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The Effect of Rootstocks and Berry Heterogeneity on the Phytochemical Properties in *Vitis vinifera* L. Papazkarası Grapes

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HIGHLIGHTS

- A total of; 144 vines, 1440 clusters and 7200 berries were evaluated in this study.
- The highest anthocyanin and phenolic concentrations were found in berries with diameters ≤12.00mm.
- None of the berry size groups or rootstocks reached full ripeness.
- A reduction in berry size may improve grape quality, offering potential benefits for viticultural practices
- Wines from berry size groups with better must quality are expected to yield clearer sensory results.

Abstract

There are differences in development and composition between the clusters on the vines and the grape berries on the clusters. Therefore, grouping berries by size in wine grape varieties can help better manage the composition of the wine to be produced. The aim of this research is to reveal the effect of berry heterogeneity on primary and secondary metabolites in the cv. Papazkarası. The Papazkarası vines are grafted onto the rootstocks 1103P, 110R, and 420A and are trained in the double Cordon Royat system. The clusters harvested from each rootstock were separated into individual berries. These berries were then grouped by size using sieves. The size groups were: ≤12.00 mm; 12.01-14.00 mm; 14.01-16.00 mm; 16.01-18.00 mm; and ≥18.00 mm, forming five groups. A control group was created by taking berries from each size group. In terms of primary metabolites in the must, the Papazkarası/1103P combination and the ≥18.00 mm size group stood out. However, the maturity indices, Brix, and % alcohol criteria were found to be insufficient for all rootstocks and berry sizes. Regarding secondary metabolites, it was found that the ≤12.00 mm and 12.01-14.00 mm size groups had high values in all graft combinations. The size group with the highest total anthocyanin and total phenolic content was the ≤12.00 mm group. Based on these results, berry size reduction practices will improve quality. The quality of the wines obtained from different size groups should also be supported by sensory analyses. Sorting the berries by size will positively impact grape and thus wine quality.

Keywords: Berry size; wine grape; Papazkarası; autochthonous variety; secondary metabolites

1. Introduction

Berry size is one of the most important factors affecting grape and wine quality (Champagnol 1998; Gil et al. 2015; Melo et al. 2015). Significant differences in composition and development exist between the clusters

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on the vine (Nicolle et al. 2023) and between the berries within a cluster (Gouthu et al. 2014). The phenolic compounds in berry composition and their ratios are controlled by genetic structure, which is a characteristic of the species and variety (Cantürk and Kunter 2019). Additionally, it has been emphasized that the amount of these compounds is shaped by terroir factors (climate, soil, berry ripening stage, cultural practices, etc.) (Kennedy 2008; Kizildeniz et al. 2015; Shi et al. 2016; Foroni et al. 2017; Ribéreau-Gayon et al. 2021). Particularly in red grape varieties, attention should be paid not only to sugar content and acidity values during ripening but also to phenolic compounds and their extractability, as they directly affect the organoleptic properties of the wine (Ramos et al. 2024). Furthermore, along with phenolic compounds in the chemical composition of berries, organic acids also play a significant role in improving the sensory properties of wines depending on the degree of berry ripeness (Silva and Queiroz 2016).

Berry size has an impact on quality, though there are differing opinions on the matter. Matthews and Nuzzo (2007) reported that smaller berries are preferred because they have a higher surface-to-volume ratio, allowing for more compounds to transfer from the skin to the must, while larger berries are less favored due to dilution of their composition. However, Roby and Matthews (2004) argued that the skin-seed to pulp ratio does not change based on berry size, and since the skin grows along with the berry, the surface-to-volume ratio does not always determine the amount of soluble substances that can be extracted from the skin (Matthews and Nuzzo 2007; Barbagallo et al. 2011). They suggested that differences in berry size can lead to changes in composition, making it more challenging to control the ripening process (Barbagallo et al. 2011).

Grapes, which contain numerous secondary metabolites, are rich in polyphenols. The main polyphenols include anthocyanins, flavonols, and stilbenes-three classes of compounds that play a crucial role in vine metabolism and exhibit unique characteristics (Flamini et al. 2013). Among polyphenols, tannins are also present in both the seeds and skin (Ramos et al. 2024). The phenolic composition is greatly influenced by grape varieties, environmental conditions, and cultural practices (He and Giusti 2010). Arozarena et al. (2002) reported that the composition of anthocyanins in grape berries is controlled by genetic factors and varies according to variety. Zhang et al. (2021) emphasized that environmental factors such as temperature, sunlight, soil, and cultural practices affect these ratios. It has also been noted that temperature plays a crucial role in the formation of phenolic compounds (Spayd et al. 2002). However, the phenolic potential of grapes is mainly determined by the phenolic composition of the skin -anthocyanins, flavonols, and tannins- and the ability of these compounds to be extracted from the must (Schwarz et al. 2005; Casassa and Habertson 2014).

