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Determination of callusing performance and vine sapling characteristics on different rootstocks of 'Merzifon Karası' grape variety (*Vitis vinifera* L.)

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ABSTRACT

Grafted vines are the only option while establishing vineyards over the sites contaminated with phylloxera. Furthermore, grafted vines used to establish new vineyards should be well-developed, healthy and compatible with the scions. In this research, we aimed to find out the most suitable rootstock for 'Merzifon Karası' using ten rootstocks (140 Ruggeri, 110 R, 99 R, 41B, 5C, 5BB, SO4, 1103 Paulsen 140 Ruggeri and Rupestris du Lot) in 2010 and 2011. The effects on the callusing performance, growth parameters and graft development characteristics were evaluated by the modified weighted-ranking method. Significant differences were obtained in terms of callus formation, rooting capacity and growth characteristics of cultivar among the rootstocks. Callus formation rate (97.7%) and callus development level (3.7) were better on 8B grafted cuttings. Rooting percentage was found the highest on 1103 Paulsen rootstock (64.8%). While the shoot length and diameter differed according to the rootstocks (P<0.01), the highest shoot length and diameter was determined on 41B grafted vines (92.3 and 9.2 cm, respectively). As a result of weighted-ranking, 1103 Paulsen rootstock got the higher scores than the other rootstocks. According to the results obtained in Samsun conditions, which may be most suitable rootstock for 'Merzifon Karası' grape varieties were evaluated as 1103 Paulsen.

Anahtar Sözcükler: Callusing cv. Merzifon Karası Graft success Rootstock Weighted ranking

Farklı anaçlar üzerinde 'Merzifon Karası' (*Vitis vinifera* L.) üzüm çeşidinin kalluslanma performansı ve fidan gelişimi özelliklerinin belirlenmesi

ÖZET

Filoksera ile bulaşık alanlarda bağ kurarken tek seçenek aşılı asma fidanı kullanmaktır. Bununla birlikte, yeni kurulacak bağlarda kullanılan aşılı asma fidanlarının iyi gelişmiş, sağlıklı ve üzerine aşılı çeşitle uyuşur olması gerekir. Bu çalışmada, 'Merzifon Karası' üzüm çeşidine en uygun olabilecek anaçların belirlenmesi için 2010 ve 2011 yılları arasında, on farklı asma anacı kullanıldı (140 Ruggeri, 110 R, 99 R, 41B, 5C, 5BB, SO4, 1103 Paulsen 140 Ruggeri ve Rupestris du Lot). Anaçların kallus oluşturma performansı, gelişme özellikleri ve aşı gelişimi üzerine etkileri tartılı derecelendirme metodu ile değerlendirilmiştir. Farklı anaçların kallus oluşturduğu belirlenmiştir. En yüksek kallus oluşum oranı (%97.7) ve kallus gelişim seviyesi (3.7) 8B anacına aşılı çeliklerden elde edilmiştir. En yüksek köklenme oranı ise 1103 Paulsen anacı üzerine aşılı çeliklerde gerçekleşmiştir (%64.8). Sürgün uzunluğu ve sürgün çapları anaçlara göre farklılık gösterirken, en yüksek sürgün uzunluğu ve sürgün çapı 41B üzerine aşılı fidanlarda tespit edilmiştir (sırasıyla 92.3 ve 9.2 cm). Tartılı derecelendirme sonuçlarına göre, anaçlar arasında en yüksek puanı 1103 Paulsen almıştır. Samsun koşularında elde edilen bu sonuçlara göre, 'Merzifon Karası' üzüm çeşidi için en uygun olabilecek anaç 1103 Paulsen olarak değerlendirilmiştir.

Keywords: Kalluslanma başarısı Merzifon Karası Aşı gelişimi Anaç Tartılı derecelendirme

