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# The Determination of Sapling Characteristics and Affinity Situations of Some Local Cultivars on Some American Rootstocks in Grafted Vine Sapling Production

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## Abstract

The Gelin, Pembe Salman, and Osmanca cultivars of *Vitis vinifera* L. have traditionally been grown on their own roots using Goblet training systems in the Aydın province. These cultivars are sensitive to *Daktulosphaira vitifoliae*, making it necessary to graft nursery plants of these cultivars in future plantings. The compatibility between cultivars and rootstocks is one of the most important factors in selecting a suitable rootstock. This study evaluated the graft affinity of the Gelin, Pembe Salman, and Osmanca cultivars with five rootstocks: 41B, 110R, 140Ru, 1103P, and Fercal. After the callusing process, grafting success and rooting ratios were assessed. In autumn, the ratios of first-grade saplings and total saplings were determined. When comparing the rootstocks, the highest first-grade sapling ratio was found in 1103P at 35.9%, while the lowest ratio was in 110R at 23.5%. The middle values were recorded for the 140Ru, 41B, and Fercal rootstocks, which had percentages of 28.2%, 32.6%, and 34.7%, respectively. Among the cultivars, the Osmanca cv. had the highest first-grade sapling ratio at 35.5%. Gelin and Pembe Salman followed with ratios of 27.9% and 29.3%, respectively. Furthermore, Osmanca cv. exhibited the thickest shoots compared to the other two cultivars. Root analyses of mature saplings indicated that 110R and 140Ru exhibited the lowest values, while Fercal and 1103P had the highest values; 41B showed intermediate results. After 260 days, anatomical examinations of the graft unions revealed continuous cambial connections and well-developed conductive tissues, including xylem and phloem.

**Keywords:** Bench Grafting, Affinity, Compatibility, Rootstock, Vine, Grape, Seedling ratio

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## INTRODUCTION

Phylloxera is harmful to *Vitis vinifera* L. and causes vines to dry over time, so the only solution is grafting onto resistant American rootstocks. However, American rootstock cultivars have different requirements in terms of soil structure, soil calcium content and water availability (Uzun,1996).

The other situation for proper rootstock selection is graft compatibility between the rootstock and the scion. Generally, as the relativity increases between plants, the compatibility also increases in parallel. *Vitis* species generally can be grafted onto each other within the *Vitis* genus. However, graft incompatibility has been met in some combinations and rootstocks such as Kober 5BB. In addition, it is known that viral diseases prevent graft success (Grief et al., 1995; Tedesco, 2023). Moreover, it is thought that partial incompatibility can happen instead of complete incompatibility. For this reason, many researchers examined the cultivar rootstock relationship in terms of graft incompatibility at the sapling production stage. After indoor grafting (bench-grafting), callusing and acclimatisation stages, grafting cuttings are planted in field or greenhouse conditions (Hartmann et al., 1990). After saplings become mature, the total sapling yield and the First grade sapling yield have been evaluated by many researchers. The highest total sapling yield and First grade sapling yield were found at Yuvarlak Seedless/Kober 5BB, Gamay/Kober 5BB (Cangı, 1996), Datal/5BB, Datal/99R (Çoban and Kara, 2003), Yalova İncisi/8B (Sabır et.al., 2005), Amasya/Kober 5BB (Dardeniz et.al., 2005), Chardonnay/41B and Merlot/Kober 5BB (Todic et al., 2005), Cardinal/5BB (Kamiloğlu, 2005) and Vrancea/140Ru (Ionica et. al., 2024). Anatomical and histological examination of graft site of grapevine saplings showed that 16 days after grafting cambial continuity began and after 25-30

days cambial continuity completed. In addition, after 45 days, it was observed that graft union of same grafts began to weak in the side of scion (Cangi, 1996). Internal connection and the differences in the graft union, can be determined by Magnetic Resonance Imaging (MRI). If the cost of MRI technology decreases in the future, MRI methods will be used in determine of connection level of grafted saplings in the future (Bahar et al. 2010; Korkutal et al, 2018).

Based on the information presented above, this research aims to assess the grafting compatibility between several local cultivars—Gelin, Pembe Salman, and Osmanca—found in the Aydın province, and various commonly used rootstocks, including 41B, 110R, 140Ru, 1103P, and Fercal combinations.

## MATERIALS AND METHODS

### Materials

The local grape cultivars *Vitis vinifera* L. 'Gelin' and 'Pembe Salman' are cultivated on the plateaus of Aydın province. These cultivars reach maturity and are harvested during October, November, and December. Due to their late ripening period and the limited grape supply in local markets during these months, they command relatively high prices. Both cultivars exhibit desirable table grape attributes, including large berry size and firm pulp texture. Vines of 'Gelin' and 'Pembe Salman' are generally trained as high Goblet (head) systems on the slopes of the plateaus. However, some vineyards in the region contain old vines that are increasingly susceptible to diseases. Since these cultivars are traditionally grown on their own roots, they exhibit high sensitivity to phylloxera (*Daktulosphaira vitifoliae*). Another local cultivar, *V. vinifera* L. 'Osmanca', is predominantly cultivated along the lower slopes and coastal areas of Aydın. This cultivar, also propagated on its own roots and trained in a low Goblet system, ripens in September and is utilized both as a table grape and for winemaking purposes. For the sustainable cultivation and preservation of these local genotypes, it is essential to establish new vineyards using grafted vines on phylloxera-resistant American rootstocks that are well adapted to local soil and climatic conditions.