When the local cv. Papazkarası was grown at an altitude of 1030 m in Tunceli province, it was found that the total phenolic content in the skin and seeds was 21323 mg GAE kg^{-1}, and the total anthocyanin content was 816.1 mg kg^{-1} (Sanyürek et al. 2018). Özdemir (2017) reported that the Papazkarası grape variety from Kırklareli had a Titratable Acidity (TA) of 6.67 g L^{-1}, pH of 3.18, and Total Soluble Solids (TSS) of 19°Brix. Faikoğlu (2014) recorded that the TSS of the hardaliye (a traditional fermented beverage) made from the cv. Papazkarası was 5.2 g 100 mL^{-1}, pH 3.37, and TA 1.167 g 100 mL^{-1}. Using the cv. Papazkarası from five different vineyards, Erseç and Demirci (2023) produced wine via spontaneous fermentation and identified nine different strains through DNA sequencing.

Additionally, rootstocks influence grape quality by altering various physiological responses, such as managing the vigor of grafted scions, changing the hydraulic capacity of the root system, and controlling stomatal closure to reduce water loss during water stress (Gambetta et al. 2012; Marguerit et al. 2012). Besides contributing to disease control, rootstocks also affect grape quality by altering phytochemical components (Chen et al. 2024). Blank et al. (2022) stated that rootstocks are a powerful tool in managing grape phenolic components; for instance, the total tannin ratio of a variety grafted onto SO4 rootstock was 15% higher than that grafted onto Riparia gloire rootstock. However, Wang et al. (2019) found that rootstocks had little effect on the phenolic components of grapes. Different rootstocks have been shown to alter the growth and development of varieties, as well as their grape quality and resistance to stress factors (Ulaş et al. 2014; Ausari et al. 2024). Rootstocks grafted onto different grape varieties such as Syrah (Walker 2019), Cabernet Sauvignon (Miele and Rizzon 2019), and Pinot Noir (Harbertson and Keller 2012) have been suggested to cause changes in pH and TA content. Moreover, it has been found that the total phenolic content, total tannin, and flavan-3-ol accumulation of Cabernet Sauvignon grapevines grafted onto rootstocks such as SO4 and 1103P are affected

(Koundouras et al., 2009). Rootstocks can change the grape ripening rate and composition, such as TSS and TA (Stevens et al. 2008; Koundouras et al. 2009). Harbertson and Keller (2012) grafted Merlot, Syrah, and Chardonnay varieties onto five different rootstocks and reported that rootstocks did not affect anthocyanin and tannin levels. All measured grape and wine components, except TSS, changed significantly with the grafted variety.

Barbagallo et al. (2011) categorized grapes from the Syrah/99R graft combination into four groups based on their weight: 1. ≤1.50 g; 2. 1.51-2.00 g; 3. 2.01-2.50 g; 4. ≥2.50 g. As a result, they concluded that reducing variability in berry weight and size is important for improving wine quality. Melo et al. (2015) separated Syrah grape berries into three groups by diameter: small (<13 mm), medium (13-14 mm), and large (>14 mm), and found that smaller berries had a higher numerical density, while larger berries had a lower density. Chen et al. (2018) categorized Cabernet Sauvignon grape berries into three different size groups: small (0.75 g), medium (0.76-1.25 g), and large (>1.25 g), and reported that more than 50% of the berry population belonged to the medium group. They found that the physicochemical and biochemical parameters of the berries were significantly affected by berry size. Ünlüsoy (2019) determined that the 8mm-10mm berry size group of the Merlot grape variety had the highest TA, total phenolic content, total anthocyanin, total tannin, and total antioxidant values. They also stated that the best quality berry sizes were 8-10 mm and 10-12 mm.

The aim of this research is to reveal the changes in primary and secondary metabolites in the must of Papazkarası grape variety grafted onto three different rootstocks according to different berry size groups. Another goal is to determine which berry size should be used to improve the phytochemical composition of grape juice to enhance quality, based on the idea that achieving quality in the wine industry depends on grape quality.

2. Materials and Methods

2.1. Location and Plant Material

The trial was conducted in the vineyards of Irem Çamlıca Vineyards and Winery Ltd. Co. in Kırklareli (41°61'23.26"N, 27°61'89"E, 304 m altitude), where Papazkarası vines trained in the Double Cordon Royat trellising system were used as plant material. The 10-year-old Papazkarası vines were grafted onto 1103P, 110R, and 420A rootstocks. The planting distance was 2×1 m with 500 vines da^{-1}, and no irrigation or fertilization was applied.

The cv. Papazkarası is native to Türkiye. As an indigenous variety, it is commonly cultivated in the Thrace Region (Korkutal et al. 2019). It ripens in mid-October, its berries are medium-sized, and its clusters are compact. It is commonly used for both winemaking and table consumption. When processed into wine, it exhibits medium to low tannin content, high acidity, and high aroma. It is registered in the VIVC catalog under number 8923 as a hybrid of Alba Imputotato x Prokupac, originating from Türkiye (VIVC 2024).

