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# Determination of Grafting Success and Carbohydrate Distributions of Foxy Grape (*Vitis labrusca* L.) Varieties Grafted on Different American Grape Rootstocks

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**Abstract:** This study aimed to determine the grafting success and carbohydrate distributions for newly registered foxy grape varieties grafted on different American grapes and grown in humid conditions in the Black Sea Region of Turkey. In the study, 'Rizessi', 'Çeliksu', 'Ülkemiz', 'Rizellim', and 'Rizpem' foxy grape varieties were grafted on 140Ru, SO4, and 110R American rootstocks. The graft success, growth, and development characteristics were determined, and carbohydrate accumulations and distributions at the graft area, under the graft area (rootstock), and above the graft area (scion) of the grafted grapevines in the dormant period were also determined. The highest graft success rate was 100.00% in the combinations of 'Rizessi'/SO4, 'Rizellim'/SO4, 'Rizpem'/SO4, 'Rizellim'/110R, and 'Rizpem'/110R. The percentage of vine saplings ranged from 86.00% ('Rizellim'/SO4) to 27.14% ('Rizpem'/140Ru). Regarding carbohydrate distribution, the 'Rizessi'/140Ru, 'Rizessi'/SO4, 'Rizessi'/110R, 'Çeliksu'/SO4, 'Çeliksu'/110R, 'Ülkemiz'/SO4, 'Rizellim'/140Ru, 'Rizellim'/SO4, 'Rizpem'/SO4, and 'Rizpem'/110R combinations showed a balanced distribution. The highest total carbohydrate accumulation (100.41 mg·L$^{-1}$) was detected in the root region of the 'Ülkemiz'/SO4 combination. On the other hand, vine sapling rate enhanced as carbohydrate accumulation increased in the grafting area. The SO4 American rootstock was found to be more successful than the others.

**Keywords:** foxy grape; *Vitis labrusca*; grafting; rootstock; graft success; carbohydrate



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## 1. Introduction

Turkey is one of the most suitable geographies for viticulture due to its gene resources and more than 1200 varieties [1,2]. The Black Sea Region has high humidity. Therefore, growing varieties of *Vitis vinifera* L. in this region is not easy. Because grape varieties of *V. vinifera* species are sensitive to fungal diseases, even if excessive spraying is carried out, quality and quantity losses are high [3]. However, among the variety richness in Turkey, there are types of *Vitis labrusca* L., which have adapted to the conditions of the Black Sea Region and are naturally grown in the region's rainiest and humid areas. Foxy grape types have been grown to meet the needs of the people living in the region. Therefore, studies carried out with five foxy grape types ('Rizessi', 'Çeliksu', 'Ülkemiz', 'Rizellim', and 'Rizpem') were conducted and registered in 2016 [4–6].

Due to the phylloxera, resistant grape rootstocks and grafted grape vines must be used in modern viticulture. Choosing the correct rootstock to establish vineyards is one of the most critical factors in viticulture. To determine the most suitable rootstock to be used in the graft, the climatic and soil characteristics of the region need to be known. There should be no anatomical, physiological, or biological compatibility problems between the rootstock and the scion [7] because rootstocks affect the growth and development of the vine, its yield, and the development of the shoots [8–10]. Internationally, rootstocks are the only consistent and effective strategy for growing grapes under abiotic and biotic stress conditions [11]. Today, there are various and many new rootstocks, so it is not easy to choose [12]. In order of importance, rootstocks are selected according to seven criteria: resistance to phylloxera and nematodes, adaptation to low pH and high pH, tolerance to saline soils, adaptation to moist or poorly drained soils, and drought resistance [13].

There are generally two types of compatibility problems in viticulture: The first one is seen in the nursery conditions and is called incompatibility. The second one emerges years later and is called affinity [14]. Measurements for the degree of incompatibility are usually based on graft success rates or well-defined physiological and morphological indicators [15]. Studies on graft success issues are carried out to reveal the reasons for the incompatibility, eliminate the incompatibility, and define the incompatibility as soon as possible [16]. Graft success is defined as a callus forming from the rootstock and scion and acting as a single plant [17]. The basic rule for the better fusion of these two plant parts is the exact coincidence of the cambium tissues of the rootstock and the scion and the formation of vascular tissues besides the callus [16]. The graft union is successful when phloem and xylem connections are established on the graft surface [18,19]. There should be a strong callus connection between the rootstock and shoot, and a good callus connection depends on the rootstock–scion relationship [20]. Graft success varies among the species and clones used. Graft success is higher when the scion and rootstock of the same species are used [21]. Grafting is the most commonly used propagation method in viticulture [22]. For vaccine success, multiple improvements are required in the graft area, such as cell recognition and communication, cell cycle initiation, cell proliferation, and cell differentiation [23,24]. Several methods aim to reveal graft compatibility. These include methods such as the phenotypic characteristics and nursery performance of vaccine combinations [7,25,26] and total protein amounts [27].

Carbohydrates, the energy storage molecules of all living organisms, form various structural components of cells [28]. Roots are the main location where nutrients are stored as well as represent the place where the effects of phylloxera and environmental conditions are removed [3,10]. The storage of carbohydrates (especially sugar and starch) is critical for plants. The collection, storage, and binding of photosynthetic products such as starch provide a buffering mechanism that allows plants to maintain cellular processes, growth, and defense functions when metabolic demands exceed the energy supply [29–31]. These reserves help perennial plants to resist against biotic and abiotic stress factors, including pests, diseases, and drought [32]. It is known that plant roots contain higher carbohydrate concentrations than other tissues and organs at the end of the vegetation period. Carbohydrates affect respiration after dormancy, and this has a positive effect on shoot and root growth and development, shoot and root diameter, root elongation, flower bud, and grape berry formation [33]. The carbohydrates that are produced in the leaves are transported to the phloem and are stored in the roots as sugar and starch. If the cambium and phloem tissues in the graft area do not form rapidly in spring, sufficient amounts of the nutrients accumulated in the root and stem will not reach the buds, shoots, and flowers [34]. Secondary xylem and phloem parenchyma cells transform into ray parenchyma and axial parenchyma [35]. The ray and axial parenchyma tissues represent the majority of the living cells in the plant and have multiple functions. One of these tasks is storing and transporting carbohydrates [36–39]. There are some articles on the transport of carbohydrates from the xylem as well, and grapevine is also included in these plants [40,41]. It is clear that the xylem is an essential carrier for the transfer of carbohydrates and other materials stored in vines [42].

In the research, the suitability of using American vines as rootstock was investigated in order to obtain high-quality and high yields in different climates and soil conditions and to control the vegetative development of strongly growing foxy grapes. In addition, the graft success performance, growth, and development characteristics of the rootstocks used and foxy grape varieties in the grafting room and nursery plots and their compatibility in the grafting region were revealed. Carbohydrate accumulations in the roots of grafted vine saplings were determined, and we hoped that their distribution in the graft region (under the graft, graft area, and above the graft) would provide us with information about the development of the vascular bundles.

## 2. Materials and Methods

### *2.1. Characteristics of Cultivars and Rootstocks*

This research was carried out in the Research and Application Area and Horticulture Laboratories of Ondokuz Mayıs University's Faculty of Agriculture between March 2020 and March 2021. The 'Rizessi', 'Rizpem', 'Rizellim', 'Ülkemiz', and 'Çeliksu' foxy grape varieties to be used as scions were collected from the vineyard area of the Faculty of Agriculture, Department of Horticulture; the American grape vines SO4, 140Ru, and 110R used as rootstocks were obtained from the Manisa Viticulture Research Institute.

