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Effects of Different Water Stress Levels, Heterogeneity, and Location on Berry Phytochemical Properties in an Organic and Conventional Vineyard (*Vitis vinifera* cv. Cabernet-Sauvignon)

Elman BAHAR[1], İlknur KORKUTAL[2]✉, Müge UZUN[3]
[1, 2, 3] Tekirdag Namik Kemal University, Agricultural Faculty, Department of Horticulture, Tekirdag-Turkiye
[1]https://orcid.org/0000-0002-8842-7695, [2]https://orcid.org/0000-0002-8016-9804, [3]https://orcid.org/0009-0006-0245-0226
✉: ikorkutal@nku.edu.tr

ABSTRACT

Investigate the effects of water stress on grape berry heterogeneity and composition in Cabernet-Sauvignon (*Vitis vinifera* L.) grapes under different farming practices (organic and conventional) based on soil structure and provide valuable information for the wine industry regarding quality. The research was conducted in two vineyards, one certified organic and the other following conventional practices. The experiment, designed with Split-Plot Experimental Design based on stress levels, was divided into two main plots, Organic and Conventional, and each of these plots was further divided into two subplots. The predawn leaf water potential results categorized the vines into two groups: those with values lower than -8 MPa and those above -8 MPa, which were labeled as Dryland-shallow soil and Baseland-deep soil, respectively, based on the location and soil type. During the harvest, grape clusters were collected and classified into three groups based on berry diameter (10mm-12mm, 12mm-14mm, 14mm-16mm). The results indicated that the 10mm-12mm berry size group generally exhibited the desired characteristics across all evaluated criteria. The total anthocyanin and total tannin content were higher in the 10mm-12mm berries from vines experiencing moderate stress (Stress 1), regardless of location. Additionally, the Dryland-shallow soil condition showed higher tannin content. On the other hand, grapes from high-stress vines displayed lower antioxidant values. The total polyphenol index content was higher in the organic vineyard. Based on the findings, it was suggested that to obtain high phytochemical compounds from Cabernet-Sauvignon grapes in the Tekirdağ region, cultivation should be carried out under Dryland-shallow soil conditions, where the predawn leaf water potential can drop as low as -0.8 MPa during the period between veraison and harvest. Moreover, berries between 10 mm and 12 mm might suit for this purpose.

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Organik ve Konvansiyonel Bağda Yetiştirilen *Vitis vinifera* Cabernet-Sauvignon Üzüm Çeşidinde; Farklı Su Stresi Seviyelerinin, Tane Heterojenitesinin ve Konumun Fitokimyasal Özellikler Üzerine Etkileri

ÖZET

Cabernet-Sauvignon (*Vitis vinifera* L.) üzüm çeşidinde farklı tarım uygulamalarındaki (organik ve konvansiyonel) toprak yapısına bağlı olarak su stresinin tane heterojenitesi ve bileşimi üzerine etkilerini araştırmak ve şarap sektörüne ham madde kalitesi konusunda öncü bilgi sağlamaktır. Araştırma organik tarım sertifikalı ve konvansiyonel bağcılık yapılan iki bağda yürütülmüştür. Stres düzeylerine göre Bölünmüş Parseller Deneme Desenine göre kurulmuş olan deneme, Organik ve Konvansiyonel olarak iki ana ve ikişer alt parsele ayrılmıştır. Ölçülen şafak öncesi yaprak su potansiyeli sonuçlarına göre -8 MPa'dan düşük olan ve -8 MPa'dan büyük olan omcalar, arazi ve toprak tipine göre Kıraç arazi-yüzlek toprak ve Taban arazi-derin toprak olarak gruplandırılmıştır. Hasat yapılan salkımlardaki taneler çaplarına göre 3 ayrı grupta toplanmıştır (10mm-12mm, 12mm-14mm,