The rootstocks used are: 1103P (Berlandieri Resseguier No:2 x Rupestris du Lot hybrid), 110R (Berlandieri Resseguier No:2 x Rupestris 110 Richter hybrid), and 420A (Berlandieri x Riparia 420A Millardet et de Grasset hybrid). The 1103P rootstock is highly resistant to the root form of phylloxera and shows 17% resistance to "active" limestone. It adapts well to dry conditions and is particularly suited to acidic soils, with fairly good tolerance to chlorides. The 110R rootstock is even more resistant to the root form of phylloxera. It also shows 17% resistance to "active" limestone and adapts very well to drought. It is especially suitable for dry, poor, stony, and schist soils. The 420A rootstock, like the other rootstocks, is highly resistant to the root form of phylloxera, with 20% resistance to "active" limestone. This rootstock is productive and well-adapted to deep, clay-limestone soils, but it has a low capacity for absorbing K from the soil (Plantgrape 2024).

2.2. Trial Design and Statistical Analysis

The trial, established with 144 vines in a Randomized Block Design, involved examining a total of 1440 clusters and 7200 berries across three rootstock groups, with 480 clusters from each rootstock. Berries were

grouped by diameter using sieves. A control sample was created selecting berries from each size group, resulting in six berry size groups:

- E0 (Control): Mixed-sized berries,
- E1: Berries with a diameter less than Ø 12 mm,
- E2: Berries with a diameter between Ø 12.01-14.00 mm,
- E3: Berries with a diameter between Ø 14.01-16.00 mm,
- E4: Berries with a diameter between Ø 16.01-18.00 mm,
- E5: Berries with a diameter ≥ 18.01 mm.

The measurement results of grape berry samples collected at harvest were analyzed using the MSTAT-C statistical software package, and the differences between berry sizes and rootstocks were determined using the Least Significant Difference (LSD) test.

2.3. Analysis of Primer and Seconder Metabolites

Changes in Total Soluble Solids (TSS), Titratable Acidity (TA), and pH were monitored between veraison and harvest. Basic must analyses were performed on berry samples taken from each size group, including the control. The TSS (°Brix) of the juice from the six size groups was measured using a hand refractometer, the alcohol content (%) was determined using the Blouin and Guimberteau (2000) method, the TA value (g-tartaric acid L^{-1}) was determined using the titrimetric method, and the pH was determined according to Cemeroğlu (2015). Maturity Indices, such as °Brix/TA and pH^2×°Brix, were calculated according to Blouin and Guimberteau (2000).

From each size group, 100 berry samples were taken to determine anthocyanin, tannin, total phenolic content, total polyphenol index (TPI), etc. To determine the total anthocyanin content (mg kg^{-1}) from secondary metabolites, a puree of crushed grape berries was transferred into glass bottles (100ml), and an acidified methanol solution (2% HCl) was added to make up the volume to 100ml. After being left in the dark for one day, the extract was filtered, transferred into plastic tubes, and stored in a deep freezer (-18°C). Total monomeric anthocyanins were determined using the pH Differential Method. In this method, the difference between absorbance values measured at pH 1.0 and pH 4.5 is directly proportional to anthocyanin concentration. pH 1.0 (0.025 M KCl buffer solution) and pH 4.5 (0.4 M NaOAc buffer solution) were used as buffers. Absorbance readings were taken with a spectrophotometer at wavelengths of 520-720 nm. To determine the total phenolic content (mg kg^{-1}), 75 ml of distilled water + 1ml of extract was added to a volumetric flask. After adding 5 ml of Folin-Ciocalteu reagent and shaking, the mixture was left to stand for 3 min, followed by the addition of 10 ml of saturated Na_2CO_3 solution and 9ml of distilled water, making up the volume to 100 ml. After 60 min, the absorbance was read at 720nm using a spectrophotometer (Cemeroğlu 2015). To determine the total tannin content (mg kg^{-1}), 5ml of methanol + 1ml of extract was added. From this solution, 100 µl was taken, 500 µl of Folin-Ciocalteu reagent was added, followed by 1ml of Na_2CO_3, and the volume was made up to 10 ml with distilled water, with absorbance readings taken at 750 nm using a spectrophotometer (Mohammed and Manan 2015). To determine the Total Polyphenol Index (TPI), 1ml of must was diluted with 50 ml of distilled water, centrifuged at 8000 rpm for 10 min, and absorbance was read at 280 nm using a spectrophotometer. The dilution factor was multiplied by the absorbance value for the final calculation (INRA 2007).

3. Results and Discussion

3.1. Grouping of Berries According to Diameter (berries)

The berries from the clusters of each rootstock group were separated according to diameter (Figure 1). Accordingly, the number of berries in clusters from the 1103P rootstock was concentrated in the E3 group (42.63 berries), with the fewest berries found in the E1 (5.83 berries) and E5 (8.48 berries) groups. For the 110R

rootstock, the highest number of berries was in the E3 group (40.77 berries), and the lowest number was in the E5 group (3.23 berries). For the 420A rootstock, the group with the highest number of berries was E3 (51.25 berries), although the E2 group (40.54 berries) also had a high berry count. The E5 group (2.50 berries) had the lowest value.