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1. Introduction

Vineyard establishment with self-rooted grapevines in

phylloxera infested areas is impossible. To establish a new vineyard, it is necessary to have qualified grafted and rooted vines (Korkutal et al., 2011). Grape rootstocks have

been very important in viticultural countries (Omer et al., 1999; Troncoso et al., 1999), and grafting of Vitis vinifera varieties on resistant rootstocks is still considered to be the most effective way of protection against phylloxera (Vršič et al., 2004). Different effects of scions on vegetative growth and vine quality of grape rootstocks have been demonstrated in several studies (Cangi, 1998; Celik, 2000; Dogan et al., 2000; Hamdan and Salimia, 2010). One of the main problems when choosing the right scion/rootstock combination is that it is fairly hard to predict how the scion and rootstock genotype will interact (Cus, 2004). Many studies proved that rootstocks affect vine growth, yield and fruit quality through the interactions between the environmental factors and the physiology of scions and rootstock cultivars utilized (Boselli et al., 1992; Ferroni and Scalabrelli, 1995; Rizk-Alla et al., 2011; Rafaat S.S. et al., 2013). In grapes, yield is dependent upon the vigor of the rootstock, and it can be a strong influencing factor (Harmon, 1949). In this regard, when deciding, any stock/scion combination should be exhibited in terms of compatibility with rootstocks, adaptation to climate and soil conditions, effects on growth and development (Celik and Odabas, 1995; Turkben and Sivritepe, 2000; Pina and Errea, 2005).

Turkey is one of the most important areas for viticulture, because of its suitable climatic conditions and it has valuable grape germplasm resources. In Turkey, more than 1200 grape varieties are grown (Ergul and Agaoğlu, 2001; Ergul et al., 2002; Uzun and Bayir, 2008). According to recent data, Turkey ranks sixth in the world in terms of grape production and fifth in terms of growing area, with an annual output of 4.296.351 tons of grapes produced on 462.296 ha of vineyard area (FAOSTAT, 2012). As the mainland of numerous grape genotypes, Turkey is one of the major centers of viniculture genetic materials in the world. 'Merzifon Karası' (Vitis vinifera L.) is one of the most valuable red wine grape cultivars in Turkey. But, this grape generally shows millerandage characters according to years, and it has not been tested thoroughly on the rootstocks yet except for 1103 Paulsen. Thus, the aim of the present study was to evaluate the suitable rootstock/scion combination on different rootstocks (140 Ruggeri, 110 R, 99 R, 41B, 5C, 5BB, SO4, 1103 Paulsen, 140 Ruggeri and Rupestris du Lot) for 'Merzifon Karası' grape variety. With this purpose, the callusing performance of each rootstock, vine sapling growth parameters, and graft union characteristics were determined in the present study. In order to determine the most suitable rootstock, growth characteristics were evaluated by the modified weightedranking method.

2. Materials and Methods

Experiments were conducted at Ondokuz Mayis University Agricultural Faculty during 2010 and 2011 in Samsun, Turkey. One year old cuttings of ten rootstocks (140 Ruggeri, 110 R, 99 R, 41B, 5C, 5BB, SO4, 1103 Paulsen, 140 Ruggeri, and Rupestris du Lot) were provided from Tekirdag and Manisa Viticultural Research Stations in February, and they were stored at +2°C, 95-100% humid conditions (Celik and Odabas, 1998). Rootstocks, 8-12 mm in thickness, were grafted with scions of 'Merzifon Karası'.

The scions of the cultivar were collected during dormant period in winter from private vineyards in the Merzifon district. Before the grafting, the rootstocks and scions were disinfected with using Captan 50 % W.P. Grafting was performed on April 4, 2010 and April 3, 2011 using omega grafting technique. Graft union area was dipped for 1 to 2 seconds into melted paraffin wax. In order to stimulate callus formation, the grafted cuttings were stored for 30 days under controlled temperature and humidity conditions. The temperature inside the stratification room was kept at 28 °C for 3 weeks and 26 °C for one week (Todic et al., 2005; Korkutal and Dogan, 2010). Relative air humidity ranged from 70 to 80 % in both years. During the callusing period, grafted cuttings were put into plastic boxes (40x70x40 cm) that include contain water and charcoal. After the callusing was completed, the grafted cuttings were stored at room temperature for a week to acclimatize to the external environment conditions. Before the plantation of grafted cuttings, sprouting shoots of the scions were shortened above one or two buds, and secondly, graft union areas were waxed. Grafted cuttings were hill planted in the nursery with a black plastic mulch to control weeds and retain heat and moisture on May 11, 2010 and May 10, 2011. Drip irrigation system was used in the nursery plots. The nursery soil was a clay-loam with 2.99% organic matter and a pH of 7.1 (Table 1).

