The average degree of callus formation obtained in both years depending on rootstock and scion compatibility was approximately 3.45. Callus formation was lower than this average value in 5C, Harmoney and Ramsey rootstocks with values of 3.25-3.30, 3.23-3.15 and 2.65-2.83 in the first and second years, respectively. On the other hand, the combination with 99R rootstock showed a callus formation of 3.75 in the first year, which was above the average callus degree for both years, but this value decreased to 3.38 in the second year, causing it to be evaluated below the average (Table 4). According to the statistically analysed data, a high degree of callus formation, i.e. almost all-around callus formation, was recorded in the 1103P combination with a calculated value of 3.80. Although almost all combinations formed callus in 3 or more directions, the percentage of callus formation in the combination with Ramsey rootstock varied on average between two and three directions (2.74), making it the combination with the lowest degree of callus formation compared to all other combinations (Figure 1.).

Table 1. Analysis of variance results for callus scale parameter of saplings of Kalecik Karası grape variety produced by different rootstock combinations

Source	Nparm		DF		Sum of squares		F ratio		Prob > F	
Years	First	Second	First	Second	First	Second	First	Second	First	Second
Rootstock	9	9	3.69866	2.06618	0.41096	0.22958	22.7466	8.9868	<,0001*	<,0001*
Replication	3	3	0.20691	0.08959	0.06897	0.02986	3.8174	1.169	0,0102*	0.3213
Error	387	387	6.99192	9.88622	0.01807	0.02555				
Total	399	399	10.8975	12.042						
CV	7.14	8.19								

*denotes a significant difference at 5%.

Çakır and Yücel (2016) created a grafting combination between the Kalecik Karası grape variety and 1103P American grapevine rootstock. In the obtained saplings, it was determined that the callus rate was 60%. In a study by Çalkan Saglam and Saglam (2020), the effect of hot water treatments on callus formation in some grape varieties and rootstock combinations was investigated. In the Ramsey×Sultani seedless combination, the highest four-sided callus formation with a rate of 13% was obtained in the treatment in which the rootstocks were kept in water for 30 minutes and the scions for 45 minutes. In another study, the degree of callus development obtained when Müşküle grape variety was grafted on 5BB, 41B and 1613C rootstocks was determined as 2.88, 3.38 and 3.11, respectively (Sivritepe and Türkmen, 2001). Eroğlu (2014) found that when Alphonse Lavallèe and Red Globe grape varieties were grafted on 110R and 1103P rootstocks, the rate of all-round callus formation was 64.90% - 84.90% for Alphonse Lavallèe and 84.94% - 92.67% for Red Globe grape varieties, respectively. Çakır et al. (2013) grafted Sultani seedless grape variety on 8 different American grapevine rootstocks, the lowest percentage of four-way callus formation was obtained from 99R rootstock with 4.8% and the highest percentage of four-way callus formation was achieved from the combination of 5BB rootstock with 82.29%. Köse et al. (2015), when Merzifon Karası grape variety was grafted to 10 different grapevine rootstocks, the callus development degree was found to be 3.5, 2.6, 1.6, 2.3, 3.1 and 3.4 on 99R, 41B, 5C, 5BB, SO4 and 1103P rootstocks, respectively. According to the findings obtained in our study, the highest degree of callus development was obtained in 1103P combination. This finding was similar to the studies carried out by previous researchers with different varieties.

Shoot length (cm)

Analysis of variance for the shoot length parameter of the Kalecik Karası grape variety grafted on different rootstocks showed that in both years there were differences between the rootstocks at 5% level of significance (Table 2).

Saplings of Kalecik Karası grape variety formed shoots with an average length of 10.90 cm in both years on different rootstocks. The highest shoot length (13.05 cm) was recorded in combination with 1616C rootstock, while the shortest shoots (8.17 cm) were found in combination with Ramsey rootstock. In addition,

only 41B, 1103P and 1616C combinations had higher values than the average shoot length values of two years with 12.29, 12.30, and 13.05 cm respectively (Figure 1). Looking at Table 4, it is seen that there are fluctuations in the shoot lengths of the combinations on a yearly basis. In both the first and second year, 1616C rootstock had the highest shoot length with 13.15 and 12.94 cm shoots, respectively, and Ramsey formed the shortest shoot length with 8.1 and 8.25 cm shoots in the same order. While Harmoney was the combination with the shortest shoot length after Ramsey with 9,43 cm in the first year, in the second year this combination was among the top three rootstock with the longest shoots by forming shoots with an average length of 12,18 cm.