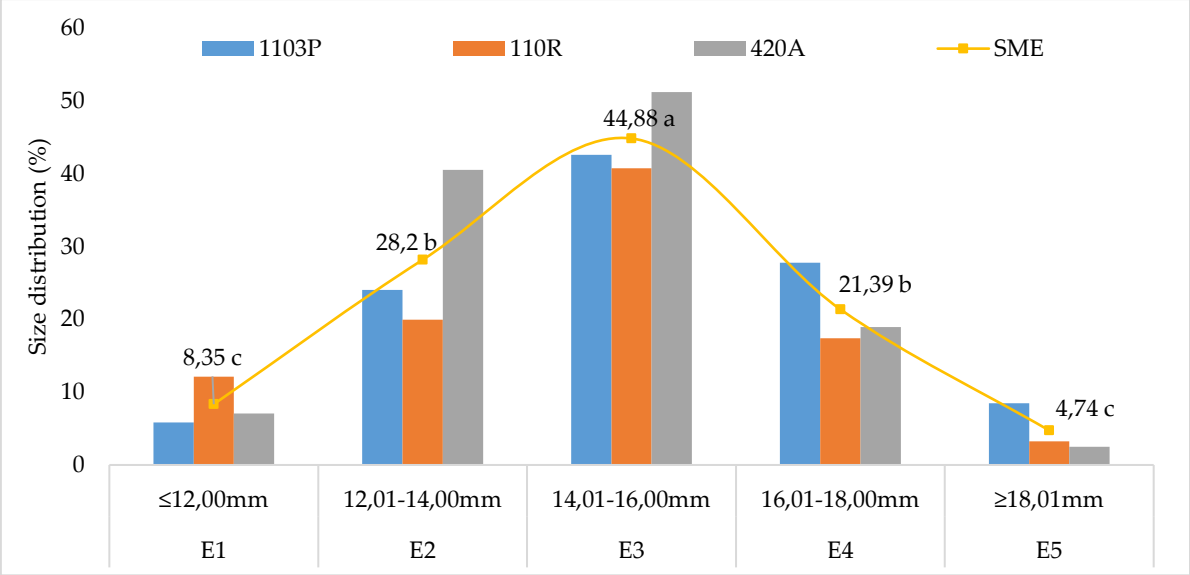

Figure 1. Size distribution of berries in the cluster [RME=Rootstock Main Effect, SME=Size Main Effect]

(RME $LSD_{0.01}$ = 8.66; SME $LSD_{0.01}$ = 5.74; Rootstock x Size Interaction $LSD_{0.01}$ = 4.39)

When examining the size distribution of the berries, the 1103P rootstock was found to be more effective in larger berries, while the 420A rootstock was more effective in smaller berries. This difference is thought to be due to the timing of pollination (Pisciotta et al. 2012; Tarter and Keuter 2005).

3.2. Proportional Distribution of Berry Size Groups (%)

In all rootstocks, berry sizes were concentrated in the E3 group. The proportion of the E2 size group in the 420A rootstock (33.09%) was higher than the E3 size group in the other rootstocks. In the 1103P rootstock, the proportion of the E4 size group (25.51%) was higher than that of the other rootstocks. Overall, 85-90% of the berries in the clusters fell within the 12.01-18.00 mm (E2, E3, and E4) size range (Figure 2).

3.3. Changes in Must Composition Between Veraison and Harvest

For wine grape varieties to reach harvest maturity, the desired values for TSS (20-25°Brix), pH (3.2-3.5), and TA (3-9 g L^{-1}) are considered (Blouin and Guimberteau 2000). The TSS value obtained from the study was 20.41°Brix, and the TA value was 7.13 g L^{-1}, which fall within the specified ranges. In summary, as expected, TSS increased and TA decreased by harvest time. However, the pH value remained almost unchanged between days of the year (DOY) 252 and 283, with a pH of 2.81 at harvest (Figure 3).