Table 1. Soil characteristics of the nursery

Soil characteristicses	рН	EC (dS/m)	Organic matter (%)	CaCO ₃ (%)	Texture
Values	7.1	0.62	2.99	0.85	43.34
Valuation	Neutral	Non salty	Medium	Less calcareous	Clay- loam

Temperatures were recorded by data logger (KIMO KH-100) in the nursery, and annual rainfall values were obtained by the Turkish State Meteorological Service (Figure 1).

2.1. Measurements of callusing performance

The following parameters were evaluated during the experiment for the callus formation rate and callus development level:

- Callus formation rate (%): Percentage of callused grafted cuttings (Celik, 2000).
- Callus development level (0 to 4): 0= no callus, 1=25%, 2=50%, 3=75% and 4=100% callus formation on graft union surface (Celik, 2000).

2.2. Measurement of grafted vine growth parameters

When the leaves fall completely in December, grafted vines were digged out with shovel and some growth parameters determined as follows. Shoot length (cm), shoot diameter (mm), internode length (cm), graft union diameter (mm), graft union/rootstocks diameter (mm), root number, root length (cm) and root development level (0-4) were measured at the end of the growth season.

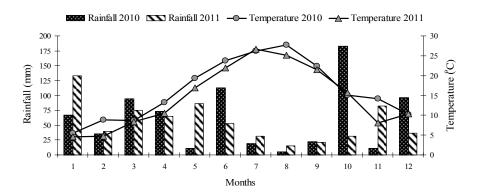


Figure 1. Monthly mean temperatures (°C) and precipitation values (mm) of the 2010 and 2011 years

The following parameters were evaluated at the end of the experiment for the Rooting percentage, Root development level and graft union/rootstocks diameter:

- Rooting percentage (%): Percentage of grafted grape vines which have a vigorous root system and matured shoots at the end of the growth season.
- Root development level (0 to 4): 0= no root formation, 1= one-sided weak root formation, 2= two-sided root formation, 3= three-sided root formation and 4= four-sided (Dardeniz et al., 2008).
 - Root number: Primary roots were measured.
- Graft union/rootstocks diameter (mm): The ratio of graft union diameter to rootstocks diameter (mm).

In the study, in order to determine suitable rootstocks, some growth characteristics were evaluated by the modified weighted-ranking method used by Soylu and Serdar (2000). In this method, relative and class scores of each characteristic were determined, and the total score of each rootstock was calculated to measurement of rootstock suitability (Table 2).

2.3. Experimental design

This research was conducted as a randomized complete block design with 3 replications, and 50 cuttings were used per replication to determine the callusing performance of each rootstock. When the callus development was completed, 30 successfully callused cuttings per replication were used in the nursery experiment for each rootstock. For comparison of the means, DUNCAN multiple range test was used. The percentage data was subjected to arc sin \sqrt{x} transformation before analyzing. All analyses were performed using the SPSS 16.0 statistical package. Results were presented as means and a pooled SEM. Differences among nursery conditions were declared at P < 0.05 and P < 0.01 level of significance.

3. Results and Discussion

Callus formation rate (%) and callus development levels (0-4) were presented in Table 3. Significant differences were determined for callus formation rate and callus development levels (P<0.01) amongst rootstocks. Callus formation rate varied from 62.0 to 97.7 % among tested

rootstocks. The highest callus formation rate (97.7 %) and callusing level (3.7) was determined on 8B grafted cuttings, whereas the lowest callus formation rate and callusing level was obtained from 5C grafted cuttings (62.0 % and 1.58). In this study, 8B grafted vines were found to be the best rootstock in terms of callus development and callus formation ratio among all rootstocks. The level of callus formation at the graft union is the main factor of good compatibility between stock and scion (Kester, 1965; Coombe and Dry, 1992; Hartman et al., 1997; Celik, 2000). In fact, many researchers emphasized that the callus formation performance of rootstocks differed according to the scion/rootstock combination (Celik and Agaoglu, 1979; Agaoglu and Celik, 1982; Tangolar et al., 1997; Turkben and Sivritepe, 2000; Coban and Kara, 2003; Dardeniz and Sahin, 2005; Sabir and Agaoglu, 2009). There are several factors which have impact on the callus formation, such as genetic composition of rootstocks and scions, supply of endogenous growth regulators, protein composition and carbohydrate reserves of graft materials, temperature and humidity during callusing, levels of oxygen (16-18%) and enzymes such as acid phosphatases and peroxidases (Astudillo and Teresa, 1993; Hunter et al., 2004). Cangi et al. (2000) stated that the callusing between grafting components was mostly established by rootstock depending on grafting method and growing conditions. Ambrosi and Kriel (1958) reported that ununiform lignifications of both the scion and stock may negatively affect the callus formation on graft union. In the present study, the scion grafted on 5C rootstock showed poor callusing performance. The poor performance of callus formation characteristics can probably be the result of physiological deficiencies or the incompatibility of 5C rootstock with this grape variety.