#### 2.1.1. Cultivars

*'Rizessi'*

This is a black and seeded grape variety. It has a hermaphroditic flower structure. It has 2–3 clusters, the length of the tendrils is short, and the leaves are three-segmented. It matures during the middle period [6].

*'Çeliksu'*

This is a blue–black-colored and seeded grape variety. Its leaves are three-segmented. The flower structure is hermaphroditic, it has 2–3 clusters, and the tendrils are short. Its fruits mature during the late period [6].

*'Ülkemiz'*

Its fruits are blue–black and have seeds. It has a hermaphroditic flower structure and 1–2 clusters. Its tendril length is medium. There are no segments in the leaves. This grape variety matures during mid-season [6].

*'Rizellim'*

This is a blue–black-colored and seeded grape variety. The flower structure is hermaphroditic and forms 2–3 clusters. The tendrils are short in length, and its leaves are tripartite. It matures in the late period [6].

*'Rizpem'*

This variety is pink in color and have seeds. Its flowers are hermaphroditic. It forms 1–2 clusters, and its tendrils are short. There are no segment in its leaves. It matures in the late period [6].

#### 2.1.2. Rootstocks

*140Ru*

This grape vine a hybrid of *Vitis berlandieri* × *Vitis rupestris*. It grows very vigorously, and its cuttings are difficult to root [34,43]. It is drought- and salinity-tolerant. It is well adapted to acidic soils [44,45].

*SO4*

*Vitis berlandieri* × *Vitis riparia* is a hybrid, weak to moderately strong rootstock, and its cuttings root well [34,43,46]. It grows well in moist and heavy soils (Steel, 1995). It performs well in acidic soils [47].

*110R*

The *Vitis berlandieri* × *Vitis rupestris* hybrid is a medium-strength hybrid, and the rooting rate of the cuttings is low [34,43]. Its roots grow deep. It is suitable for all types of soils, including acidic soils [44,46].

### 2.2. Production of Grafted Vine Saplings

Before grafting, fungicide was applied to all rootstocks and scions. Grafting was carried out in April using an omega grafting machine. In grafted cuttings, paraffin was applied to cover the rootstock's scion, graft area, and upper part. Then, the grafted cuttings were placed in plastic cases using thin, moist sawdust and were covered with plastic sheets. Grafted vine cuttings were kept at 28 °C for the first three weeks to ensure healthy callus formation at the graft area. During the last week, the callusing room was brought to a temperature of 26 °C and to a humidity level of 80–85%. At the end of 30 days, the grafted vine cuttings were kept in the room conditions for 3–4 days, allowing them to become used to the environment. Evaluations were carried out to determine the success of the graft. Then, the shoots were shortened to 5–7 cm in length, and the second paraffin process was applied [48]. Before planting, the raised bed nursery pilots were mulched to control weeds and prevent water loss. A drip irrigation system was installed for irrigation. Grafted cuttings were planted on 29 May 2020 at 10 cm intervals in double rows on a raised bed (Figure 1).

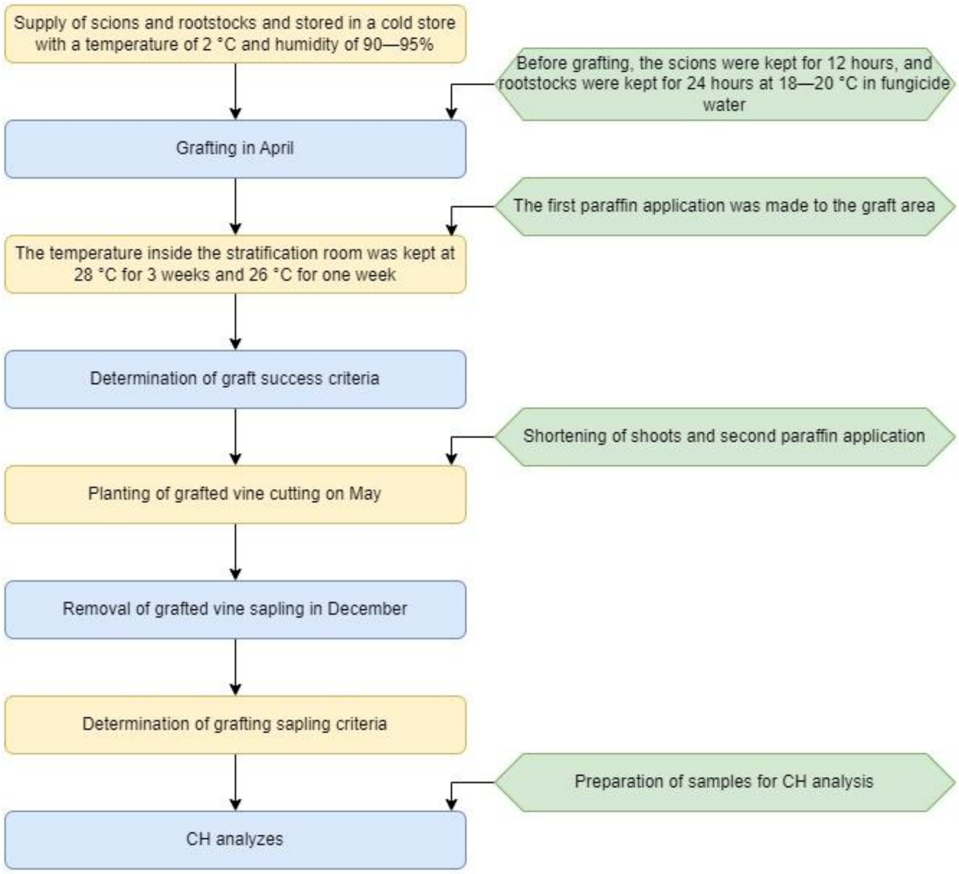



**Figure 1.** Grafted vine sapling production and analysis stages.

### 2.3. Graft Success Criteria

In determining the graft success rate in the grafted cuttings:

*Callus formation rate (%):* Percentage of callused grafted cuttings [49];

*Bud burst rate (%):* Percentage of grafted cuttings forming shoots [50];

*Callus development level (0–4):* 0 = no callus, 1 = 25%, 2 = 50%, 3 = 75%, and 4 = 100% [48];

*Root formation rate (%):* Rooting rate of grafted vine cuttings [48].

### 2.4. Grafted Vine Sapling Criteria

Grafted vine saplings removed during the dormant period:

*Grafted vine sapling rate (%):* The percentage of grafted vines that a root system and shoot at the end of the growing season [51];

*First-class grafted vine sapling rate (%):* Percentage of saplings with a vigorous root system and shoots from grafted vine saplings [48];

*Primary shoot diameter (mm) and length (cm):* The primary shoot was measured. Diameter was determined between second and third nodes, and length was measured from bottom to tip [50];

*Shoot development level (0–4):* 0 = no shoot, 1 = poor shoot, 2 = medium shoot, 3 = strong shoot, 4 = very strong shoot [48];

*Graft area diameter (mm):* Measured with a digital compass from the graft area [48];

*Rootstock diameter (mm):* Measured with a digital compass from the stem diameter 5 cm under the graft area [48];

*Root development level (0–4):* 0 = no root formation, 1 = one-sided weak root formation, 2 = two-sided root formation, 3 = three-sided root formation, and 4 = four-sided root formation [52];

*Root number and root length (cm):* Primary roots were counted, and their lengths were measured [52].

### 2.5. Carbohydrate Analyses

The grafted vine saplings used in the carbohydrate analysis were removed during the dormancy period (December), and samples were taken as 2–4 cm cuttings from the roots, under the grafting area, above the grafting area, and from the grafting area for at least 2–4 cm pieces. The samples were dried in an oven at 60 °C for 48 h. The dried samples were ground in the mill [53]. Total sugar and starch amounts were analyzed according to the 'Anthrone' method [54]. Total carbohydrate amounts were determined according to Candolfi-Vasconcelos and Koblet [55]. Total sugar and starch readings were taken with a spectrophotometer (Thermo Multiskan Go, Thermo Fisher, Vantaa, Finland) at a wavelength of 620 nm when the samples came to room temperature.