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14mm-16mm). Deneme sonucunda 10mm-12mm tane boyut grubunun genel olarak incelenen tüm kriterlerde istenilen özellikleri taşıdığı belirlenmiştir. Toplam antosiyanin miktarı ve toplam tanen miktarı konumdan bağımsız olarak orta stresteki (Stres 1) omcalarda 10mm-12mm arasındaki tanelerde yüksek değerler elde edilmiştir. Kıraç arazi-yüzlek toprakta tanen miktarı daha fazla bulunmuştur. Yüksek stresteki omcalardan düşük antioksidan değerleri kaydedilmiştir. TPI miktarı organik bağda daha yüksek bulunmuştur. Tekirdağ ilinde Cabernet-Sauvignon üzüm çeşidinden yüksek fitokimyasal bileşenler elde edilmesi için ben düşme-olgunluk arası dönemde şafak öncesi yaprak su potansiyelinin -0,8 MPa'a kadar düşebildiği Kıraç arazi-yüzlek toprak koşullarında yetiştiricilik yapılması ve 10mm-12mm arasında çapa sahip tanelerin kullanılmasının uygun olabileceği düşünülmüştür.

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INTRODUCTION

Grape quality is a general term that represents the levels of various fruit chemical compounds within a grape berry. These are commonly categorized as fruit flavonoids (anthocyanins, tannins, and total phenolics), titratable acidity (TA), pH, and total soluble solids (TSS). The concentrations of these components are determined by the combined effects of various factors in the vineyard. These factors include plant-related characteristics (vine, berry, and canopy), physicochemical properties of the soil in the root zone, and mesoclimate-microclimate attributes of the vineyard. The interactions among these factors must be considered because they contribute to the desired levels of grape chemical components known as "grape quality" (Zerihun et al., 2015). Additionally, Kontoudakis et al. (2011) stated that the most crucial factor at harvest time is the degree of grape maturity. It has been observed that high-quality wines come from grapes with optimal ripeness.

Along with vineyard soil, climate, vine, and cultural practices also affect the vineyard's performance and grape berry composition (Deloire & Rogiers, 2014; Candar et al., 2021). Even in regions with sufficient annual rainfall, irrigation may be necessary depending on soil structure (Tardáguila et al., 2011). It should be considered that some plots in the same vineyard may require irrigation, leading to differences in yield, quality, and grape heterogeneity. In red varieties and under dry conditions, vine water status has been found to have positive and negative effects on TSS, total acidity, pH, malic and tartaric acid concentrations, phenolic compounds, anthocyanins, and tannins (van Leeuwen et al., 2009; Cheng et al., 2014).

Organic viticulture is becoming widespread worldwide (Calderone et al., 2022). However, it has been reported that organic vineyards increased soil compaction, inability to replenish deficient nutrients, and increased disease pressure (Provost & Pedneault, 2016).

Berry size is a quality factor for wine production (Melo et al., 2015). Kontoudakis et al. (2011) found that when grouping berry heterogeneity based on berry density (NaCl solution), the group with the highest density had higher levels of pH, total phenolic content, total anthocyanins, and proanthocyanins. Zouid et al. (2013) reported a negative correlation between berry size and anthocyanin content, while Liu et al. (2016) found that the group with the highest density had the highest antioxidant content. Rolle et al. (2015) determined that high-density groups had higher total phenolic content and aromatic profile.

Temperature, drought, and light intensity determine the veraison process and affect the polyphenol content in the berries (Vilanova et al., 2015). Due to soil properties and spatial variations in topography, all soils do not retain water to the same extent. If irrigation is applied without considering these differences, it can reduce yield and quality in areas with excessive water stress. Similarly, in over-irrigated areas, the desired grape quality may not be achieved (Bellvert et al., 2021; Valdés et al., 2022). Echeverria et al. (2017) found that shallow vineyard soils have limited water access, leading to low yield and quality. Mirás-Avalos and Intriglio (2017) found that the variety, timing of water stress, and must composition have a significant impact, while Caruso et al. (2023) determined that different rootstocks and irrigation regimes did not affect yield, despite climate variations in trial years.