Scions were collected from the vineyards in January and February. For rootstocks, commonly used varieties in Turkey, such as 41B, 110R, 1103P, 140Ru, and Fercal American rootstocks, were sourced from the Manisa Viticulture Research Institute during the same months.

### Methods

#### The experiment design

The experiment was planned as a two-factor, completely randomized block design with three replications. Three cultivars, five rootstocks, three blocks, and 40 cuttings per block were used. A total of 1,800 saplings were planted under field conditions. Three blocks were created based on grafting time, box number and location in the field (Doğan, 1996; Cangı, 1996). The experiment was repeated for two years.

#### Grafting, stratification and callusing periods

The grafting process was carried out using a bench-grafting (Omega) machine (Nicholas et. al., 1992). After that, the grafted cuttings were immersed in the paraffin for 1 or 2 seconds (Cangi, 1996). Grafted cuttings were put in boxes filled with sawdust and perlite. Perlite was used only on the grafted side. Boxes were placed in a climate room at 85-90% relative humidity and 26°C temperature, and kept for three weeks. After that, before moving to the boxes outside, the air conditioning was closed and the temperature decreased to 22°C for one week (Çelik et al., 1998; Çelik et al.,2019).

#### Planting

In the middle of April, grafted cuttings were planted within 20 x 30 cm distances on raised beds covered with black mulch and two drip irrigation pipes with 20 cm drippers passing under the mulch in fields. (Çelik, 1998). Shade net (50%) is also used during the summer months (Yağcı and Gökkaynak, 2016). Grafted cuttings before planting were dipped in a 3000 ppm IBA solution for 1 or 2 seconds, and two-thirds of the grafted cuttings were inserted into the soil. Grafted rooted grapevine were pulled out in December.

#### The examining characteristics after callusing

After callusing, grafting success ratio (%), sprouting ratio (%), callusing degree (1-4), and rooting ratio were determined (Çelik and Ağaoğlu, 1980).

#### The examining characteristics in mature saplings

Total sapling yield (%), first grade sapling yield (%), node numbers on shoots (unit), shoot diameter (mm), and average root number (unit) were observed (Çelik, 1982; Dardeniz et al., 2005).

#### Taking of grafting site samples

According to the results of the experiment, grafting combination samples where a successful omega section grafting technique was applied were taken after 160 days from grafting and held in 70% ethyl alcohol (Tekintaş et al., 1988). Cross and longitudinal section samples of the grafting site were taken at 10 to 30 micron thickness and in different regions. The samples were stained with methylene blue. Staining provides recognition of tissues as clearly as possible and forms contrast on sections. After the permanent slides were prepared, they were examined for the anatomical structure of the graft union under the microscope (Ertan, 1999).

#### Anatomical and histological examination of the graft union

After 260 days from grafting, the callus tissue situation, which came from rootstocks and scions; the necrotic layers situation between rootstock and scion; cambial continuity; cambial differentiation and formation of new xylem and phloem tissues were examined (Tekintaş, 1988; Balta, 1993; Cangı, 1996; Dolgun,1998; Ertan, 1999).

#### The experiment analysis

Variance analysis was carried out at as a three-factor (year x cultivar x rootstock) in completely randomised blocks. Means were separated with the Duncan test (P≤%5). The original data for grafting success ratio, sprouting ratio, rooting

ratio, total sapling yield and first grade sapling yield were transformed to the arcsine transformation before being subjected to variance analysis (Gomez and Gomez, 1984). The data were analyzed using IBM SPSS Statistics (Version 21).