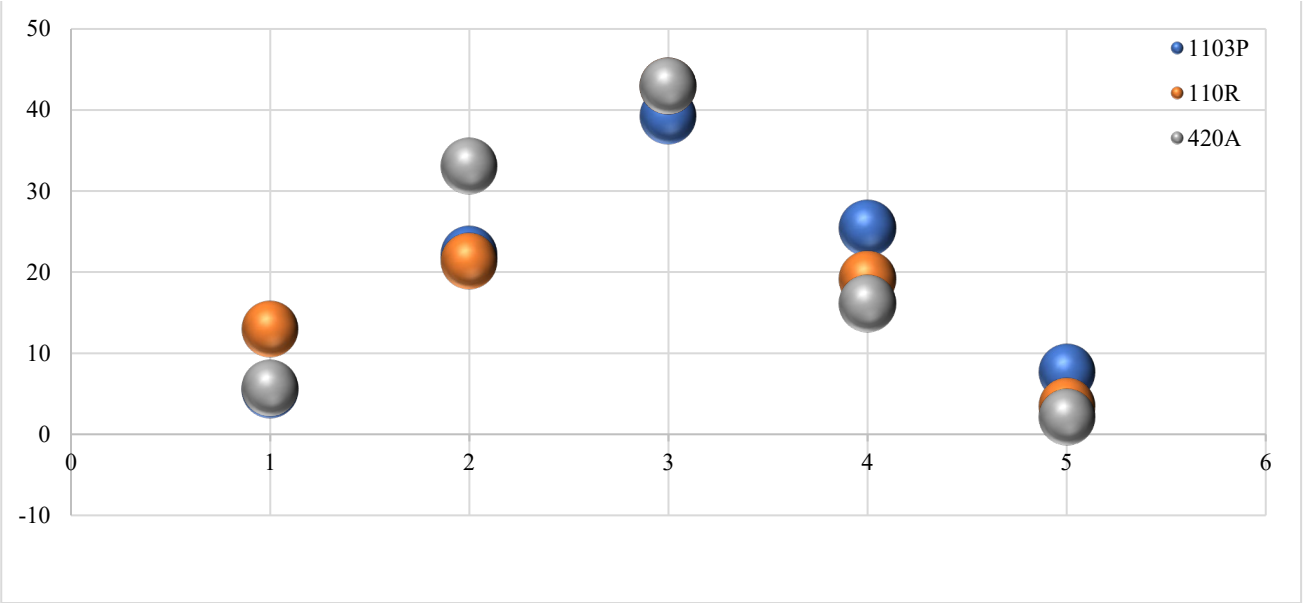

Figure 2. Proportional distribution of berry sizes in 100 berries

(SME $LSD_{0,01}$=10,71; Rootstock x Size Interaction $LSD_{0,01}$=8,18)

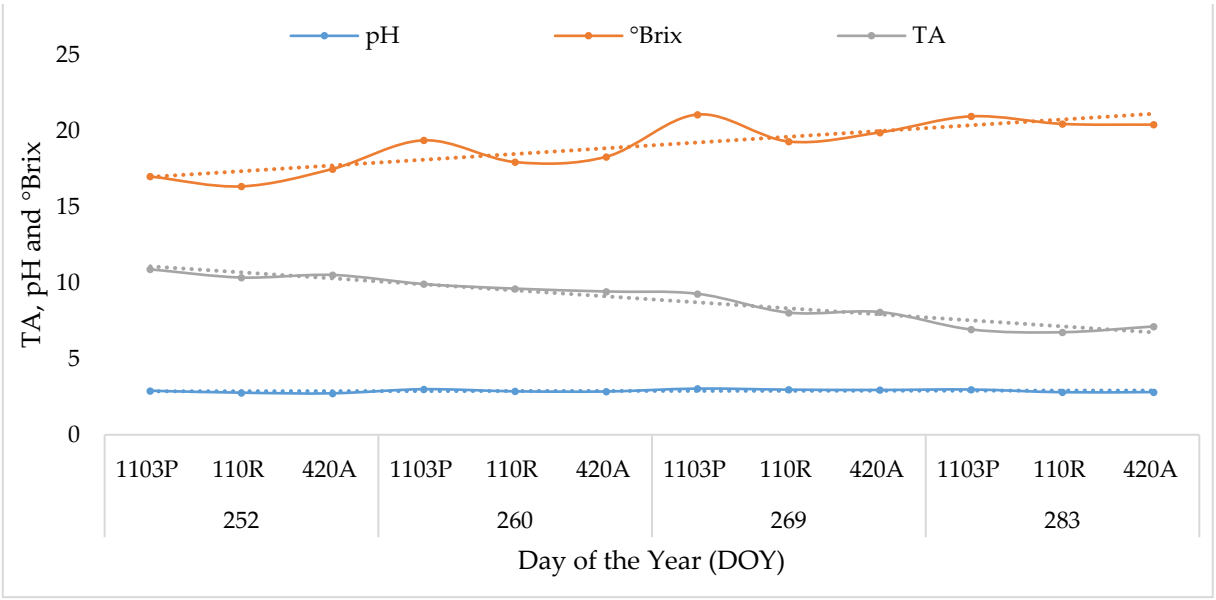

Figure 3. Weekly changes in must composition between veraison and harvest

3.4. Total Soluble Solids (TSS) (°Brix)

The factor that statistically significantly affected the TSS value in the cv. Papazkarası was the Size Main Effect (SME). When berry size was E5 (≥18.00 mm), the TSS value reached 21.76°Brix, and it was found that only this value was very close to the optimal range for wine grape varieties, 22-24°Brix (Cox 2015), while other berry sizes did not reach the optimum value (Table 1). The finding from Ünlüsoy (2019) that the 14-16mm berry size group in the Merlot/1103P combination gave the highest TSS value (21.51°Brix) aligns with the results of this study. Additionally, the TSS value of cv. Papazkarası grafted onto the 110R rootstock in the 2015 harvest season was found to be 19.00°Brix (Özdemir 2017), which is very close to the results of this study (20.46°Brix). Moreover, the findings are similar to those of Barbagallo et al. (2011), who reported a positive correlation between berry size and TSS. However, the findings contradict those of Chen et al. (2018) and Melo et al. (2015), who found a negative correlation between berry size and TSS. This difference is thought to be due

to factors such as vineyard location, grape variety, etc. The finding that the E4 and E5 groups had higher TSS values than the control is also consistent with Bahar et al. (2024). The Rootstock Main Effect (RME) was not statistically significant, and these values were quite close. This finding contradicts the results of Korkutal et al. (2023), who stated that rootstocks affect TSS values, and this discrepancy is thought to be due to the characteristics of the location where the research was conducted.