This study aims to observe the effects of water stress on grape berry heterogeneity and composition due to long-term exposure to high water stress in some plots of an organic vineyard, by grouping the grapes based on leaf water potentials at harvest and their berry sizes. As a control, the study also aims to monitor the effects of water stress resulting from the difference in water stress between Dryland-shallow soil and Baseland-deep soil, by classifying the grape berry sizes based on the average leaf water potentials of two different soil depths within a conventional vineyard. The main focus is to investigate the effects of water stress on grape berry heterogeneity and composition in Cabernet-Sauvignon (*Vitis vinifera* L.) grapes under different farming practices (organic and conventional) based on soil structure and provide valuable information for the wine industry regarding quality.

MATERIAL and METHODS

Vineyard site and climate

This study was conducted with the berries of cv. Cabernet-Sauvignon, grown in two different vineyards: ŞatoNuzun Vineyard and Winery Llc. was organic (41° 2' 20.74" N, 27° 48' 41.90" E) and Umurbey Vineyards Llc. Was conventional (40° 55' 50.23" N, 27° 25' 19.16" E) selected as the "Control" vineyard. During the period from veraison (EL 35) to harvest (EL 38), only 16 mm of rain has fallen. The average temperature during this period was 25.2°C, and the average relative humidity was 71.5%. The Index Winkler (IW) value for this region was recorded as 2235 days.

The phenological development dates for the first vineyard were recorded as follows: bud break on 15th April (EL 4), flowering on 25th May (EL 23), veraison on 24th July (EL 35), and harvest on 31st August (EL 38). As for the second vineyard (Control vineyard), the dates were as follows: bud break on 10th April (EL 4), flowering on 28th May (EL 23), veraison on 26th July (EL 35), and harvest on 17th September 2018 (EL 38).

Vineyard management

The organic vineyard, planted in 2006, consists of Cabernet-Sauvignon grafted on 1103P rootstock. It is oriented N-S and located at 130 m altitude with an 18% slope. Row and vine spacing is 2 x 2.5 m. The vine training system is VSP, and the soil composition includes gravel, sand, and clay. The conventional vineyard was planted in 1993, and the row spacing and vine spacing are 1.5 x 2.5 m. It is located 5 km from the sea at an altitude of 200 m. The vineyard is trained using the bilateral Cordon Royat system, and the soil composition is clayey and sandy. In winter pruning, 2 buds above 5 heads are left on each main branch, in short, each spur carries 10 buds, a total of 20 buds per vine. No cluster thinning process has been performed on the spurs. Both vineyards on sloped terrain, exhibit soil structure differences between the S and N-facing slopes. The N slope has a gravel + sand + limestone topsoil layer with low organic matter, leading to dry soil. Water retention is poor due to shallow soil tillage and an impermeable limestone layer, resulting in high evaporation rates and significant water scarcity. Conversely, the S slope has a fertile top layer rich in clay + sand and organic matter, with a lower layer consisting of gravel + sand + clay, providing high water holding capacity.

Berry sampling

Shoot and cluster numbers were not equalized in the application clusters. Harvested clusters from both vineyards were placed in plastic coolers and quickly transported to the laboratory. The clusters were divided into four berry size groups: 10mm-12mm, 12mm-14mm, 14mm-16mm, and 16mm-18mm. The berries, classified according to their sizes, were stored at -20°C until the analyses were performed. However, it was impossible to find samples belonging to the 16mm-18mm group in some criteria.

Measuring by Scholander Pressure Chamber

At harvest time, pre-dawn leaf water potential (Ψ_{leaf}) was measured using the Scholander pressure chamber, and stress levels were determined according to Carbonneau (1998) and Deloire & Rogiers (2014).