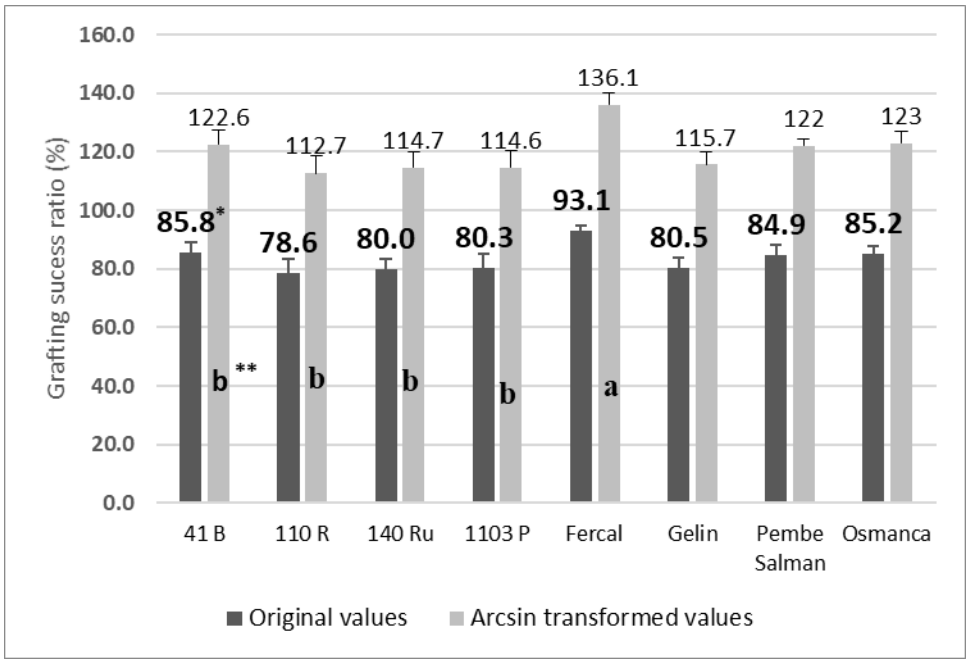
## RESULTS AND DISCUSSION

In this research, except for callusing degree, for all other characteristics, the effects of year, year x cultivar, year x rootstock, year x cultivar x rootstock, and cultivar x rootstock interactions were found to be non-significant. After the callusing period, callusing degree were significant concerning year and year x rootstock interactions. Callusing degree values increased at the second year. So, for all other characteristics except callusing degree were illustrated in the same graphics, highlighting the main effects of cultivar and rootstock.

### Results After Callusing Period

The grafting success ratio:

The interaction effects of cultivar and rootstock on the grafting success ratio were not significant (Figure 1). The grafting success ratio ranged from 78.6% to 93.1% across different rootstocks, with only the rootstocks influencing this ratio. Notably, the Fercal rootstock exhibited a higher grafting success ratio compared to other rootstocks, such as 41B, 110R, 140Ru, and 1103P. The cultivars themselves did not affect the grafting success ratio, which varied between 80.5% and 85.2% (Figure 1).



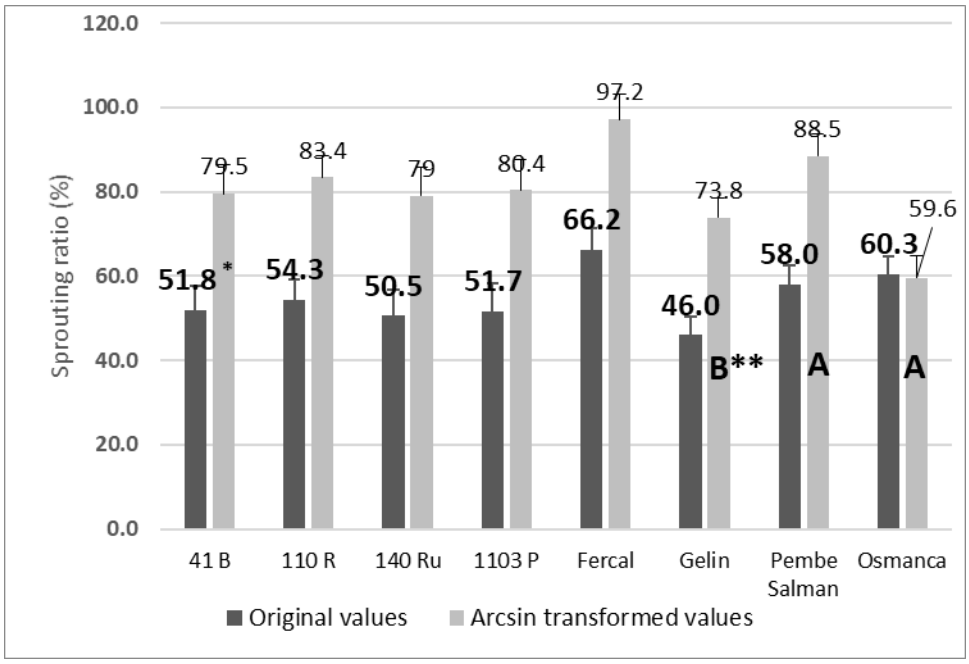

** Variance analyses were applied on percentage values transformed arcsin(sqrt). Transformed data and original data was shown on figures as means and standard error.*

*** The small letters used for comparing means of rootstocks and the big letters used for comparing means of cultivars according to the Duncan test (P≤0.05). Duncan critical values at transformed data (p=0.05) for rootstocks: 11.5, 12.1, 12.5, 12.7 for Q1,Q2,Q3, Q4 respectively. There are no differences between the similar letters.*

**Figure 1**. Grafting success ratios after callusing period (%)

### Sprouting ratios after callusing period:

After callusing, the sprouting ratio among the rootstocks ranged from 50.5% to 66.2%, with no significant differences observed. Among the cultivars, Pembe Salman and Osmanca exhibited a higher sprouting ratio compared to Gelin (Figure 2).