Table 1. TSS, Alcohol, TA, pH, °Brix/TA, and pH²×°Brix Values

	Rootstock	E0 (K)	E1	E2	E3	E4	E5	RME
TSS (°Brix)	1103P	20.78	20.30	20.40	20.68	21.13	22.45	20.96
	110R	20.73	19.90	20.18	20.40	20.48	21.10	20.46
	420A	20.18	19.70	20.20	20.25	20.38	21.73	20.41
SME		20.56 b	19.97 b	20.26 b	20.44 b	20.66 b	21.76 a	
SME $LSD_{0.01}$ = 1.91								
Alcohol (%)	1103P	11.94	11.60	11.67	11.86	12.17	13.08	12.05
	110R	11.90	11.34	11.52	11.67	11.73	12.15	11.72
	420A	11.52	11.21	11.54	11.57	11.65	12.58	11.68
SME		11.79	11.38	11.58	11.70	11.85	12.60	
NS								
TA (g L^{-1})	1103P	8.12	7.09	6.90	6.81	6.54	6,11	6.93
	110R	6.79	7.44	7.13	6.90	6.42	5.83	6.75
	420A	6.43	8.29	7.58	7.40	7.22	5.84	7.13
SME		7.11 a	7.61 a	7.20 a	7.04 a	6.73 ab	5.93 b	
SME $LSD_{0.01}$=2.67								
pH	1103P	3.01	2.77	2.86	2.95	3.11	3.17	2.98 a
	110R	2.78	2.68	2.78	2.81	2.87	2.89	2.80 b
	420A	2.73	2.78	2.75	2.79	2.86	2.94	2.81 ab
SME		2.84 ab	2.74 b	2.80 b	2.85 ab	2.94 ab	3.00 a	
RME $LSD_{0.01}$=0.38; SME $LSD_{0.01}$=0.38								
°Brix/TA	1103P	2.56	2.86	2.96	3.04	3.21	3.67	3.05
	110R	3.05	2.67	2.83	2.96	3.19	3.62	3.05
	420A	3.14	2.38	2.66	2.74	2.82	3.72	2.91
SME		2.92 b	2.64 b	2.82 b	2.91 b	3.07 ab	3.67 a	
SME $LSD_{0.01}$= 1.05								
pH²×°Brix	1103P	187.52	155.48	166.57	180.23	202.79	225.24	186.30 a
	110R	159.60	142.40	155.64	161.37	168.36	175.92	160.55 b
	420A	149.81	152.25	153.04	157.31	166.37	187.53	161.05 ab
SME		165.64 ab	150.04 b	158.42 b	166.30 ab	179.17 ab	196.23 a	
RME $LSD_{0.01}$=51.99; SME $LSD_{0.01}$=67.76								

3.5. % Alcohol

In the cv. Papazkarası, the effects of the Rootstock Main Effect (RME) and the berry Size Main Effect (SME) on alcohol percentage were not statistically significant (Table 1). It is known that as the TSS in grapes increases, the alcohol level in wine will also increase to a certain level (Feifel et al. 2024). In the study, a numerically high alcohol value was observed in the 1103P x E5 interaction (13.08%), which was thought to be due to the 22.45°Brix berry size of E5. This value was determined to be low based on Cox's (2015) finding that wine grape varieties should have a 22-24°Brix value. Other size groups did not reach the desired alcohol level. For

producing high-quality wine, the stage with the highest sugar content and consequently the highest alcohol level is the harvest stage (Conde et al., 2007). However, the findings of the study revealed that the desired full maturity was not achieved at harvest, contrary to the researchers' expectations. On the other hand, similar to the findings of Ausari et al. (2024), it was concluded that the effects of rootstocks on alcohol content were not statistically significant.

3.6. Titratable Acidity (TA) (g-tartaric acid L^{-1})

In terms of titratable acidity (TA), the berry Size Main Effect (SME, $LSD_{0.01}$) was statistically significant (Table 1). The highest TA values were obtained from berry sizes E0, E1, E2, and E3 (7.04-7.61 g L^{-1}). This was followed by E4 with 6.73 g L^{-1}. However, the finding from Cox (2015) that this value should be 0.60-0.8g mL^{-1} for red wine grape varieties does not match with the E5 (5.93 g L^{-1}) size. On the other hand, it was observed that TA decreases as berry size increases. This finding was found to be consistent with the results reported by Chen et al. (2018) and Ünlüsoy (2019), which indicate that smaller berries have higher TA values. However, the results contradict the findings of Melo et al. (2015), who reported that TA is not affected by berry size. This difference is thought to be due to varietal differences. Additionally, the finding that the TA value of Papazkarası/110R vines in the vineyard where the study was conducted was 6.67 g L^{-1} in the 2015 harvest is similar to the study results (6.75 g L^{-1}) of Özdemir (2017).