Table 2. Analysis of variance results for the length of shoot parameter of saplings of Kalecik Karası grape variety obtained by different rootstock combinations

Source	**Nparm**		**DF**		**Sum of squares**		**F ratio**		**Prob > F**	
Years	**First**	**Second**	**First**	**Second**	**First**	**Second**	**First**	**Second**	**First**	**Second**
Rootstock	9	9	20.6376	19.3944	2.29306	2.15494	8.0073	8.2528	<,0001*	<,0001*
Replication	3	3	1.79096	0.13032	0.59699	0.04344	2.0846	0.1664	0.1017	0.919
Error	387	387	110.826	101.052	0.28637	0.26112				
Total	399	399	133.255	120.576						
CV	16.45	15.59								

* denotes a significant difference at 5%.

In a study in which Uslu and Yalova Incisi grape varieties were grafted on 5BB, 140Ru, 41B and 1103P American grapevine rootstocks, the main shoot length was 34.10-28.97 cm for Uslu and Yalova Incisi varieties in 41B, 32.20-26.41 cm in 140Ru, 41.99-39.53 cm in 1103P and 33.50-32.56 cm in 5BB combination (Dardeniz and Sahin, 2005). In a study conducted by Arik and Altindisli (2019), scions of the Sultani seedless grape variety were grafted on 1103P, 41B and Ramsey American grapevine rootstocks with omega and chip-budding grafting methods. According to the analyses, the longest average shoot length was 19.5 cm in the 1103P × omega combination. This parameter was determined as 11 cm in 1103P × chip-budding combination. According to the study in which five different table grape varieties were grafted on six different American grapevine rootstocks, the average shoot length values for 41B, 5BB, SO4, 420A, 5C and 1613C rootstocks were 12.22, 12.68, 19.62, 15. 37, 19.57 and 17.88 cm; and 15.51, 19.29, 15.30, 15.22 and 15.81 cm for Razakı, Yalova İncisi, Italia, Alphonse Lavellee and Hamburg misketi (Değirmenci Karataş et al, 2023). The findings obtained in our study were not found to be similar in all other studies except the mean value of 41B combination in the study conducted by Değirmenci Karataş et al.

Number of leaves (n)

According to the analysis of variance for the leaf number parameter of Kalecik Karası grape variety grafted on different rootstocks, there were differences between rootstocks at 5% level of significance in the first year, while a significant difference (5%) was detected between both rootstocks and replicates in the second year (Table 3).

When the number of leaves formed by the saplings was analysed, the lowest number of leaves was found in the Ramsey combination with an average of 3.625, while the highest number of leaves was found in the 1616C combination with 7.58, and these ranks did not change in the second year. Although the number of leaves of the saplings formed by the combinations of 5C, SO4, 1103P and 5BB increased compared to the first year, the 41B combination with an increase of 2.85 in the number of leaves had most leaf increase. It thought that the cuttings used in second year have different carbonhydrate levels. In contrast the rest of the combinations were in the group of combinations that showed a decrease in the number of leaves compared to the second year.

Table 3. Analysis of variance results for leaf number parameter of saplings of Kalecik Karası grape variety obtained by different rootstock combinations

Source	Nparm		DF		Sum of squares		F ratio		Prob > F	
Years	First	Second	First	Second	First	Second	First	Second	First	Second
Rootstock	9	9	30.3123	21.4376	3.36804	2.38196	27.2036	18.7794	<,0001*	<,0001*
Replication	3	3	0.26185	1.22722	0.08728	0.40907	0.705	3.2251	0.5495	0,0226*
Error	387	387	47.9139	49.0868	0.12381	0.12684				
Total	399	399	78.4881	71.7516						
CV	15.15	14.7								

* denotes a significant difference at 5%.

A study using three different grape varieties grafted on 41B, SO4 and 1103P American grapevine rootstocks showed that the number of leaves was 9.71 on 1103P rootstock, 9.10 on 41B rootstock and 8.68 on SO4 rootstock (Kamiloğlu and Güler, 2014). In another study in which Sire grape variety was grafted on several rootstocks, the highest number of leaves obtained in the grafting combinations was found in the 1613C rootstock with 8.91. This combination was followed by 41B (8.54) and 1103P (7.89). All the other combinations (5BB, 420A, 99R and 110R) were statistically in the same group, and the number of leaves varied between 7.52 and 7.04 (Kaya and Karatas, 2023). The Red Globe grape variety was grafted on several American rootstocks in a research carried out by Yildirim and Dardeniz in 2021. The number of leaves observed in the saplings for the 5BB, 41B, 110R, 1613C, and 1103P rootstocks were 6.32, 4.80, 5.13, 5.83, and 6.37, respectively. While the findings of this study are in line with the findings of Yildirim and Dardeniz, the similarity could not be observed with the other two studies.