Location and soil types

Dryland – Shallow soil (D): Refers to the vines in the region with arid and highly absorbent, gravelly soil, Baseland – Deep soil (B): Includes vines in the area with high clay content and deep subsoil.

Stress levels

Conventional vineyard (Control): Represents the vineyard cultivated using traditional methods, Organic vineyard (Stress 1): Vines with low pre-dawn leaf water potential were grouped, Organic vineyard (Stress 2): Vines were grouped based on high Ψ_{leaf} values.

Statistical evaluation

The field experiment was conducted in a Split-Plot Design with two types of land (Dryland-shallow soil and Baseland-deep soil), three different stress levels (Control, Stress 1, and Stress 2), and three replications with two vines in each plot. The data obtained were analyzed using the MSTAT-C statistical program, and the LSD test (1% and 5%) was applied to reveal the differences. In some statistical analyses, the 16mm-18mm berry size group was not used due to the insufficient number of berries in this size group.

Data collection

After harvest, clusters were separated into individual berries and sorted into size classes using sieves with openings of 10mm, 12mm, 14mm, 16mm, and 18mm. Each cluster's berries were destemmed, and 200g of grape berries were blended. 50ml of resulting puree was transferred to a light-proof container, and 62ml of 80% (v/v) acidified methanol was added. After 24 hours in a dark room, the mixture was filtered with Whatman No. 1 paper to obtain grape extract and stored in air-tight containers. This process was applied to each size group separately. 1 ml was taken from the bottled samples, and 5 ml of methanol was added (dilution factor 1/6). For all other analyses, extracts were taken from this diluted extract and used.

Analysis of sugars

The TSS was measured using a refractometer and recorded as °Brix (Cemeroğlu, 2007). Sugar concentration (g L^{-1}) was determined based on the °Brix values. The sugar content in the berry (mg $berry^{-1}$) was calculated using the following formula (Carbonneau & Bahar, 2009):

$$Sugar\ content\ in\ the\ berry\ (mg/berry) = [1/1.3\ x\ Sugar\ (g/L)]\ x\ [1/100\ x\ 100\ berry\ weight\ (g)] \quad (1)$$

Additionally, the sugar content per gram of grape (mg g-$berry^{-1}$) was calculated using the following formula:

$$Sugar\ content\ per\ gram\ of\ grape\ (mg/g - berry) = Sugar\ content\ in\ the\ berry\ /\ berry\ fresh\ weight \quad (2)$$

Total acid content (TA) (g L^{-1})

Samples from the must were measured using 1N NaOH solution and phenolphthalein indicator. The amount of NaOH consumed with phenolphthalein indicator on 5 ml of must solution was recorded as tartaric acid (Cemeroğlu, 2007).

the pH of the must

The pH was determined using a digital pH meter (Cemeroğlu, 2007).

Analysis of total phenolic compounds

The Folin-Ciocalteau method was used for spectrophotometric readings (Waterhouse, 2002; Sánchez-Rangel et al., 2013). 1 ml of the diluted extract (1/6 ratio) was transferred to a 100 ml volumetric flask using a micropipette. Then, 5 ml of Folin-Ciocalteau reagent and 10 ml of Na_2CO_3 solution (20g L^{-1}) were added, and the mixture was shaken. After leaving it for 2 hours at 75°C in a water bath with 70 ml of distilled water, the volume was adjusted to 100 ml with distilled water, and the absorbance was measured at 765 nm using a spectrophotometer.

$$Formula\ (mg/kg) = Read\ Value\ x\ 11197.6 \quad (3)$$

Analysis of total anthocyanin compounds (mg kg^{-1})