3.7. pH

In the cv. Papazkarası, both the RME and the SME were found to be significant (Table 1). When examining the effect of rootstocks, the highest pH value was found in the 1103P rootstock (2.98), followed by the 420A rootstock (2.81) and the 110R rootstock (2.80). The pH value of 2.80 recorded for the 110R rootstock of the Papazkarası grape variety in 2021 from the same vineyard is thought to differ from the 3.18 pH value reported by Özdemir (2017) in 2015 due to differences in the trial years. According to the SME, the E5 size had the highest pH value (3.00). E0, E3, and E4 sizes were in the same significance group, while E1 (2.74) and E2 (2.80) sizes were in the last significance group. In general, there is a positive correlation between berry size increase and pH increase (Chen et al. 2018). However, the study did not reach Cox's (2015) finding that the optimum pH value for red varieties is 3.4. Since higher pH values can negatively affect wine quality by promoting unwanted bacterial growth, as reported by Cox, it is thought that the obtained values will not negatively impact quality.

3.8. Maturity Indices

°Brix/TA

The berry Size Main Effect was significant for °Brix/TA (Table 1). In terms of SME, the E5 group (3.67) stood out. It was found that the E4 group (3.07) followed the E5 group, and the other berry size groups were in the last significance group. Dardeniz and Kısmalı (2002) provided guidance on measures to be taken in regions with ripening issues, based on their finding that they achieved 5-9 days earlier ripening with 30-60% cluster thinning.

pH^2x°Brix

Among the ripening indices, both RME and SME were found to be significant for pH^2×°Brix (Table 1). Among the rootstocks, 1103P had the highest value (186.30), 110R had the lowest (160.55), and 420A was between the two (161.05). The E5 size group (196.23) had the highest value, while E0, E3, and E4 (165.64-179.17) sizes were in the middle, and E1 and E2 (150.04-158.42) had the lowest values. Contrary to Blouin and Guimberteau's (2000) definition that full ripeness is achieved when this index exceeds 260°Brix, it can be said that none of the berry size groups or rootstocks reached full ripeness.

3.9. Secondary Metabolites

Total Anthocyanin Content (mg kg^{-1})

Total anthocyanin content was influenced by both rootstock and berry size (Table 2). The 110R rootstock had the highest total anthocyanin content (852.70 mg kg^{-1}), followed by the 420A rootstock (744.88 mg kg^{-1}),

with the 1103P rootstock (565.43 mg kg^{-1}) in the last group. This finding is similar to Blank et al. (2022), who reported that the 110R rootstock had a higher anthocyanin concentration than the 125AA rootstock. The lowest total anthocyanin content was found in berries of size E5 (535.94 mg kg^{-1}), while the highest value was obtained from berries of size E1 (973.30 mg kg^{-1}). There was no difference between the control (E0), E3, and E4 berry sizes. The finding that small berries (<13 mm) had higher anthocyanin values than other sizes is consistent with Melo et al. (2015) and Ünlüsoy (2019). When the total anthocyanin content in grape berry skin is expressed in mg kg^{-1}, Barbagallo et al. (2011) stated that increasing berry weight reduces anthocyanin levels. The findings of this study are similar; as berry size increased (≥18.01 mm = 535.94 mg kg^{-1}), the anthocyanin content decreased. Bahar and Kurt (2015) reported that increasing the number of berries per cluster and reducing berry size led to an increase in anthocyanin content. A similar finding was obtained in this study, as smaller berries (12.00 mm, 12.01–14.00 mm, and 14.01–16.00 mm) had higher anthocyanin levels. Sanyürek et al. (2018) determined that the total anthocyanin content of the Papazkarası grape variety grown under Tunceli conditions was 816.1 mg kg^{-1}. This finding was found to be nearly identical to the anthocyanin content of E2-sized berries in the study (833.92 mg kg^{-1}).

Table 2. Total anthocyanins, Total phenolic content, Total tannins, and TPI values

	Rootstock	E0 (K)	E1	E2	E3	E4	E5	RME
T. Anthocyanins (mg kg^{-1})	1103P	548.20	828.11	680.61	550.53	444.84	340.30	565.43 b
	110R	731.33	1145.58	1025.95	871.47	717.39	624.47	852.70 a
	420A	658.54	946.19	795.21	780.11	646.15	643.06	744.88 ab
SME		646.02 bc	973.30a	833.92 ab	734.04 bc	602.79 bc	535.94 c	
SME $LSD_{0.01}$=3.99; RME $LSD_{0.01}$=3.99								
T. Phenolic Content (mg kg^{-1})	1103P	5092.98	9512.10	6823.64	6473.71	4773.05	3110.38	5964.31 b
	110R	6867.63	10514.90	9050.19	6617.34	6801.97	6328.73	7696.79 a
	420A	5666.87	8848.23	7422.52	5609.88	5331.93	4663.07	6257.08ab
SME		5875.82 bc	9625.07 a	7765.45 b	6233.64 bc	5635.65 bc	4700.73 c	
SME $LSD_{0.01}$=3.57; RME $LSD_{0.01}$=3.57								
T. Tannins (mg kg^{-1})	1103P	3400.1421	4844.7273	3992.7351	3085.9560	3830.6106	3022.2243	3696.07
	110R	3439.2756	3624.8802	4077.7107	3091.5465	2334.5928	1990.2180	3093.04
	420A	2988.6813	4031.8686	3950.2473	3334.1742	2993.1537	2900.3514	3366.41
SME		3276.03 abc	4167.16 a	4006.90 ab	3170.56 ab	3052.79 c	2637.60 c	
SME $LSD_{0.05}$=1.59								
TPI	1103P	12.87	23.68	14.53	13.45	11.60	8.25	14.06 b
	110R	13.80	25.76	18.42	16.16	15.03	9.58	16.46 a
	420A	14.38	22.21	15.80	12.23	13.46	11.31	14.90 ab
SME		13.68 bc	23.88 a	16.25 b	13.94 b	13.37 bc	9.71 c	
RME $LSD_{0.01}$=4.30; SME $LSD_{0.01}$=8.94								