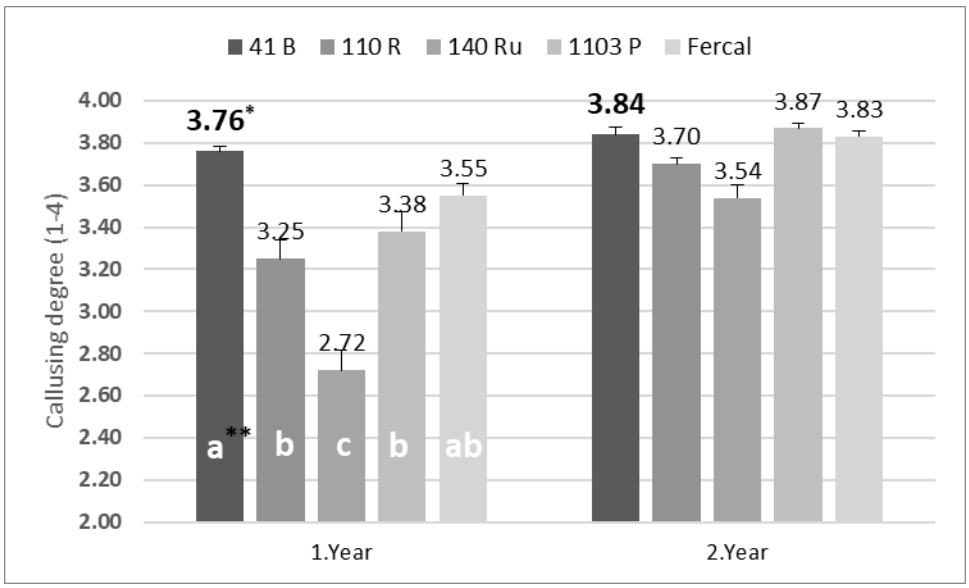

**Variance analyses were applied on percentage values transformed arcsin(sqrt). Transformed data and original data was shown on figures as means and standard error.*

*** The small letters used for comparing means of rootstocks and the big letters used for comparing means of cultivars according to the Duncan test (P≤0.05). Duncan critical value (p=0.05) for cultivars: 11.4, 12.0 for Q1,Q2 respectively. There are no differences between the similar letters*

**Figure 2**. Sprouting ratios after callusing period.

**Callusing degrees after callusing period:**

After callusing, the year-rootstock interaction effects on callusing degree were found to be significant. Callusing degrees for all rootstocks in the second year increased compared to the first year. In both years, the callusing degree of rootstocks exhibited a similar. While 41B had the highest callusing degree, 140Ru had the lowest callusing degree and the other rootstocks had medium values (Figure 3).

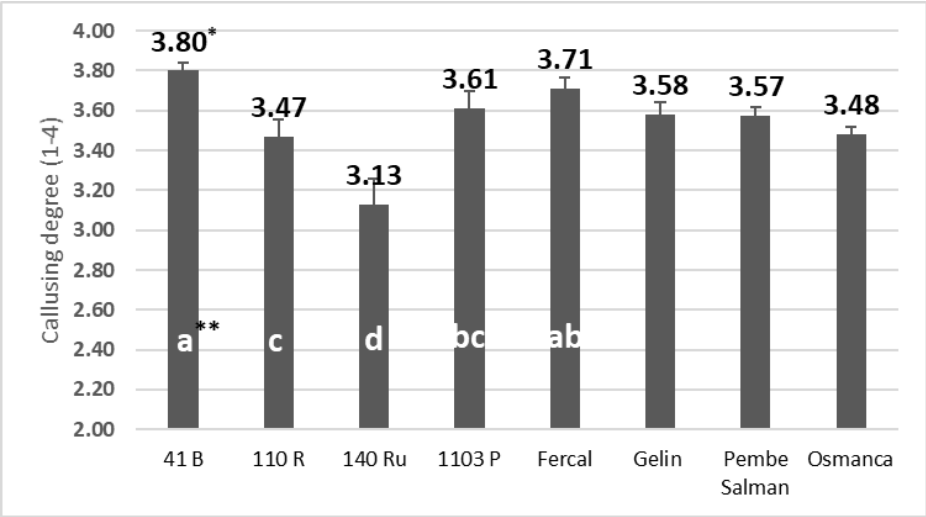



*** Data was shown on figures as means with standard error.*

*** The small letters used for comparing means of rootstocks and the big letters used for comparing means of cultivars according to the Duncan test (P≤0.05). Duncan critical value (p=0.05) for rootstocksin 1st year: 0.35, 0.36, 0.37, 0.38 for Q1,Q2,Q3,Q4 respectively. There are no differences between the similar letters*

**Figure 3**. Callusing degrees according to years after callus period

Average year values in terms of callusing degree was found that there were significant differences among the rootstocks. While Fercal and 41B rootstocks had the highest callusing degrees, 140Ru had the lowest values. 1103P and 110R had the medium values. No significant differences were observed among the callusing degrees of the cultivars (Figure 4).