### 2.6. Statistical Analysis

The experiment was set up in randomized blocks according to the factorial trial design, with 50 grafted vine cuttings in each replicate with 3 replications. In total, the 40 grafted cuttings with completed callus development were planted in the nursery for each combination. The "JMP-8" statistical package program was used to evaluate the data, and the differences between the means were determined by the LSD ($p < 0.05$) test. The "arsin$\sqrt{x}$ transformation" was applied to the data and determined as a percentage (%) in the experiment, and the differences between the averages were examined over the transformed values. The graphics of the data obtained from the trials were drawn in the "Microsoft Office Professional Plus 2016 Excel" program.

## 3. Results

### 3.1. Graft Success

The callus growth rate (%), bud burst rate (%), callus development level (0–4), and root formation rate (%) in grafted cuttings obtained from foxy grape varieties grafted on different rootstocks are given in Table 1. There were no statistical differences between the combinations in terms of callus formation and bud burst rates, but significant (5%) differences were found in the callus development level and root formation rates. The highest callus growth rate was obtained from the combinations of 'Rizessi', 'Rizellim', and 'Rizpem' (100.00%) grafted on SO4 rootstock and of 'Rizellim' and 'Rizpem' (100.00%) grafted on 110R rootstock. The lowest callus growth rate was obtained from the combination of 'Rizessi'/140Ru

(82.00%). Bud burst rates with no statistically significant differences between the combinations ranged from 75.00% to 98.00% ('Rizpem'/140Ru and 'Rizellim'/SO4, respectively). The highest level of callus development was determined in 'Rizessi'/110R (3.91), and the lowest level was observed in 'Ülkemiz'/140Ru (1.95). The cuttings of the American vine rootstocks show different rooting properties. Therefore, root formation rates were examined to obtain preliminary information about rooting status. In terms of the root formation rate, the highest values were 90.00% ('Rizpem'/110R), 88.33% ('Rizpem'/SO4), and 86.00% ('Ülkemiz'/110R), while the lowest value was 33.33% ('Rizellim'/140Ru).

**Table 1.** Grafting success criteria in foxy grape (*V. labrusca* L.) cultivars grafted on different rootstocks.

| Cultivar | Rootstock | Callus Formation Rate (%) | Bud Burst (%) | Callus Development Level (0–4) | Root Formation Rate (%) |
|---|---|---|---|---|---|
| Rizessi | 140Ru | 82.00 | 75.33 | 2.18 gh | 41.33 ef |
| | SO4 | 100.00 | 93.33 | 3.55 cde | 82.67 abcd |
| | 110R | 99.33 | 95.33 | 3.91 a | 50.00 e |
| Çeliksu | 140Ru | 90.67 | 90.67 | 2.31 fg | 51.33 e |
| | SO4 | 99.33 | 90.67 | 3.71 abcd | 80.00 bcd |
| | 110R | 98.67 | 96.67 | 3.68 abcd | 73.33 cd |
| Ülkemiz | 140Ru | 85.33 | 78.00 | 1.95 h | 71.33 d |
| | SO4 | 99.33 | 80.67 | 3.83 ab | 84.67 abcd |
| | 110R | 99.33 | 89.33 | 3.65 bcd | 86.00 abc |
| Rizellim | 140Ru | 92.00 | 90.67 | 2.52 f | 33.33 f |
| | SO4 | 100.00 | 98.00 | 3.53 de | 84.67 abcd |
| | 110R | 100.00 | 96.00 | 3.34 e | 80.67 bcd |
| Rizpem | 140Ru | 95.00 | 75.00 | 2.47 f | 45.00 ef |
| | SO4 | 100.00 | 85.00 | 3.80 abc | 88.33 ab |
| | 110R | 100.00 | 88.33 | 3.67 abcd | 90.00 a |
| LSD | | N.S | N.S | 1.14 $p < 0.01$ | 9.72 $p < 0.01$ |

N.S: non-significant. a–h: differences between results shown with different letters in the same column are statistically significant.

### *3.2. Grafted Vine Sapling*

The sapling rate (%), first-class sapling rate (%), shoot development level (0–4), primary shoot diameter (mm), and main shoot length (cm) obtained from foxy grape varieties grafted on different rootstocks and statistical differences are given in Table 2. The highest sapling rates were 86.00%, 85.62%, and 81.94% and were found in the combinations of 'Rizellim'/SO4, 'Çeliksu'/SO4, and 'Rizellim'/110R, respectively. The lowest sapling rate value was found in the combination of 'Ülkemiz'/140Ru, with 22.87%. For the first-class sapling rate, the highest value was obtained in the combination of 'Rizellim'/SO4, with 75.33%. Furthermore, 'Rizellim'/110R had a first-class sapling rate of 70.83%, and 'Çeliksu'/SO4 had a first-class sapling rate of 67.90%. The lowest first-class sapling rate was obtained from the combination of 'Rizpem'/140Ru, with 11.27%. There were no statistical differences between the combinations, and the shoot growth levels were found to be between 2.62 ('Rizpem'/140Ru) and 3.59 ('Rizellim'/140Ru). The shoot growth level generally did not fall below 3.0 in the combinations, except for 'Rizpem', and this could have been due to the weaker development of the 'Rizpem' cultivar compared to the others. When the primary shoot diameter (mm) and length (cm) values in the grafted vine saplings obtained were examined, the longest primary shoot diameters were obtained from the 'Rizellim'/140Ru (6.95 mm) and Rizessi'/140Ru (151.79 cm) combinations, which also gave the highest primary shoot length. On the other hand, the lowest primary shoot diameter and length were obtained from 'Rizpem'/140Ru (4.71 mm) and 'Rizpem'/110R (59.84 cm), respectively.

**Table 2.** Sapling rate and quality criterias of foxy grape varieties (*V. labrusca* L.) grafted on different rootstocks-I.

| Cultivar | Rootstock | Sapling Rate (%) | First-Class Sapling Rate (%) | Shoot Development Level (0–4) | Primarily Shoot Diameter (mm) | Primarily Shoot Length (cm) |
|---|---|---|---|---|---|---|
| Rizessi | 140Ru | 46.24 d | 35.72 c | 3.55 | 6.50 ab | 137.15 ab |
| | SO4 | 72.79 bc | 65.31 ab | 3.04 | 6.11 abcd | 93.13 cde |
| | 110R | 48.98 d | 38.24 c | 3.26 | 6.30 abc | 104.45 cd |
| Çeliksu | 140Ru | 48.57 d | 38.10 c | 3.26 | 6.30 abc | 141.55 ab |
| | SO4 | 85.62 a | 67.90 ab | 2.92 | 5.04 ef | 75.95 def |
| | 110R | 70.39 c | 60.83 b | 3.48 | 5.64 cde | 110.24 bc |
| Ülkemiz | 140Ru | 22.87 e | 16.31 d | 3.05 | 5.40 def | 95.86 cde |
| | SO4 | 45.67 d | 37.61 c | 3.10 | 5.40 def | 84.39 cdef |
| | 110R | 47.22 d | 34.72 c | 3.30 | 5.88 bcde | 93.24 cde |
| Rizellim | 140Ru | 45.89 d | 36.08 c | 3.59 | 6.95 a | 151.79 a |
| | SO4 | 86.00 a | 75.33 a | 3.35 | 5.53 cdef | 87.05 cdef |
| | 110R | 81.94 ab | 70.83 ab | 3.44 | 5.65 bcde | 101.68 cd |
| Rizpem | 140Ru | 27.14 e | 11.27 d | 2.62 | 4.71 f | 67.28 ef |
| | SO4 | 53.33 d | 36.67 c | 2.74 | 5.61 cde | 76.28 def |
| | 110R | 28.33 e | 15.00 d | 2.81 | 5.98 bcd | 59.84 f |
| LSD | | 8.33 $p < 0.01$ | 7.71 $p < 0.05$ | N.S | 0.85 $p < 0.05$ | 32.07 $p < 0.05$ |

N.S: non-significant. a–f: differences between results shown with different letters in the same column are statistically significant.