Root development level, root length, root number and rooting percentage of rootstocks were presented in Table 4. Root characteristics and rooting performances were found significantly different amongst rootstocks (P<0.01). The highest root development level and root length were obtained at 41B (3.0 and 40.3 cm, respectively), whereas cuttings grafted on 5C (2.0 and 19.8 cm, respectively) showed poor performance in the present study (Table 4). Root numbers changed from 9.6 to 20.8 among rootstock genotypes. The highest root numbers were determined from

Table 2. The scores of the some growth characteristics and their relative values in weighted-ranking method

Parameters	Classsification	Relative Scores	
Callus formation rate (%)	≤ 50: 1 51-60: 3 61-70: 5	71-80: 7 81-90: 9 ≥ 91: 10	15
Callus development level (0-4)	≤ 1.0: 1 1.1-2.0:3 2.01-2.5: 5	2.51-3.0 : 7 3.01-3.5: 9 ≥ 3.51: 10	15
Rooting percentage (%)	≤ 10: 1 11-30: 3 31-50: 5	51-70: 7 71-90: 9 ≥ 91: 10	20
Shoot length (cm)	≤ 10 cm: 1 10.1-20.0 cm: 3 20.1-30.0 cm: 5	30.1-40.0 cm: 7 40.1-50.0 cm: 9 ≥ 50.1 cm: 10	10
Shoot diameter (mm)	≤ 2.0 mm: 1 2.1-3.0 mm: 3 3.1-5.0 mm: 5	5.1-7.0 mm: 7 7.1-9.0 mm: 9 10≥ : 10	10
Graft diameter /rootstocks diameter (mm)	≤ 1.5mm: 10 1.51-1.60 mm: 9 1.61-1.70 mm: 7	1.71-1.80 mm: 5 1.81-1.90: 3 ≥ 1.91: 1	10
Root length (cm)	≤ 5.0 cm: 1 5.1-10.0 cm: 3 10.1-15 cm: 5	15.1-20.0 cm: 7 20.1-25.0 cm: 9 ≥ 25.1 cm: 10	10
Root development level (0-4)	≤ 1.0: 1 1.1-2.0: 3 2.01-2.5: 5	2.51-3.0 : 7 3.01-3.5: 9 ≥ 3.51: 10	10
	100		

Table 3. Effects of different rootstocks on callus formation rate (%), callus development level (0-4)

Rootstocks	Callus formation rate	Callus development level
140 Ru	97.0a	3.4bcd
99 R	94.5a	3.5ab
41B	81.6ab	2.6e
5 C	62.0d	1.6g
110 R	87.7ab	2.6ef
8 B	97.7a	3.7a
5 BB	76.3bc	2.3f
SO4	89.3ab	3.1d
Du Lot	85.0ab	3.2cd
1103 P	96.3a	3.4bc
Rootstock	**	**
Pooled SEM*	1.290	0.025

^{*} Pooled SEM*. Pooled standart error of the means (ns. P>0.05; *. P<0.05; **. P<0.01)

Table 4. Effects of different rootstocks on root development level (0 to 4), root length (cm), root number and rooting percentage (%)

Rootstocks	Root development	Root length	Root number	Rooting percentage
140 Ru	2.5ab	23.7bc	16.1b	36.8bc
99 R	2.6a	21.4cd	17.8ab	50.8ab
41B	3.0a	40.3a	15.0b	17.1d
5 C	2.0c	19.8d	10.2c	9.7d
110 R	2.1bc	20.3cd	9.6c	24.1cd
8 B	2.7a	25.3b	18.7ab	31.2bc
5 BB	2.5ab	22.3bcd	16.0b	34.1bc
SO4	2.7a	24.8bcd	20.8a	21.9cd
Du Lot	2.7a	21.8bcd	11.4c	54.2ab
1103 P	2.8a	25.6b	17.3ab	64.8a
Rootstock	**	**	**	**
Pooled SEM*	0.037	0.327	0.299	1.081