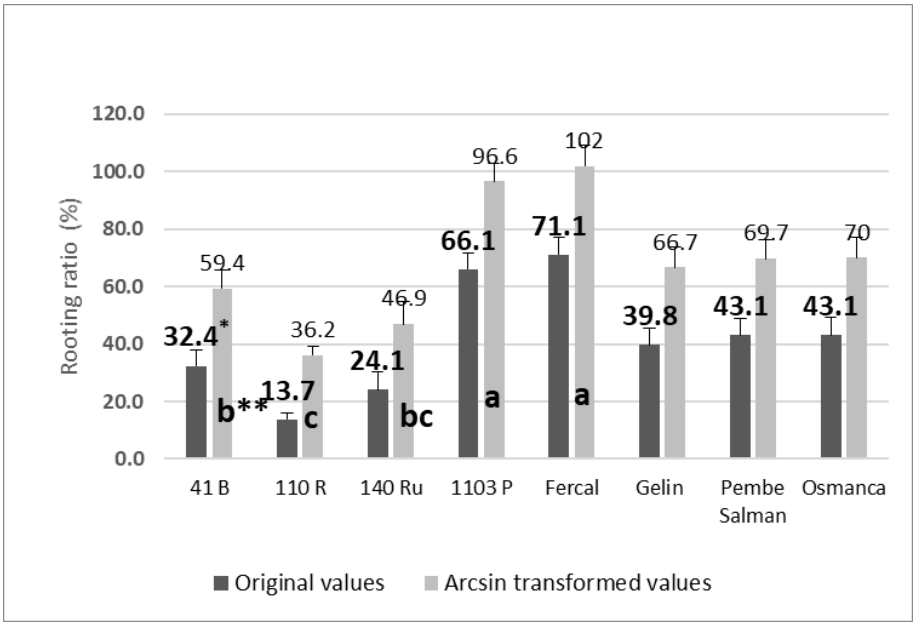

** Data was shown on figures as means with standard error.*

*** The small letters used for comparing means of rootstocks and the big letters used for comparing means of cultivars according to the Duncan test (P≤0.05). Duncan critical value (p=0.05) for rootstock=0.17, 0.18, 0.19, 019 for Q1, Q2, Q3, Q4 respectively. There are no differences between the similar letters*

**Figure 4.** Callusing degrees for average years after callusing period

**Rooting ratios after callusing period*:***

The rooting ratio also showed differences among the rootstocks, similar to the callusing degree. When the highest rooting ratio was seen in 1103P and Fercal rootstocks, the lowest rooting ratios were obtained from 110R rootstock. Medium values were observed for 41B and 140Ru rootstocks. Cultivars did not show the differences in terms of rooting ratio (Figure 5).




** Variance analyses were applied on percentage values transformed arcsin(sqrt). Transformed data and original data was shown on figures as means and standard error.*

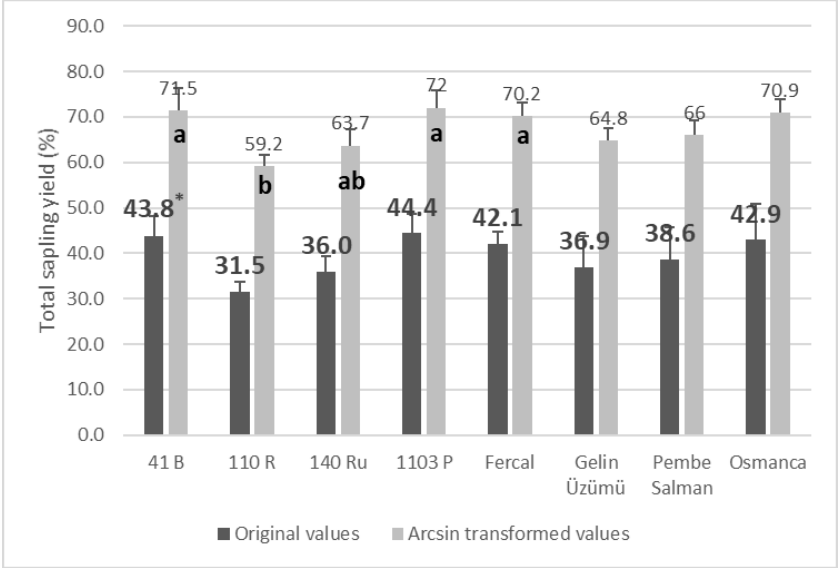
*** The small letters used for comparing means of rootstocks and the big letters used for comparing means of cultivars according to the Duncan test (P≤0.05). Duncan critical value (p=0.05) for rootstock=19.1, 20.0, 20.6, 21.1 for Q1, Q2, Q3, Q4 respectively. There are no differences between the similar letters*

**Figure 5.** Rooting ratios after callusing period.

**Field Growing and Uprooting of Saplings**

Total sapling yield (%) after the field growing period:

At the end of the growing season, the characteristics of mature saplings were examined. All data about saplings were evaluated as rootstocks and cultivar effects separately because interaction effects were not significant. As seen in Figure 6, the total sapling yield was affected by rootstocks. 110R had the lowest value. Cultivars did not show differences. Total sapling yield ranged between 31.5 % and 44.4 % for rootstocks and 36.9 % and 42.9 % for cultivars.



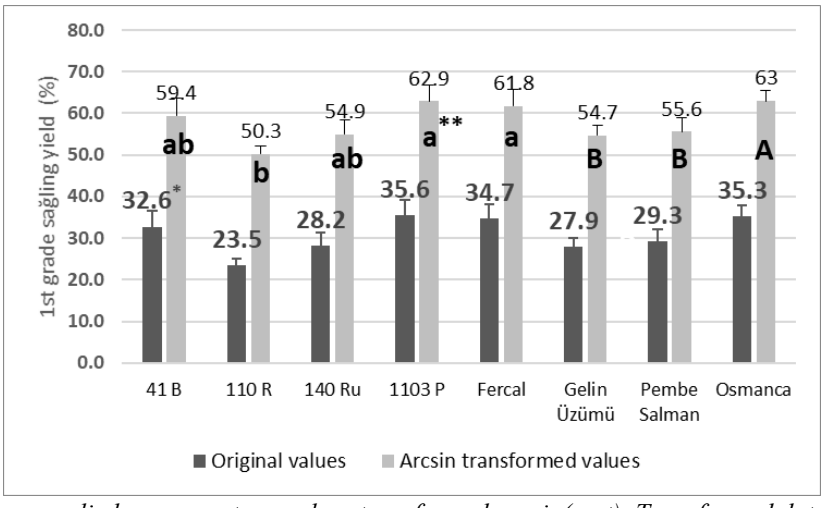

**Variance analyses were applied on percentage values transformed arcsin(sqrt). Transformed data and original data was shown on figures as means and standard error.*