Different pH methods were used for anthocyanin determination (Cemeroğlu, 2007). The buffer solution (696.5 ml citric acid + 303.5 ml disodium monophosphate solution) is a mixture. Monometric anthocyanins in black-colored grapes, extracted using disodium monophosphate, were determined using the pH-Differential method and expressed as malvidin-3-glucoside (mg kg^{-1}). Methanol was used for the preparation of extracts in the determination of total anthocyanins. Two tubes were prepared for each sample: Tube 1: 1 ml of the extract + 1 ml of 80% (v:v) methanol diluted with distilled water and 10 ml of 2% HCl solution. The reading was taken at 520 nm using a spectrophotometer. Tube 2: 1 ml of the extract + 1 ml of methanol + 10 ml of buffer solution. After shaking, the reading was taken at 520 nm using a spectrophotometer.

$$Formula\ 4645.8\ x\ (High\ reading - Low\ reading) \quad (4)$$

Analysis of total monomeric anthocyanins by pH differential method

Potassium chloride buffer ($pH_{1.0}$) and Sodium Acetate buffer ($pH_{4.5}$) solutions were prepared, and samples were compared with predetermined ratios in preliminary tests to establish equilibrium after waiting for 30 min. The absorbances of both buffer solutions for each sample were then measured at 520 nm and 720 nm using a spectrophotometer. The number of anthocyanins in the samples was determined using the following equation. $pH_{1.0}$ Buffer: In a container, 250 ml of 0.2 N KCl (14.9 g L^{-1}) and 650 ml of 0.2 N HCl (17 ml L^{-1}) solutions are combined and mixed. The pH of the solution should be adjusted to 1.0. If it is not, it is adjusted using an HCl solution. $pH_{4.5}$ Buffer: 1.64 g of Sodium Acetate ($CH_3CH'Na.3H_2O$) is dissolved in 100 ml of distilled water, and then 1 N HCl (83 ml of concentrated HCl per L) is added to adjust the pH to 4.5 ± 0.1.

$$A = (A520 - A720)\ pH\ 1.0 - (A520 - A720)\ pH\ 4.5$$

$$Total\ Anthocyanin\ Content\ (mg/kg) = (A)\ x\ (MW)\ x\ (Sf)\ x\ 1000\ /\ (\varepsilon)\text{^}1 \quad (5)$$

(ε): Molar absorption coefficient for Malvidin-3-glucoside: 28,000

MW: Molecular weight of Malvidin-3-glucoside: 493.5

Sf: Dilution factor

l: Cuvette layer thickness: Set as 1.

Analysis of total tannin compounds

From the 1/6 diluted extract, 1 ml was transferred into a 100 ml volumetric flask. Then, 10 ml of Folin-Denis reagent was added, and it was filled up to 100 ml with $NaCO_3$ solution (%35 (m:v) in distilled water)

and thoroughly mixed. After waiting for 30 min and ensuring no turbidity, the samples were transferred into the spectrophotometer cuvette and read at 750 nm. If the 100 ml volumetric flask and the sample did not align precisely due to differences in micropipette and volumetric flask diameters, the sample was transferred to another container after shaking. After waiting for 30 min and without disturbing the sediment, the sample was taken.

$$Formula\ (mg/kg) = 13417.2\ x\ Read\ value \quad (6)$$

Analysis of total phenolic compounds (TPC) for antioxidant content

Total phenolic compounds in grape methanol extracts were determined using the Folin-Ciocalteau reagent (FCR) method (Kupina et al., 2017). The FC reagent, a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$), undergoes a colour change to blue compounds during phenol oxidation. This color change read at 760 nm on a spectrophotometer, is proportional to the amount of polyphenolic compounds, and is expressed as gallic acid or pyrocatechol equivalents. The calculation is based on the formula obtained in terms of gallic acid.