Pooled SEM*. Pooled standard error of the means (ns. P>0.05; *. P<0.05; **. P<0.01)

SO4 (20.8) grafted vines, whereas the lowest was on 110 R (9.6). Very significant differences (P<0.01) were determined amongst rootstocks in terms of the rooting percentage. In this study, rooting percentage of grafted cuttings changed from 9.7 to 64.8 % (Table 4). The best rootstock of rooting percentage was determined on 1103 Paulsen (64.8 %), whereas the lowest ones were obtained on 5C (9.7 %) grafted vines in the present study. Dardeniz and Sahin (2005) found that length, levels and numbers of roots were high at 1103 Paulsen grafted vines. In the present study, we obtained that 1103 Paulsen has the best rooting ability, but 5C has poor rooting capacity amongst the tested rootstocks. The poor rooting performance of 5C can as well be probably the result of scion/ rootstock incompatibility. In the present study, average temperatures of the second year were lower than the previous year, especially in the 45 days after the planting of grafted cuttings to nursery. Besides, rainfall in May was higher in the second year compared to the previous year (Figure 1). We considered that low temperature and high rainfall may be negatively affecting the rooting of all grafted cuttings in the second year. Sengel (2005) reported that the structure of the nursery soil and climatic conditions in the following period of 2-3 weeks after the planting of grafted cuttings greatly affected the grapevine efficiency. Richards (1983) emphasized that root anatomy and morphology, development and distribution may show difference among rootstock species.

Significant differences were found in the shoot length and diameter of grafted vines amongst rootstocks (P<0.01). Shoot length and shoot diameter varied among rootstocks. The longest shoots (92.3 cm) and the highest shoot diameter (6.2 mm) were found on 8B rootstock, whereas the lowest shoot length was on 99R (33.3 cm) and the lowest shoot diameter was on 1103 Paulsen rootstocks (4.5 mm) (Table 5). In this study, the longest shoot length and diameter was obtained from 8B grafted vines, but the

shortest was at 99R grafted vines. Because the 8B grafted vines have the best callusing and rooting performance, they may have the longest shoot growth. A healthy grafted vine should have at least a 30-40 cm well-developed shoot. In the present study, all scion/rootstock combinations had a minimum of 40 cm main shoot lengths. Many of researchers stated that rootstocks affect vine vigor (Striegler and Howell, 1991; Williams and Smith, 1991; Smart et al., 2006; Tandonnet et al., 2010). The vigour of the root structure of rootstocks can affect the development of the scions (Jogaiah et al., 2013). Hartmann et al. (1997) also stated that a rootstock found to be useful for one cultivar may not be proper for others, as the interaction of scion/rootstock affects the vine performance more than the stock or scion alone. In the present study, 41B rootstock had a very strong root development and structure among others. So, the scion on the 41B has grown more vigorously than the other rootstocks.

In this study, graft union diameter and the graft union/rootstock diameter ratio were found significantly different (P<0.01) amongst rootstocks (Table 5). The highest graft union diameter (20.7 mm) and rootstock diameter (11.3 mm) were obtained from 41 B grafted vines. Although the highest graft union diameter was found at 41 B rootstocks, the highest graft union / rootstock diameter ratio was calculated at 99R grafted vines. A swelling occurring at the graft union site can be an indication of incompatibility. Similarly, the highest graft union / rootstock diameter ratio can be an indication of incompatibility. According to the obtained results, it seems that 5C and 1103 Paulsen will have the most appropriate graft compatibility for 'Merzifon Karası' in the future. Graft incompatibility can also be caused by anatomical mismatching, poor craftsmanship, environmental conditions, and disease (Hartmann et al., 2002). For grafted plants to grow successfully, the combined plant parts (rootstock and scion) should be compatible with each other.