*** The small letters used for comparing means of rootstocks and the big letters used for comparing means of cultivars according to the Duncan test (P≤0.05). Duncan critical value (p=0.05) for rootstocks: 9.9, 10.4, 10.7, 10.9 for Q1, Q2, Q3, Q4 respectively. .There are no differences between the similar letters*

**Figure 6.** Total sapling yield (%) after the field growing period

**First grade sapling yield (%) after field growing period:**

The 1st-grade sapling yield ratio showed significant differences for rootstocks and cultivars separately. The highest 1st grade sapling yield was taken from 41B, 1103P and Fercal rootstocks, while the lowest one was taken from 110R. 140Ru gave the medium value. Among the cultivars, Osmanca had the highest 1st-grade sapling yield (Figure 7).



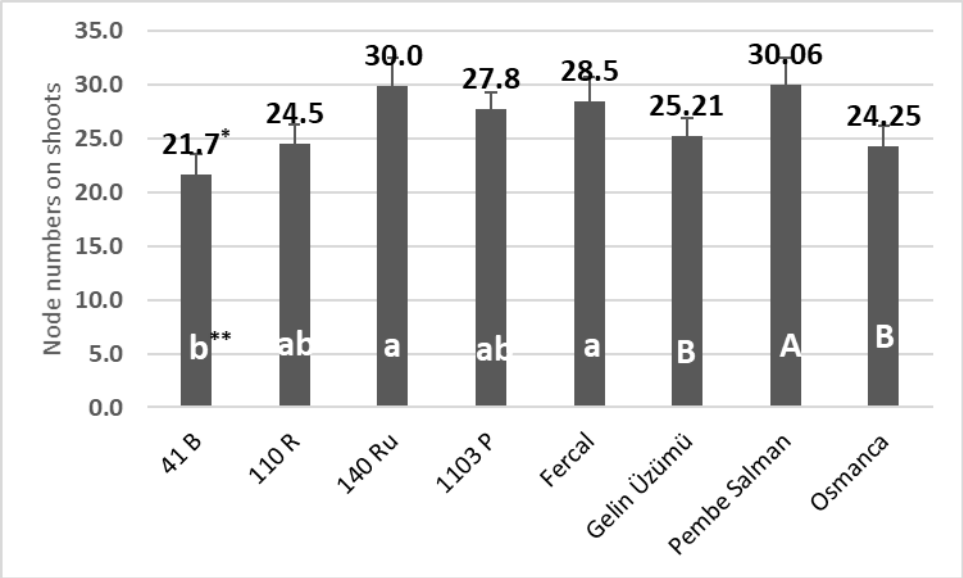

** Variance analyses were applied on percentage values transformed arcsin(sqrt). Transformed data and original data was shown on figures as means and standard error.*

*** The small letters used for comparing means of rootstocks and the big letters used for comparing means of cultivars according to the Duncan test (P≤0.05). Duncan critical value (p=0.05) for rootstocks: 8.7, 9.1, 9.3, 9.5 and for cultivars: 6.5, 6.8. There are no differences between the similar letters.*

**Figure 7**. First grade sapling yield (%) after field growing period

**Node numbers on shoots after field growing period:**

Both the rootstock and cultivar significantly affected the total number of nodes. Among the rootstocks, Fercal and 41B exhibited the highest node counts, while 41B had the lowest. The rootstocks 110R and 1103P demonstrated medium values. When evaluating the cultivars separately, the Pembe Salman cultivar had a higher node number compared to the Osmanca and Gelin cultivars (Figure 8).