Statistically significant (5%) differences were obtained between the combinations in the grafting area diameter (mm), rootstock diameter (mm), and root length (cm) values in the grafted vine saplings. However, no significant differences were found between the root development levels (0–4) and root number values (Table 3). The graft area diameter of the grafted vine saplings was 14.14–20.49 mm ('Ülkemiz'/140Ru and 'Rizessi'/110R), and the rootstock diameter was 9.02–11.62 mm ('Rizpem'/SO4, 'Çeliksu'/110R, respectively). In the results obtained from the combinations, root growth levels were between 2.61 ('Ülkemiz'/140Ru) and 3.40 ('Ülkemiz'/SO4); root length was between 35.07 cm ('Rizpem'/140Ru) and 56.46 cm ('Rizessi'/140Ru); and root number was between 9.06 ('Rizellim'/110R) and 16.97 ('Rizellim'/SO4).

**Table 3.** Sapling rate and quality criterias of foxy grape varieties (*V. labrusca* L.) grafted on different rootstocks-II.

| Cultivar | Rootstock | Graft Area Diameter (mm) | Rootstock Diameter (mm) | Root Development Level (0–4) | Root Length (cm) | Number of Roots (pcs) |
|---|---|---|---|---|---|---|
| Rizessi | 140Ru | 19.92 abc | 10.20 bcd | 3.35 | 56.46 a | 13.32 |
| | SO4 | 19.66 abc | 10.56 bcd | 3.26 | 52.07 abc | 13.59 |
| | 110R | 20.49 a | 10.21 bcd | 3.00 | 43.97 def | 11.82 |
| Çeliksu | 140Ru | 19.83 abc | 9.75 def | 2.87 | 50.62 abcd | 9.84 |
| | SO4 | 18.46 cde | 10.13 cde | 3.23 | 55.65 ab | 15.37 |
| | 110R | 20.31 a | 11.62 a | 3.16 | 37.89 fg | 9.46 |
| Ülkemiz | 140Ru | 14.14 f | 9.31 ef | 2.61 | 48.35 cd | 9.68 |
| | SO4 | 17.71 de | 10.56 bcd | 3.40 | 51.60 abc | 13.22 |
| | 110R | 18.59 bcd | 9.96 de | 3.10 | 46.11 cde | 9.28 |
| Rizellim | 140Ru | 20.30 a | 10.12 cde | 2.76 | 47.05 cde | 10.25 |
| | SO4 | 19.68 abc | 10.98 ab | 3.39 | 48.99 bcd | 16.97 |
| | 110R | 20.16 ab | 10.79 abc | 2.95 | 40.09 efg | 9.06 |

**Table 3.** *Cont.*

| Cultivar | Rootstock | Graft Area Diameter (mm) | Rootstock Diameter (mm) | Root Development Level (0–4) | Root Length (cm) | Number of Roots (pcs) |
|---|---|---|---|---|---|---|
| Rizpem | 140Ru | 14.25 f | 9.09 f | 2.77 | 35.07 g | 12.04 |
| | SO4 | 16.95 e | 9.02 f | 3.06 | 50.99 abcd | 16.76 |
| | 110R | 17.12 de | 9.32 ef | 3.11 | 49.19 bcd | 11.69 |
| LSD | | 0.92<br>$p < 0.01$ | 0.83<br>$p < 0.05$ | N.S | 7.18<br>$p < 0.01$ | N.S |

N.S: non-significant. a–g: differences between results shown with different letters in the same column are statistically significant.

### *3.3. Carbohydrate Accumulations*

The differences between the cultivar/rootstock combinations were determined in the carbohydrate accumulations in the root, under the graft area (rootstock), at the graft area, and above the graft area (scion) of the grafted vine saplings obtained by grafting foxy grapes on different rootstocks.

The differences between the total sugar amounts and the total carbohydrates accumulated in the roots of grafted vine saplings were statistically significant (5%), and the differences between starch amounts were insignificant. The accumulation values were between 17.25 and 28.46 mg·L$^{-1}$ ('Çeliksu'/110R and 'Çeliksu'/140Ru) for the total sugar, between 62.67 and 77.62 mg·L$^{-1}$ ('Rizellim'/SO4 and 'Ülkemiz'/140Ru) for the total starch, and between 80.09 and 100.41 mg·L$^{-1}$ ('Rizellim'/SO4 and 'Ülkemiz'/SO4) for the total carbohydrates (Figure 2).

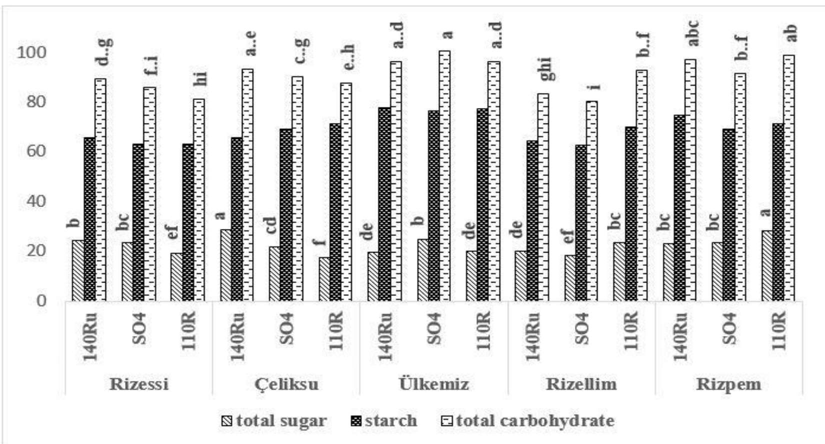



**Figure 2.** Root carbohydrate accumulations in grafted vine saplings. total sugar $p < 0.01$ LSD: 2.20, total carbohydrates $p < 0.01$ LSD: 7.35. a–i: differences between results shown with different letters in the same designs are statistically significant.

The differences between the total sugar, starch, and total carbohydrate values under the graft area (rootstock) in the grafted vine saplings were statistically significant at the 5% level. The highest total sugar and total carbohydrate values were in 'Çeliksu'/110R (32.82 mg·L$^{-1}$ and 74.50 mg·L$^{-1}$, respectively), and the highest starch content was found from the combination of 'Rizessi'/110R (45.30 mg·L$^{-1}$). The lowest accumulation was obtained in 'Ülkemiz'/110R (23.25 mg·L$^{-1}$) for total sugar, in 'Ülkemiz'/140Ru (30.69 mg·L$^{-1}$) for starch, and in 'Rizellim'/SO4 (59.34 mg·L$^{-1}$) for total carbohydrates (Figure 3).