Viability ratio (%)

When the viability rate, which represents the conversion rate of the grafted cuttings of Kalecik Karası grape grafted on different rootstocks to saplings after rooting in the greenhouse, was analysed, the 99R and Ramsey combinations had the lowest viability rates with 20% and 48% in the first year, 33% and 45% in the second year, respectively. All other rootstocks showed remarkable success. Although there were decreases and increases between the combinations from the previous year, these changes were not enough to cause a statistical difference. The highest viability rate obtained in the combinations was achieved in 41B rootstock with an average of 94%. This combination was followed by the 1613C combination with 92.5% (Figure 1.).

Table 4. Mean values of some sapling quality features examined in the first and second year in the combinations of Kalecik Karası grape variety grown on different American grapevine rootstocks

Rootstock	Years	Callus scale (0-4)	Shoot length (cm)	Number of leaf (n)	Viability ratio (%)
41B	First	3.63 a	12.48 ab	3.68 c	0.93 a
	Second	3.5 a-c	12.1 ab	6.53 a-c	0.95 a
1103P	First	3.85 a	12.1 ab	5.9 b	0.93 a
	Second	3.75 a	12.5 ab	6.18 b-d	0.93 a
SO4	First	3.75 a	10.45 b-d	4.4 c	0.9 a
	Second	3.65 ab	10.33 b-d	5.95 b-d	0.88 a
Harmoney	First	3.23 b	9.43 c-d	6.3 b	0.88 a
	Second	3.15 cd	12.18 ab	5.45 cd	0.75 a
99R	First	3.75 a	10.48 b-d	5.78 b	0.2 c
	Second	3.38 a-c	9.48 cd	5.05 d	0.33 b
1616C	First	3.65 a	13.15 a	7.63 a	0.95 a
	Second	3.38 a-c	12.94 a	7.53 a	0.9 a
1613C	First	3.65 a	10.06 b-d	5.58 b	0.83 a
	Second	3.58 ab	11.54 a-c	5.48 cd	0.9 a
5C	First	3.25 b	10.44 b-d	6.38 b	0.88 a
	Second	3.3 bc	10.53 b-d	6.43 a-c	0.78 a
Ramsey	First	2.65 c	8.1 d	3.65 c	0.48 b
	Second	2.83 d	8.25 d	3.6 e	0.45 b
5BB	First	3.55 ab	10.84 a-c	6.15 b	0.83 a
	Second	3.53 a-c	10.56 a-d	6.68 ab	0.85 a

*A significant variation between rootstocks is shown by different letters in the same column.

Kavak (2006) examined the effect of mycorrhiza and humic acid in different graft combinations and found that in the control group, Kalecik Karası had a sapling yield of 52.67% in 1103 P and Fercal combinations. Gursoz et al. (2017) in their study, the total sapling yield of 99R in 3 different grape varieties varied between 66.7% and 80%. Cangi and Deveci (2018) reported that the total sapling yield in the grafting combination of Royal grape variety with several different rootstocks was 78% on 5BB rootstock and 70% on 1613C rootstock. Gunen and Altindisli (2017) stated that when they grafted Cabernet Sauvignon, a wine grape variety, on 99R, 110R and 1103P American grapevine rootstocks, the total sapling yield was 78%-46.03%-90.48% in the field and 84.12%-60.32%-89.68% under the greenhouse, depending on the rootstock order in the first year of the study. Sucu and Yagci (2017) examined the sapling yield of 10 different grapevine rootstocks (Rup.Du Lot, 420A, 5BB, SO4, 8B, 110R, 1103P, 140Ru, 41B, Ramsey) both grafted with Sultani seedless grape variety and without grafting. The highest sapling yield was observed in Du Lot and 5BB combinations (54%), while the lowest sapling yield was found in 140Ru combination. The yield of non-grafted saplings varied between 84-60% and the highest yield of saplings was obtained when Sultani seedless grape variety was grown in their roots. The reason why the yield values in our study differed between years is thought to be due to the diameter (Etker, 2015) and length (Guler et al., 2017) of the cuttings as mentioned by other researchers.