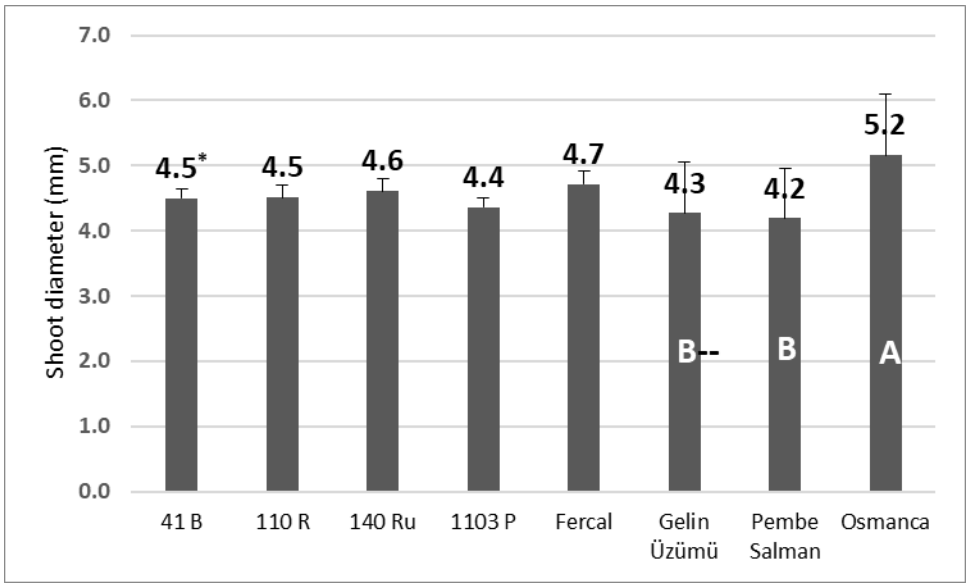

** Data was shown on figures as means standard error.*

*** The small letters used for comparing means of rootstocks and the big letters used for comparing means of cultivars according to the Duncan test (P≤0.05). Duncan critical value (p=0.05) for rootstocks: 5.3, 5.5, 5.7, 5.8 and for cultivars: 3.97, 4.16. There are no differences between the similar letters*

**Figure 8**. Node numbers on shoots after field growing period

**Shoot diameters after field growing period:**

As seen in Figure 9, while shoot diameter did not show the differences between rootstocks, among the cultivars, Osmanca had the highest shoot diameter.



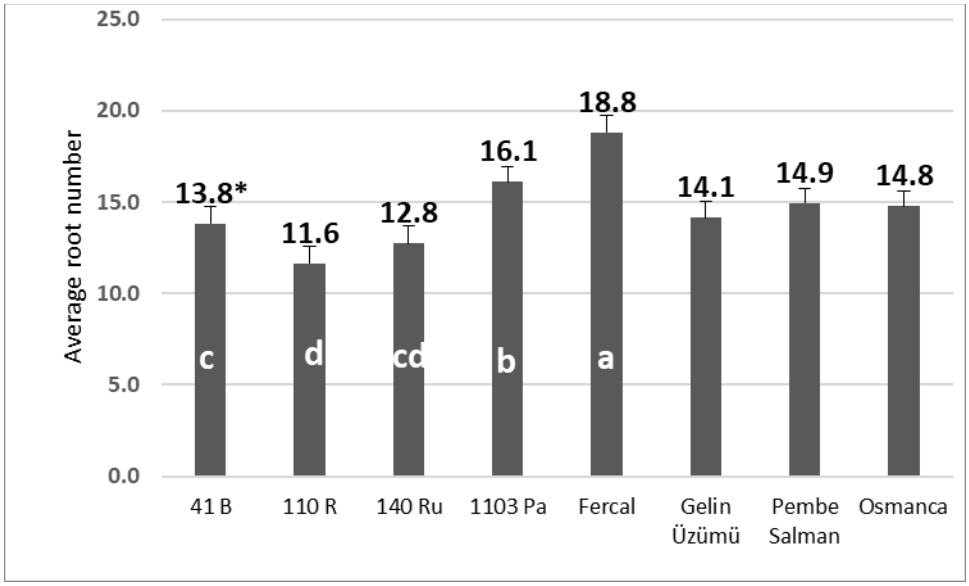

*** Data was shown on figures as means with standard error.*

*** The small letters used for comparing means of rootstocks and the big letters used for comparing means of cultivars according to the Duncan test (P≤0.05). Duncan critical value (p=0.05) for cultivars: 0.3, 0.3.There are no differences between the similar letters*

**Figure .9**. Shoot diameters after field growing period

**Average root number after the field growing period:**

As shown in Figure 10, the choice of rootstock significantly influenced the number of roots formed. Fercal and 1103P produced the highest root counts, while 110R and 140Ru had the lowest. The root number for 41B was average.




** Original data was shown on figures as means standard error.*

*** The small letters used for comparing means of rootstocks and the big letters used for comparing means of cultivars according to the Duncan test (P≤0.05).Duncan critical value (p=0.05) for rootstocks: 1.9, 2.0, 2.0, 2.1 for Q2, Q3, Q4, Q5. There are no differences between the similar letters*

**Figure 10.** Average root number after the field growing period

**Results About Anatomical Examination**

Two hundred sixty days after bench grafting, grafting samples, examined anatomically. It was seen that grafting union between rootstock and scion by callusing (Figure 11). It was also observed that callus tissue differentiated into new cambium tissues and cambial continuity was completed between the rootstock and scion, and from the new cambium tissues, new conductive tissues such as xylem and phloem developed. It was determined that translocation of water and mineral nutrients was successful, and that graft union between scion and rootstock was established.

After cutting, necrotic layers formed as results of the enzymatic reactions of phenolic compounds at the cutting surface was observed. They were not absorbed in some sample sections, but the necrotic layer areas stayed in small sections and did not prevent the formation of cambial continuity. Between scion and rootstock, the graft union becomes settled and growing and developing continues as one unit plant.