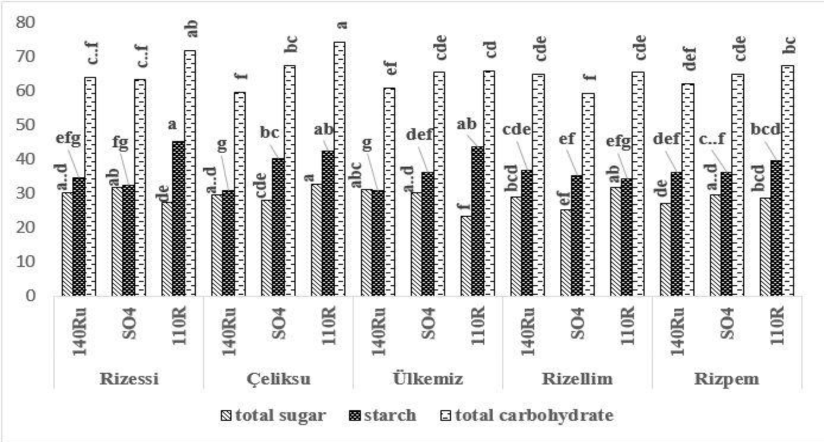



**Figure 3.** Under the graft area (rootstock) carbohydrate accumulations in grafted vine saplings. total sugar $p < 0.01$ LSD: 3.41, starch $p < 0.01$ LSD: 4.14, total carbohydrates $p < 0.01$ LSD: 5.01. a–g: differences between results shown with different letters in the same designs are statistically significant.

The differences between total sugar, starch, and total carbohydrate values in the graft area were statistically significant (5%). The highest accumulation values were found to be 27.05 mg·L$^{-1}$ ('Rizessi'/140Ru) for total sugar, 43.06 mg·L$^{-1}$ ('Rizellim'/110R) for starch, and 64.01 mg·L$^{-1}$ ('Rizellim'/110R) for total carbohydrates. The lowest was 16.54 mg·L$^{-1}$ ('Ülkemiz'/140Ru) for total sugar, 25.79 mg·L$^{-1}$ ('Ülkemiz'/140Ru) for starch, and 41.50 mg·L$^{-1}$ ('Ülkemiz'/140Ru) for total carbohydrates (Figure 4).

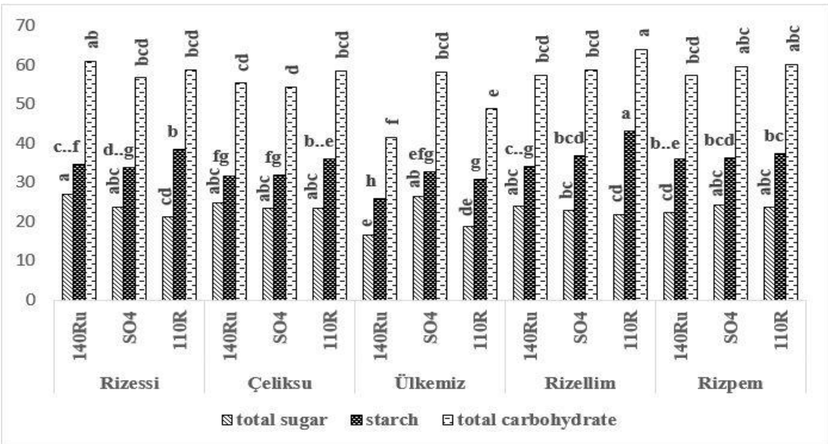



**Figure 4.** Graft area carbohydrate accumulations in grafted vine saplings. total sugar $p < 0.01$ LSD: 3.88, starch $p < 0.01$ LSD: 3.48, total carbohydrates $p < 0.01$ LSD: 5.06. a–g: differences between results shown with different letters in the same designs are statistically significant.

Statistically, the differences between the total sugar and total carbohydrate accumulations above the graft area (scion) were significant at 5%, and the differences between starch accumulations were nonsignificant. The highest sugar accumulation in the above the graft area was found in the 'Rizellim'/110R (33.82 mg·L$^{-1}$) combination, and the lowest was found in 'Rizpem'/110R (19.19 mg·L$^{-1}$). The highest numerical value for starch accumulation was obtained in 'Ülkemiz'/SO4 (43.56 mg·L$^{-1}$), and the lowest was obtained in 'Rizellim'/140Ru (36.70 mg·L$^{-1}$). The highest total carbohydrate value was found in 'Ülkemiz'/110R (72.10 mg·L$^{-1}$), while the lowest value was found in 'Rizpem'/110R (57.75 mg·L$^{-1}$) (Figure 5).

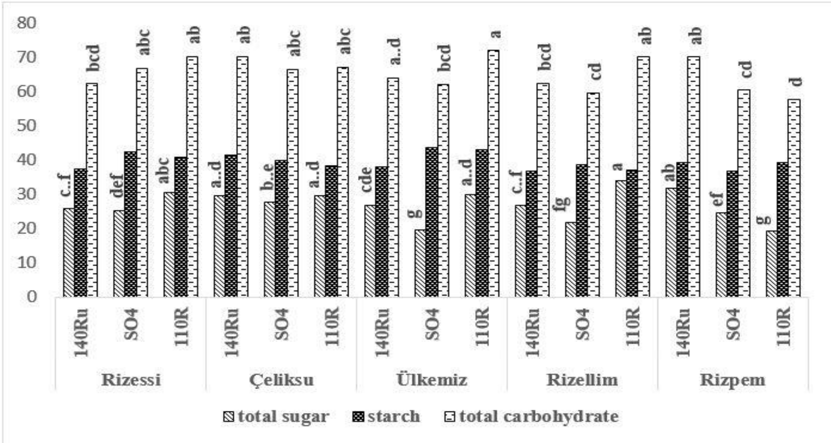
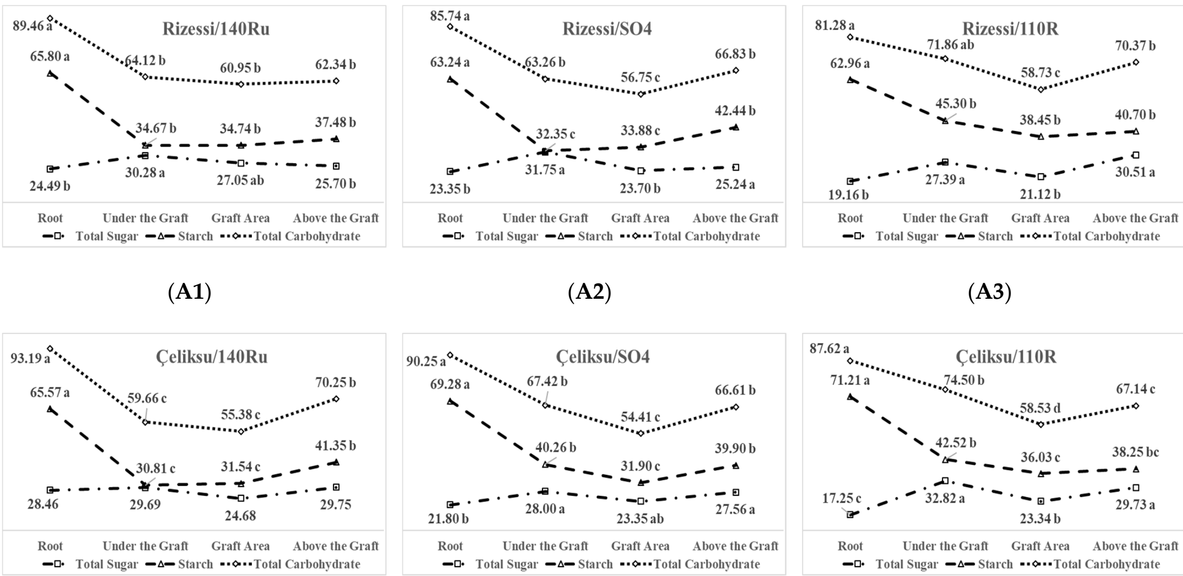



**Figure 5.** Above the graft area (scion) carbohydrate accumulations in grafted vine saplings. total sugar $p < 0.01$ LSD: 4.95, total carbohydrates $p < 0.05$ LSD: 8.31. a–g: differences between results shown with different letters in the same designs are statistically significant.