Table 5. Effects of different rootstocks on shoot length (cm), internodes length (cm), graft union diameter and shoot diameter (mm)

Rootstocks	Shoot length	Shoot diameter	Graft union diameter	Rootstock diameter	Graft union / rootstock diameter	
140 Ru	48.2b	5.1bc	19.3b	10.8ab	1.8b	
99 R	33.3c	4.9bc	19.5b	9.5cd	2.1a	
41B	92.3a	6.2a	20.7a	11.3a	1.8b	
5 C	42.3bc	4.9bc	17.2cd	10.0cd	1.7b	
110 R	44.1bc	5.1bc	18.3bc	9.9cd	1.9b	
8 B	53.3b	5.3b	17.9bcd	9.6cd	1.9b	
5 BB	50.2b	5.0bc	17.1cd	9.3d	1.9b	
SO4	42.8bc	5.0bc	17.9cd	9.9cd	1.9b	
Du Lot	49.9b	5.0bc	18.6bc	10.4bc	1.8b	
1103 P	41.7bc	4.5c	16.7d	9.7cd	1.7b	
Rootstock	**	**	**	**	**	
Pooled SEM*	1.262	0.055	0.126	0.064	0.013	

^{*} Pooled SEM*. Pooled standard error of the means (ns. P>0.05; *. P<0.05; **. P<0.01)

Table 6. The evaluation of rootstocks with weighted-ranking method

Rootstocs		Scores of formation		Scores of develop		Scores of leng		Scores of shoot diameter	
		CS	CS*RS	CS	CS*RS	CS	CS*RS	CS	CS*RS
140 Ru		10	150	9	135	9	90	7	70
99 R		10	150	10	150	7	70	5	50
41B		9	135	7	105	10	100	7	70
5 C		5	75	3	45	9	90	5	50
110 R		9	135	7	105	9	90	7	70
8 B		10	150	10	150	10	100	7	70
5 BB		7	105	5	75	10	100	5	50
SO4		9	135	9	135	9	90	5	50
Rup. du Lot		9	135	9	135	9	90	5	50
1103 P		10	150	9	135	9	90	5	50
Rootstocs	Rootstocs Scores develo			Scores of root length		Scores of rooting percentage		Scores of graft union/rootstock diameter	
		CS	CS*RS	CS	CS*RS	CS	CS*RS	CS	CS*RS
140 Ru		5	50	9	90	5	100	5	50
99 R		7	70	9	90	7	140	1	10
41B		7	70	10	100	3	60	3	30
5 C		3	30	7	70	1	20	5	50
110 R		5	50	9	90	3	60	3	30
8 B		7	70	10	100	5	100	3	30
5 BB		5	50	9	90	5	100	3	30
SO4		7	70	9	90	3	60	3	30
Rup. du Lot		7	70	10	100	7	140	5	50
1103 P		7	70	9	90	7	140	5	50
				Rootst	tock Scores	5			
140 Ru	99 R	41B	5 C	110 R	8 BB	5 BB	SO4	Du Lot	1103 P
735	730	670	430	630	770	600	660	770	775

CS: Classification Score, RS: Relative Score

When they show incompatibility, rootstock and scions may grow at different rates. Therefore, the trunk diameter can vary above and below the graft union. 99 R rootstocks had a higher ratio of graft union diameter to rootstock diameter. Also, the lowest shoot length was obtained at 99 R grafted vines. So, this rootstock may have graft incompatibility problem in the future. Dardeniz and Sahin (2005) reported that higher graft union diameters were determined at Uslu grape variety on 140 Ruggeri, and they mentioned that the swelling of graft diameter could be a sign of incompatibility. But, the ratio of graft union diameter to rootstock diameter is a better parameter than graft union diameter to determine graft compatibility. Similar results reported by Reynier (1982) show that if the yield and growth performance of grapevine appear normal, the differences that occur below and above the graft point may not always be a symptom of an incompatibility.

Since it is more difficult to decide suitable rootstocks with using tested growth parameters, there are used the weighted ranking method for selecting suitable rootstocks using some growth parameters (Table 6). According to the weighted ranking result, 1103 Paulsen has taken higher scores (775), and it was determined as the best/a better rootstock for 'Merzifon Karası' grape variety (Table 7). In this study, we evaluated rootstocks for the selection of best rootstocks for the 'Merzifon Karası' grape variety. Since it is difficult to estimate the appropriate rootstock in early time, we used weighted ranking method to the suitable combination. In the tested parameters, 1103 Paulsen received the highest score among other rootstocks.

4. Conclusions

In the present study, ten rootstocks were tested with the weighted-ranking method to determine the suitability of different rootstocks for the 'Merzifon Karası' grape variety. According to the tested parameters, 1103 Paulsen was determined as the most suitable rootstock for 'Merzifon Karası'. However, the impact of rootstocks on this grape variety should be monitored in vineyard conditions for a long time period.

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