Total Phenolic Content (mg kg^{-1})

The Rootstock Main Effect was statistically significant, and the 110R rootstock had the highest total phenolic content (7696.79 mg kg^{-1}) (Table 2). This was followed by the 420A and 1103P rootstocks. According to SME, the phenolic content was also statistically significant. The highest total phenolic content was found in E1 size berries (9625.07 mg kg^{-1}). The lowest amount was recorded in the E5 size group (4700.73 mg kg^{-1}), while no difference was found between the E3 and E4 size groups. This finding aligns with Ünlüsoy (2019), who reported the highest phenolic content in the smallest berry size group (8–10 mm). Similarly, in this study, the berry size group E5 had the lowest phenolic content. However, the results contradict the findings of Melo et al. (2015), who reported that berry size did not affect phenolic content. This discrepancy is thought to be due to differences in rootstock, variety, and location.

Total Tannin Content (mg kg^{-1})

Berry size significantly affected total tannin content in the cv. Papazkarası (Table 2). The highest total tannin content was found in the smallest berry size group (E1=4167.16 mg kg^{-1}). No difference was found between E2, E3, E4, and E5 berry sizes in terms of tannin content. As expected, the E0 size (3276.03 mg kg^{-1}) had a total tannin content value between E2, E3, E4, and E5. The smallest tannin values were found in the E4 (3052.79 mg kg^{-1}) and E5 (2637.60 mg kg^{-1}) berry sizes. The findings are consistent with Ünlüsoy (2019). In short, as berry size decreased, tannin content increased. The finding that the tannin content in the 110R rootstock was lower than in other rootstocks is consistent with the findings of Ausari et al. (2024).

Total Polyphenol Index (TPI)

Berry size and rootstock types had significant effects on the Total Polyphenol Index (TPI), which indicates the degree of phenolic maturity in grapes (Table 2). The 110R rootstock had the highest TPI value (16.46), while the 1103P rootstock had the lowest (14.06). The 420A rootstock had a value between these two rootstocks (14.90). According to the SME, E1-sized berries had the highest TPI value (23.88), while the E5 size had the lowest TPI value (9.71). The other berry size groups and the control group were between these two values.

4. Conclusions

The three different rootstocks onto which the cv. Papazkarası was grafted were generally insufficient in terms of the criteria examined in this terroir and ripeness. The 1103P rootstock had a high °Brix value, reduced titratable acidity, increased pH, and achieved the highest alcohol content, but it did not reach the desired level. The pH^2×°Brix should be 260, but it was 186.03, indicating that full ripeness was not achieved. The 1103P rootstock exhibited higher total tannin content compared to the other rootstocks. It should be noted that a high total tannin content can result in an astringent taste in the wine. The 110R rootstock had moderate sugar accumulation, low pH, and TA values, and the alcohol content was also recorded as moderate. This rootstock had the lowest ripeness index value. Full ripeness was not achieved with this rootstock either, and small and late-ripening berries negatively affected the quality of the must. Although small berries increased phenolic components, they reduced the total tannin content. The 420A rootstock, despite having the lowest sugar content, did not show a significant difference compared to the other rootstocks. Due to the sugar accumulation, the pH value was low, and the acid content was high. Although this rootstock did not reach the required level of ripeness, its homogeneous berries show promise in terms of must quality. However, studies with different rootstocks need to be continued.

For all rootstocks, the highest number of berries was observed in the size range of 12.01 mm to 18.00 mm (E2, E3, E4, and E0). Although these berries were not of high quality, they are considered suitable for winemaking. The other size groups, E1 and E5 (≤12.00 mm and ≥18.00 mm), were rich in primary and secondary metabolites but did not reach sufficient berry numbers. The highest levels of anthocyanins and phenolic compounds were recorded in berries sized ≤12.00 mm. These findings suggest that reducing berry size could improve quality. It is believed that sensory evaluation of wines produced from the berry size groups that improve must quality will provide more definitive results.

To enhance the phytochemical composition, efforts should be made to reduce berry sizes through various techniques and to increase ripeness, focusing on methods that raise alcohol content. Sorting berries by size manually is a long and costly process but using mechanization could speed up this process and reduce costs. It is anticipated that this method will be beneficial in improving grape and, consequently, wine quality.

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