*3.4. Carbohydrate Distributions*

In the combinations obtained with the 'Rizessi' cultivar and 140Ru, SO4, and 110R rootstocks, there were statistically significant (5%) differences in the total sugar, starch, and carbohydrate levels among the carbohydrate distributions by region. The highest starch and total carbohydrate accumulations were in the roots. In the 'Rizessi'/140Ru combination, the total carbohydrate and starch distributions were close under the graft area (rootstock), the graft area, and above the graft area (scion). However, the total sugar distribution was the greatest under the graft area (Figure 6A1). In the 'Rizessi'/SO4 combination, sugar accumulation under the graft area was higher, while starch accumulation remained lower than in other areas. Since the starch accumulation above the graft area was higher than under the graft area, it ensured that the total carbohydrate distributions remained at the same statistical level. There was less accumulation at the graft area compared to other parts (Figure 6A2). In the 'Rizessi'/110R combination, in the carbohydrate distributions in the graft region, the total sugar and total carbohydrates accumulations were higher above and under the graft area than at the graft area, and the starch accumulations showed a balanced distribution (Figure 6A3).




(A1) (A2) (A3)

(B1) (B2) (B3)

**Figure 6.** *Cont.*

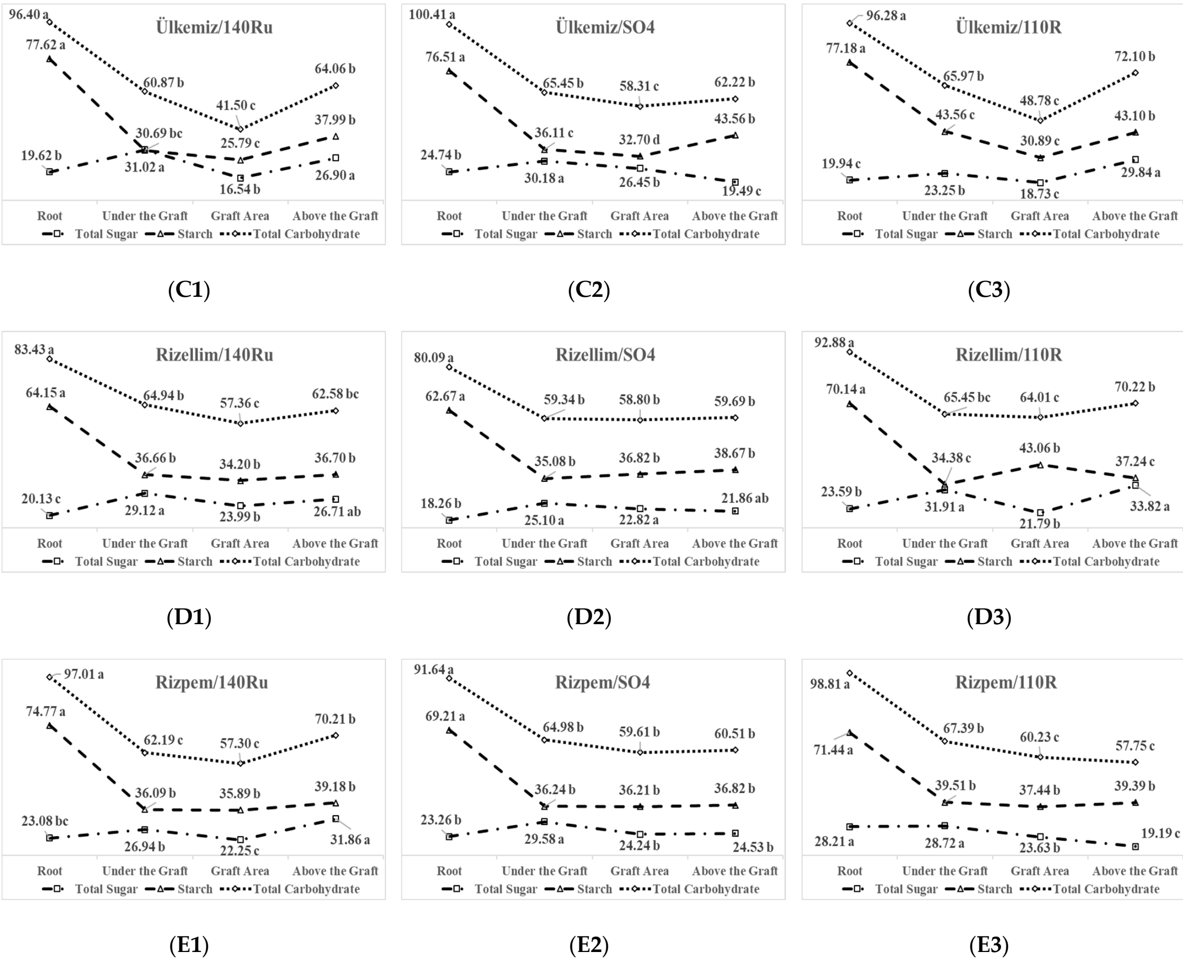



**Figure 6.** Carbohydrate distributions in different combinations of the *V. labrsuca* cultivars. (**A1**) 'Rizessi'/140Ru $p < 0.01$ total sugar LSD: 3.82, starch LSD: 8.11, total carbohydrates LSD: 7.58. (**A2**) 'Rizessi'/SO4 $p < 0.01$ total sugar LSD: 2.86, starch LSD: 3.32, total carbohydrates LSD: 5.17. (**A3**) 'Rizessi'/110R $p < 0.01$ total sugar LSD: 4.85, starch LSD: 7.39, total carbohydrates LSD: 9.78. (**B1**) 'Çeliksu'/140Ru $p < 0.01$, starch LSD: 8.27, total carbohydrates LSD: 8.76. (**B2**) 'Çeliksu'/SO4 $p < 0.05$ total sugar LSD: 5.41, $p < 0.01$ starch LSD: 5.51, total carbohydrates LSD: 8.25. (**B3**) 'Çeliksu'/110R $p < 0.01$ total sugar LSD: 3.85, starch LSD: 6.46, total carbohydrates LSD: 7.04. (**C1**) 'Ülkemiz'/140Ru $p < 0.01$ total sugar LSD: 3.82, starch LSD: 8.11, total carbohydrates LSD: 7.58. (**C2**) 'Ülkemiz'/SO4 $p < 0.01$ total sugar LSD: 3.09, starch LSD: 3.21, total carbohydrates LSD: 3.82. (**C3**) 'Ülkemiz'/110R $p < 0.01$ total sugar LSD: 4.63, starch LSD: 8.06, total carbohydrates LSD: 10.65. (**D1**) 'Rizellim'/140Ru $p < 0.01$ total sugar LSD: 3.57, starch LSD: 6.57, total carbohydrates LSD: 6.95. (**D2**) 'Rizellim'/SO4 $p < 0.01$ total sugar LSD: 4.16, starch LSD: 3.90, total carbohydrates LSD: 5.77. (**D3**) 'Rizellim'/110R $p < 0.01$ total sugar LSD: 4.09, starch LSD: 3.73, total carbohydrates LSD: 5.63. (**E1**) 'Rizpem'/140Ru $p < 0.01$ total sugar LSD: 4.39, starch LSD: 5.09, total carbohydrates LSD: 7.34. (**E2**) 'Rizpem'/SO4 $p < 0.01$ total sugar LSD: 2.97, starch LSD: 5.30, total carbohydrates LSD: 6.16. (**E3**) 'Rizpem'/110R $p < 0.01$ total sugar LSD: 2.86, starch LSD: 3.77, total carbohydrates LSD: 3.91. a–d: differences between results shown with different letters on the same lines are statistically significant.