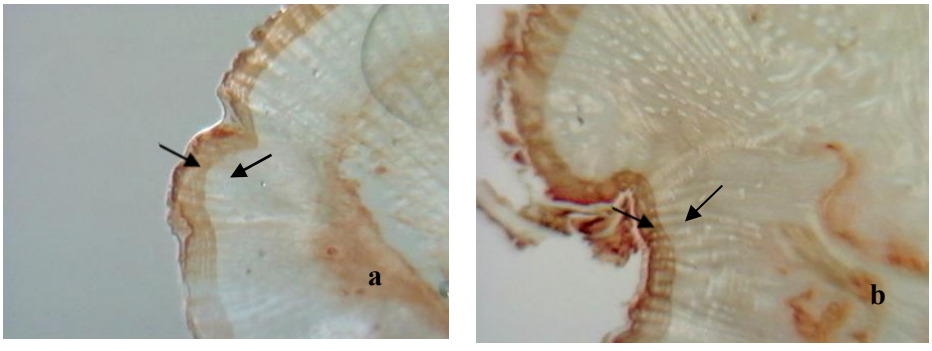



**Figure 11.** After 260 days from grafting at Pembe salman/41B (a) and Osmanca/110R grafting combinations (b), formation of cambium continuity and new vascular tissues indicated with arrow

**DISCUSSION**

In this research, the graft success ratio varied between 78.6% and 93.1% among the grafting combinations. Teker et al. (2014) obtained the graft success ratio between 46.7% and 80.0% in different grafting combinations. According to graft combinations, at graft success ratios, different results have been obtained. In this research, the total sapling yield ranged between 31.5% and 44.4%. The lowest value was obtained as 31.3% from one grafted on 110R. Another study used 110R

rootstock, which came from 10 different areas, grafted by *V. Vinifera* L. cv. Tempranillo. Total sapling yield, obtained from field conditions, varied between 76.3 and 91.5 (Villa-llop, 2025). The reason for the differences between the two studies using 110R as a rootstock for total sapling yield could be nursery conditions, soil structure, etc.

In this research, when compared, 1st grade sapling ratio was higher at vines grafted on 1103P than that of 110R. Similarly, mature saplings, at vines grafted on 1103P, root development level and root number were higher than that of 110R. Another research used 5BB, 110R and 1103P rootstocks with King's Ruby, Cardinal and Italy cultivars for bench grafting and greenhouse and misting conditions. Sapling quality was examined, and it was found that rooting ratio, rooting degree and root number values were the highest at 1103P and Kober 5BB. While the lowest values were obtained from vines grafted on 110R. These results are compatible with our research.

In this research, although after callusing, besides the 110R, 140Ru and 41B also showed the weak rooting characteristics, the mature saplings that were grafted on 140Ru and 41B compensated for the weak rooting partially. Before planting, a 3000 ppm IBA application could increase the rooting when grafted on 140 Ru and 41B, but not 110R. In another research, Çelik and Gargın (2009) used 0, 3000 and 4500 ppm IBA applications to increase the rooting ability of 420A, 41B and 110R rootstocks and 3000 ppm IBA improved the rooting ability at 41B, but not 110R.

Kısmalı (1978) searched the grafting compatibility of grafting combinations of the Yuvarlak Seedless cv. *Vitis vinifera* L. and 99R, 41B, Harmony and Ramsey American rootstocks and did not observe the incompatibility for the graft combinations. It was stated that the most important factor for the production of saplings was the rooting ability of rootstocks, and that the solution was the use of rooted cuttings. While unrooted cuttings of 41B and Ramsey provide sapling yield ratios of 19.92% and 18.83%, rooted cuttings of the same rootstocks gave the sapling ratios of 64.67% and 60.20%. The rooted cuttings could be used for difficult rooted rootstocks in bench grafting.

In this research, when comparing the 140Ru rootstock effects with the other rootstocks in terms of characteristics, the callusing degree at the grafting site was observed as weak in vines grafted on 140Ru rootstock, while the 1st grade and total sapling yield in vines grafted on 140Ru did not show any differences. The differences could be due to the field conditions.

Many researchers have noted that 5BB rootstock was superior in terms of sapling yield (Cangı, 1996; Borgo et al., 2001; Çoban and Kara, 2003; Dardeniz et al., 2005; Todic et.al., 2005; Kamiloğlu, 2005). However, Bisof (1995) claimed that there was graft incompatibility between Kober 5BB and Red Traminer cv. Grief et al. (1995) indicated that there was no incompatibility at the graft combinations with 5BB and that the reason for the negative development in graft union was virus contamination of grafting cuttings. In addition, Kober 5BB is known as sensitive to drought, so it was not used in this research.

## CONCLUSION

In this research, 1103P and Fercal are rootstocks that came first in terms of first-grade sapling yield. It is advised to use 3000 ppm IBA for 41B and 140Ru. It is required to improve the root conditions for 110R. Difficult rooted cuttings such as 110R can be rooted after grafting in greenhouse conditions controlled moisture and temperature instead of field conditins.

Osmanca cv. (*Vitis vinifera* L.) is adapted to the Aydın province conditions. In this research, when compared with the others, Osmanca cv. gave the thickest shoots, and had the highest 1$^{st}$ grade saplings. Osmanca, Gelin and Pembe Salman cvs (*Vitis vinifera* L.) were found to be compatible with 1103P, Fercal, 41B, 140Ru, and 110R American rootstocks. In Aydın region, the local cultivars, Osmanca, Gelin and Pembe Salman cvs. can be propagated with bench grafting method with using 110R, 1103P, 140Ru, 41B and Fercal rootstocks.

**Compliance with Ethical Standards**

**Peer-review**

This article has been reviewed by independent experts in the field using a rigorous double-blind peer review process.

**Declaration of Interests**

On behalf of all authors, the corresponding author states that there is no conflict of interest.

**Author contribution**

The contribution of the authors to the present study is equal.

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