From the 1/6 diluted extract, 1 ml was transferred into a 100 ml volumetric flask. Then, 5 ml of FCR and 10 ml of $NaCO_3$ solution (20 g L^{-1}) were added and shaken. After that, 70 ml of distilled water was added, and the flask was kept in a water bath at 75°C for 2 hours. After, the flask was filled up to 100 ml with distilled water, and a sample was taken from this solution for reading at 760 nm on a spectrophotometer to calculate the antioxidant content in terms of gallic acid.

$$Absorbance\ (\lambda: 760\ nm) = 0.0011[Gallic\ acid] - 0.0022 \quad (7)$$

Analysis of antioxidant enzyme activities by H_2O_2 method

The antioxidant content of grape extracts was determined by measuring their hydrogen peroxide (H_2O_2) removal activity using the method described by Benmeziane (2017). A specific amount of hydrogen peroxide solution was added to the reaction medium, and the breakdown by the sample extract was monitored by measuring the absorbance change at 230 nm. A 0.1M Phosphate buffer (pH=7.4) was prepared, and a 0.40mM H_2O_2 solution. The sample extract was added to the solution, and after a 10-minute waiting, the absorbance at 230 nm was measured. A control determination was also performed without H_2O_2.

$$\%\ Inhibition = [(Acontrol - Asample) / Acontrol]\ x\ 100 \quad (8)$$

A control = Absorbance of the control, A sample = Absorbance of the sample.

Analysis of total polyphenol index (TPI)

Grape juice was first passed through a coarse filter and then centrifuged at 8000 rpm for 5 minutes in a conical tube. Afterwards, it was filtered again through a coarse filter. From this filtrate, 1 ml was taken and diluted with 50 ml of pure water in a volumetric flask, and then measurements were made using a spectrophotometer. The obtained values were multiplied by the dilution factor and used for further analysis.

RESULTS

Leaf water potential (Ψ_{pd}, MPa)

The grapevine water status Ψ_{pd} (Pre-dawn leaf water potential) was determined through measurements (Data not shown). According to the Location x Stress interaction, in the Dryland-shallow soil x Stress 1 interaction, the average value was measured as -0.77 MPa. In the Dryland-shallow soil x Stress 2 interaction, the average value was -1.22 MPa, and in the Dryland-shallow soil x Control interaction, it was -0.92 MPa. The Dryland-shallow soil interactions were found to be in the high-stress and severe-high-stress groups according to Carbonneau (1998) and Deloire and Rogiers (2014). On the other hand, Baseland-deep soil reduced the Ψ_{pd} values, indicating a stress reduction. They were recorded as Control (-0.29 MPa) for low-moderate stress, Stress 1 (-0.77 MPa) for severe-high stress, and Stress 2 (-0.92 MPa) for high stress (Korkutal et al., 2023). Ojeda et al. (2002) stated that the grape berry quality slightly increased with moderate water stress.

Sugar Contents

In terms of Total Soluble Solids (TSS) values, the Stress Main Effect (STME) and Location x Stress interactions, as well as the main effect of Location (LOME), were found to be significant. Regarding the LOME, Dryland-shallow soil (D) had a value of 23.94°Brix, and Baseland-deep soil (B) had a value of 22.83°Brix. Concerning the STME, the lowest value was obtained from the Stress 2 group (23.12°Brix), and the highest value was from the Control group (23.85°Brix). Stress 1 (23.18°Brix) fell between these two values. In terms of the Location x Stress interaction, the Dx Control interaction (24.81°Brix) had the highest value, while the B x Stress 1 interaction (22.50°Brix) had the lowest value. These findings are consistent with the observation made by Lafontaine et al. (2013) and Melo et al. (2015) that as the berry size decreases, the °Brix ratio increases. Additionally, in Dryland-shallow soil (Stress 2 < -0.8 MPa), the effect of water deficiency in the vine, as noted by Koundouras et al. (2006), led to a reduction in sugar accumulation in the berries during the ripening process (Data not shown). Romero et al. (2010), and Zúñiga et al. (2018) contradict each other

in their research findings regarding the effect of moderate water stress on increased TSS in red grape varieties. It is thought that this discrepancy may have resulted from differences in soil structure.