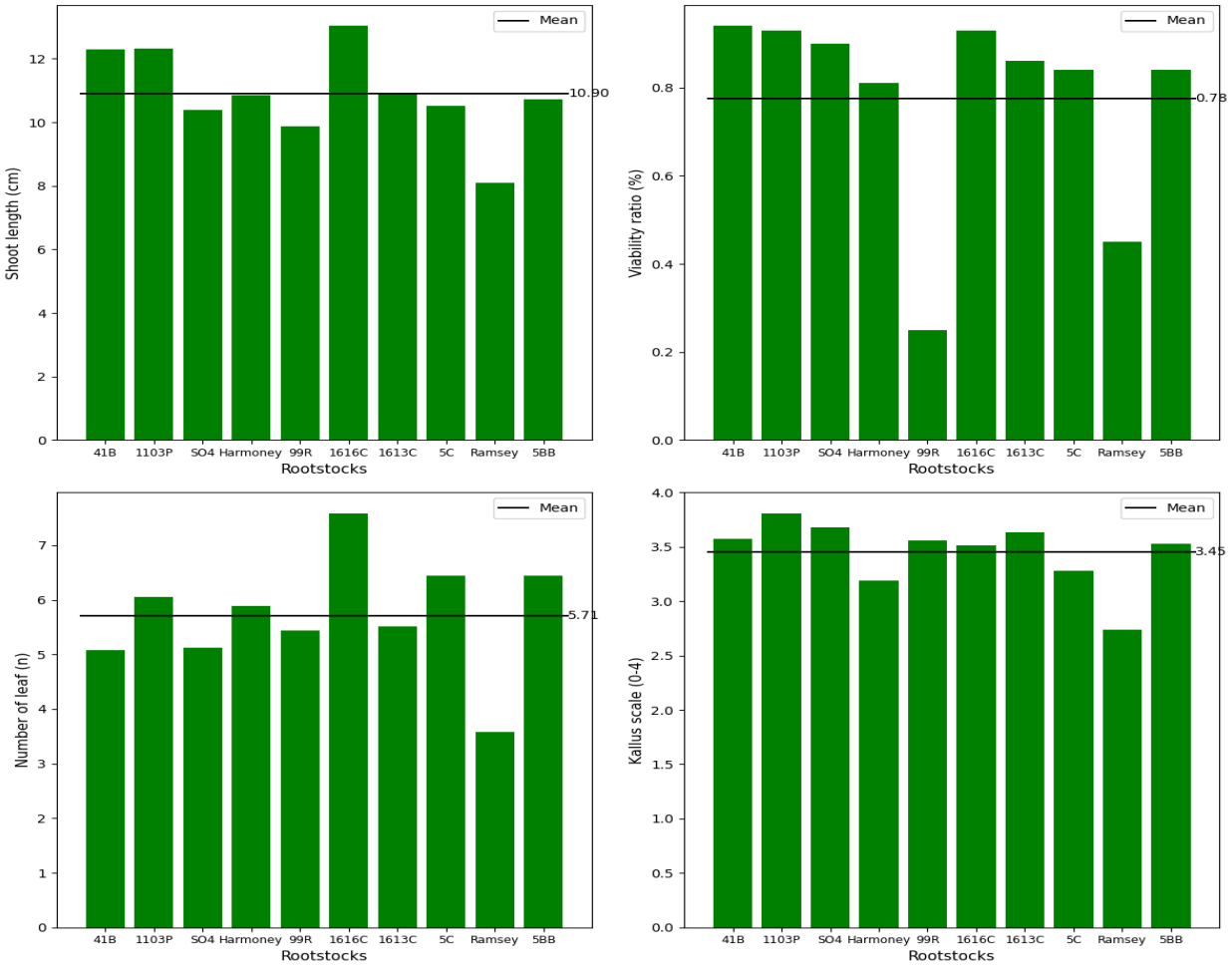

Figure 1. Average values of affinity and sapling quality characteristics of combination for both years

CONCLUSION and SUGGESTIONS

In this study, scions of Kalecik Karası grape variety were grafted on different rootstocks and their affinity was analysed. According to the statistical analysis of the data obtained after the growth period was completed, it can be said that no compatibility problem was observed in all rootstocks except 99R and Ramsey rootstocks and remarkable success was obtained. When the sapling quality values are analysed, it is an undeniable fact that lower quality saplings were observed compared to the previous studies conducted by the researchers. Among all combinations, 1616C rootstock stands out in terms of sapling quality measurements. As a result, it is

recommended that the scions belonging to the Kalecik Karası grape variety should be grafted again on the varieties that show affinity in our study and treatments should be carried out to improve the quality of the saplings, as well as the soil structure that each rootstock needs varies, and also the appropriate combination should be selected according to the soil structure in which the cultivation will be performed.

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