Statistically significant (5%) differences were determined between all combinations in terms of their total sugar, starch, and total carbohydrate distributions, except for the total sugar amount in 'Çeliksu'/140Ru in Çeliksu' combinations. In the 'Çeliksu'/140Ru combination, the total sugar distributions in the graft region were close, and the starch and total carbohydrate contents were higher above the graft area (scion) (Figure 6B1). While the distribution of total sugar, starch, and total carbohydrates under the graft area (rootstock) and above the graft region of 'Çeliksu'/SO4 was equally distributed, there was a lower accumulation at the graft area (Figure 6B2). In 'Çeliksu'/110R, the carbohydrate distributions in the graft region and the starch and total carbohydrate accumulations under the graft area were higher. There was a balanced distribution under and above the graft area regarding total sugar accumulation. In this combination, the lowest accumulations in the carbohydrate distributions were determined at the graft area (Figure 6B3).

Statistically, 5% differences were found in the total sugar, starch, and total carbohydrates among the carbohydrate distributions in the combinations obtained from the 'Ülkemiz' variety. In 'Ülkemiz'/140Ru, the distribution of total sugar and total carbohydrates under the graft area (rootstock) and above the graft area (scion) was in the same importance group, while a difference was observed in the starch distributions. The accumulations at the graft area remained at the lowest level (Figure 6C1). In 'Ülkemiz'/SO4, total sugar accumulation was higher under the graft area, and starch accumulation was higher above the graft area. Values under and above the graft area for the total carbohydrate distributions were at the same statistical level. The starch and total carbohydrate distributions in the graft area remained at the lowest level (Figure 6C2). In 'Ülkemiz'/110R, the distribution of total sugar was higher above the graft area, while the starch and total carbohydrate distributions were statistically similar under and above the graft area. The lowest accumulations were observed in the carbohydrate distributions in the graft area (Figure 6C3).

Statistically, 5% differences were determined in the total sugar, starch, and total carbohydrates among the carbohydrate distributions in the 'Rizellim' combinations (Figure 6D1–D3). In 'Rizellim'/140Ru, the highest values in the distributions of total sugar and total carbohydrates were under the graft area (rootstock), while there was a balanced distribution for starch (Figure 6D1). In 'Rizellim'/SO4, the distributions of total sugar, starch, and total carbohydrates were balanced in the parts under the graft area, graft area, and above the graft area (Figure 6D2). In 'Rizellim'/110R, the total amount of sugar was balanced under and above the graft area and less accumulated at the graft area, while the amount of starch was more accumulated at the graft area. For the total carbohydrate distributions, accumulations above the graft area were higher than those below the graft area. The lowest accumulation was detected at the graft area (Figure 6D3).

The carbohydrate distributions in the combinations obtained with the 'Rizpem' cultivar showed statistically significant 5% differences in the total sugar, starch, and total carbohydrate levels. In 'Rizpem'/140Ru, the total sugar distribution in the graft region was the highest above the graft area (scion), while the starch accumulations were evenly distributed. It is observed that the total carbohydrate accumulations were higher above the graft area (Figure 6E1). In 'Rizpem'/SO4, total sugar accumulation under the graft area (rootstock) was higher, and starch and total carbohydrate distributions were in the same importance group (Figure 6E1). In 'Rizpem'/110R, the total sugar and total carbohydrate distributions were higher under the graft area. The starch distribution was balanced (Figure 6E1).

The relationships between the findings obtained for the grafted vine saplings and the distributions of total carbohydrates are given in Table 4. There were negative correlations between sapling yield and first-class sapling yield and total carbohydrates in the root and positive correlations with total carbohydrates in the graft area (GA). Significant negative correlations were determined between the shoot development level and total carbohydrates in the root. As the graft area diameter increased, the total carbohydrate amount in the graft area also increased. There is a negative relationship between the rootstock diameter and root total carbohydrate content. There were significant positive correlations between the root development level and total carbohydrates in the graft area (GA), and negative correlations with total carbohydrate levels in above the graft (ATG).

**Table 4.** Correlation between sapling rate criteria and carbohydrate accumulations.

| | Root Total Carbohydrates | UTG Total Carbohydrates | GA Total Carbohydrates | ATG Total Carbohydrates |
|---|---|---|---|---|
| Sapling rate | −0.361 * | 0.133 | 0.387 ** | 0.083 |
| First-class sapling rate | −0.400 * | 0.137 | 0.358 * | 0.072 |
| Shoot development level | −0.335 * | 0.063 | 0.164 | 0.060 |
| Primarily shoot diameter | −0.279 | 0.074 | 0.155 | −0.037 |
| Primarily shoot length | −0.281 | −0.085 | −0.020 | 0.076 |
| Graft area diameter | −0.434 * | 0.182 | 0.423 * | 0.082 |
| Rootstock diameter | −0.352 * | 0.261 | 0.261 | 0.168 |
| Root development level | −0.121 | 0.143 | 0.339 * | −0.351 * |
| Number of roots | −0.157 | −0.078 | 0.288 | −0.443 * |
| Root length | 0.071 | −0.291 | −0.046 | −0.310 * |

UTG: Under the graft, GA: Graft area, ATG: Above the graft. * There are statistically significant differences at the $p < 0.05$ level. ** There are statistically significant differences at the $p < 0.01$ level.

## 4. Discussion

The formation of callus tissue at the graft area is the first response to grafting [19]. Most previous studies determined that callus development levels differ according the variety/rootstock combination [56–58]. Although research varies according to the varieties and rootstocks used in the callus development path, studies generally focus on grafting *V. vinifera* varieties to American grapevine rootstocks. Very little work has been carried out on grafting varieties or types of *V. labrusca* on American grapevines rootstocks. Köse, Ateş, and Çelik [51] grafted foxy grape varieties to different rootstocks (5BB, SO4, and 110R) and determined callus development rates between 84.44% and 85.55%. While the highest result obtained by the researchers was 85.55%, the study obtained a 100% callus development rate (Table 1). Similar to the results obtained in studies on grafting *V. vinifera* cultivars to American grapevine rootstocks, generally high rates of callus development have been obtained [26,48,59,60]. During fusing in the grafting room, bud burst may occur on the grafted cuttings. Because of the humidity and temperature, the grafting room creates a suitable environment for the buds to burst [16,61]. When the Kalecik Karası and Narince grape varieties were grafted onto the 1103P rootstock, Çakır and Yücel [62] obtained bottom root formation at a rate of 55.00% and 49.00%, respectively. In a similar study, the rates of bottom root formation after folding in cultivar/rootstock combinations were determined to be between 20.00% and 90.00% (for Hatun Parmağı/110R Çiloreş/140Ru) [63]. Although the callus growth rate, bud burst, callus development level, and root formation rate vary according to the grape and rootstock varieties used, stratification conditions, grafting type, time, and methods [20,64], the results of these researchers are similar to the results that we obtained (Table 1).

Cultivars and rootstocks have different growth forces, shoot rates, and callus and root formation abilities. In addition, the compatibility of the rootstock and scion, affinity, and anatomical structures are also effective in determining the sapling rate. Therefore, different sapling production rates and qualities are obtained from different combinations [65]. These differences were also seen in the results for the sapling rate (Table 2). The highest values of the effects of the rootstocks on the sapling production rate of the Müşküle grape variety were determined to be 73.75% (1616C) and 71.14% (1613C) [66]. Köse, Ateş, and Çelik [51] determined sapling production rates between 5.56% and 36.67% in their study using *V. labrusca* types grafted on 5BB and 110R, respectively. On the other hand, Tunçel and Dardeniz [58] found first-class sapling rates between 57.00% and 68.50% in their research, in which they grafted different grape varieties on 5BB rootstock. In the study in which Börner rootstock was grafted with different varieties, the first-class sapling rate obtained was between 49.00% (Riesling/Börner) and 70.20% (Weschriesling/Börner) [67]. Our findings that the sapling rate differs according to the cultivar/rootstock combinations align with the studies conducted in this field. In a study in which Razakı, Victoria, and Alphonse Lavallee cultivars were grafted on 5BB, the thickness of the primary shoot diameter changed to be between 5.72 and 6.34 mm (between the second and third nodes), and the length of the primary shoot was between 32.9 and 47.8 cm [32]. Jogaiah et al. [68] determined primary shoot diameters between 7.22 mm (110P) and 8.85 mm (Dogridge) and primary shoot lengths between 98.13 cm (1103P) and 129.00 cm (St. George) in a study in which the Thompson seedless grape variety was grafted onto different rootstocks. The lengths and diameters of the primary shoots differed according to the cultivar and rootstock characteristics or climatic conditions. As a result of the research, the primary shoot diameters and shoot lengths obtained from SO4 rootstock were generally lower than those obtained for other rootstocks (Table 2).

Tedesco, Pina, Fevereiro, and Kragler [22] inoculated the same and different genotypes and determined graft area diameters in the range of 13.88–19.28 mm (for TN112/TN112 and SY470/110R, respectively) and rootstock diameters in the range of 10.14 mm–14.00 mm (for TN112/110 and, SY470/SY470, respectively). On the other hand, they determined graft area diameters in the range of 19.15–23.31 mm (1613C and 5BB) in a study in which they grafted the Red Globe grape variety to different rootstocks [69]. Although graft area swelling is usually due to the discontinuation of vascular connections, it can also be caused by differences in the scion and rootstock growth rates [70]. In the results obtained in the study, an excessive graft area diameter and thickness indicated that there was no inconsistency. Since the cultivar and rootstock had the same diameter, differences in rootstock diameters between the combinations were obtained (Table 3). Root formations in the production of grafted vine saplings vary according to the rootstock, variety, amount of irrigation, mulch, and rooting environment [71]. In the present research, negative correlations were determined between the number of roots in the grafted vine saplings and the total carbohydrate values above the graft area. In other words, as the number of roots increased, carbohydrate accumulation above the graft area decreased (Table 4). According to this result, it can be concluded that strong root formation in the rootstock positively affects the vascular bundles in the graft region and is more effective in transporting carbohydrates.

Starch is generally a storage material found in carbohydrate accumulations [72,73]. At the same time, total sugar amounts significantly contribute to carbohydrate content [74]. In the results, it was seen that total sugars affect the total carbohydrate amounts (Figures 2–6). In grafted saplings, rootstocks affect the amount of starch stored in the plants [75]. Similarly, Prats-Llinàs et al. [76] stated that the roots were the primary organ in which starches are accumulated and retained. According to the data we obtained as a result of the study, it was determined that the roots are the largest storage organ. Concord roots in the winter rest period contain 84.00% of the total starch due to higher concentrations, and at the end of the study, it was stated that the primary storage organ of the carbohydrates necessary to support shoot and root development was the roots [77]. In the study, it was determined that the highest accumulations were in the roots (Figures 2 and 6). Starch accumulation occurs in the stem as well as in the roots [78–80]. Indeed, Earles et al. [81] determined that the amount of starch accumulated in the trunk (radial and axial parenchyma) could vary between 3 $mg \cdot g^{-1}$ and 84 $mg \cdot g^{-1}$. The energy required for successful callus formation is provided by inherent carbohydrates [28], and starch can directly affect callus formation [82]. In the results, the carbohydrate accumulations at the grafting area were generally found at low levels in the grafted vine saplings of the 'Ülkemiz' variety (Figure 6C1–C3). Bahar et al. [83] stated that the retention rate in the field increased as the carbohydrate content in the saplings increased. Similarities were found between the subjects stated by the researchers and the results obtained. As a matter of fact, in Table 4, positive correlations were determined between the sapling yields and the total carbohydrate values of the graft area. As the amount of carbohydrates in the grafting area increased, the sapling rate also increased.

The accumulation, transformation, and transport of carbohydrates in separate vine parts are essential for healthy sapling growth [84]. When the carbohydrate distributions in the grafting region were examined, the sapling yield values were found to be high in the combinations with a generally balanced distribution (Figure 6). Xylem and phloem are the main tissues involved in transporting substances in plants [85]. The success of grafting in plants is variable, and the development of vascular bundles affects grafting success [86]. In combinations where the carbohydrate distribution in the grafting region is equal or close, it can be concluded that vascular bundles develop healthily, and the substances synthesized by the plant are transported from these vascular bundles, as stated by the researchers. In the results obtained, we tried to obtain information about the newly formed cambium tissue in the graft region of the carbohydrate distributions according to the cultivar/rootstock combinations and the functionality of the vascular bundles. According to these results, the combinations that were stored equally or in excess by passing from the above of the graft area to the under the graft area were 'Rizessi'/140Ru, 'Rizessi'/SO4, 'Rizessi'/110R, 'Çeliksu'/SO4, 'Çeliksu'/110R, 'Ülkemiz'/SO4, 'Rizellim'/140Ru, 'Rizellim'/SO4, 'Rizpem'/SO4, and 'Rizpem'/110R.

## 5. Conclusions

This study investigated the graft success status, sapling production rate, and growth and development status of foxy grape varieties grafted on different rootstocks, carbohydrate accumulations in graft combinations, and distribution in the graft area. Successful grafting is a complex biochemical and structural process that begins with callus formation and continues with the formation of the cambium and the establishment of the vascular system [19,87]. The first stage of this process is the formation of a successful callus at the graft site. In the study, the best callus growth was generally seen in combinations grafted with SO4 and then with 110R rootstock. The second important step in the production of grafted vine saplings is to produce grafted vine saplings of the best possible quality. In order to obtain quality seedlings, the scion and rootstock must show good physiological development. In the nursery plots, the highest seedling yield and first-class seedling yield were obtained from the 'Rizellim'/SO4 combination and from those grafted with the SO4 rootstock in other cultivars. Carbohydrates are the most important storage material in plants. These products are produced from leaves and are transported to the areas where they are needed and to the places where they will be stored by transmission bundles [88]. The formation of vascular bundles in the graft site is important for the delivery of carbohydrates to the necessary places. Therefore, carbohydrate distributions and accumulations in the root and graft region were determined, and information was obtained about the functionality of the vascular bundles. In general, carbohydrates in combinations grafted with SO4 showed a balanced distribution. In combinations with balanced distributions, seedling yield values were also found to be high in general. Sapling yield values and carbohydrate distributions were determined to support each other. According to the values obtained for graft success, sapling rate, and carbohydrate distribution, SO4 was the superior rootstock for all characteristics and was more suitable for newly registered foxy grape cultivars.

The most important factor affecting the production of grafted vine saplings is the selection of the variety/rootstock combination. The results may differ depending on the climatic conditions of the nursery field. Therefore, according to these results obtained from variety/rootstock combinations, it is necessary to examine their performance in vineyards in the coming years. According to the results obtained here, before deciding on any combination, the adaptation of rootstocks to the climate and soil conditions in the region to be bonded, their effects on yield and quality, and their effects on the growth and development of the variety should be fully investigated. It is thought that the findings obtained as a result of this study will help the development of viticulture in the Black Sea Region and the real vineyard potential of the region by establishing new *labrusca* grape vineyards.

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