When evaluating the sugar concentration (g L^{-1}) the STME, LOME, and Location x Stress interactions were found to be significant (Data not shown). For the STME sugar concentration values, it was observed that the Control vines (236.34 g L^{-1}) had higher values, followed same group by Stress 1 (228.52 g L^{-1}) and Stress 2 (227.87 g L^{-1}). The sugar concentration in the D (237.42 g L^{-1}) was found to be higher than in the B (224.40 g L^{-1}). The obtained values are consistent with the findings of Matthews & Nuzzo (2007), which suggest that sugar concentration in berries decreases as berry size increases.

Regarding the sugar amount in berries (mg $berry^{-1}$), only the BSME showed statistical significance (Figure 1). It was observed that the 14mm-16mm size group had the highest sugar amount (137.48 mg $berry^{-1}$). For STME, the amount of sugar in berry values between 112.74 mg $berry^{-1}$ (Control) to 103.77 mg $berry^{-1}$ (Stress 2) were observed. This finding is consistent with the study by Ojeda et al. (2002), which reported that water deficiency after veraison reduces sugar content in berries. On the other hand, the research findings of Zarrouk et al. (2012) are parallel with the result that there was no difference in sugar content in the berries between non-irrigated and regulated water restriction conditions.

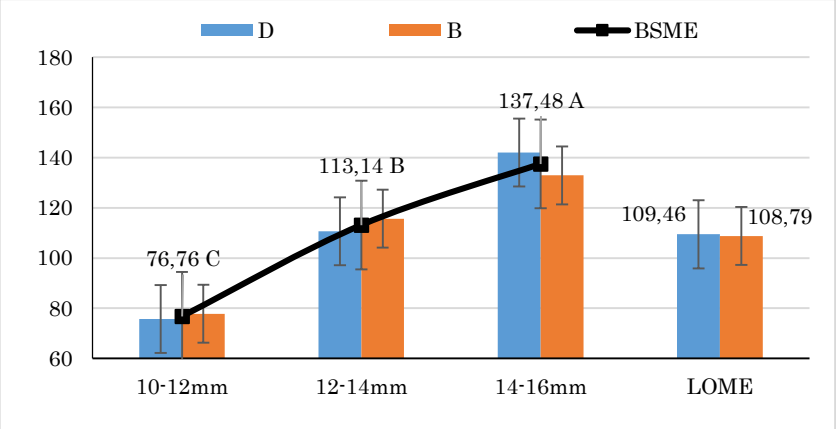

Figure 1. Effects of different berry sizes depending on the land-soil type on the sugar amount in berries
Şekil 1. Arazi ve toprak tipine bağlı olarak farklı tane boyutlarının tanedeki şeker miktarına etkileri
BSME LSD %1 = 13,41675

The amount of sugar per gram of grape berry (mg g-$berry^{-1}$) showed statistical significance in terms of berry size groups, LOME, STME, and Location x Stress interactions (Data not shown). In terms of LOME, values of 79.14 mg g-$berry^{-1}$ for D and 74.80 mg g-$berry^{-1}$ for B were obtained. The results are consistent with Bahar et al. (2017), who stated that the lowest sugar content in a single grape berry is obtained when Ψ_{pd} falls below -0.7 MPa, in terms of Location.

Total acidity (TA) (g L^{-1})

In terms of TA criteria, significant differences were found only for LOME among different berry size groups based on location and soil type. A TA value of 7.71 g L^{-1} was obtained for B. This value was followed by D with a TA value of 6.50 g L^{-1}. Kontoudakis et al. (2011) reported that the group with higher density (smaller berries) had higher TA content. This finding is in line with results where smaller berries were observed to have higher TA values (Data not shown). Furthermore, the results are consistent with the findings of Koundouras et al. (2006) and Caruso et al. (2023) that water deficiency determined based on Ψ_{pd} values disrupts the accumulation of malic acid in the must and reduces TA (Romero et al., 2010).

Grape must pH

In terms of grape must pH, STME, Location x Stress interaction, and LOME have shown significant effects (Table 1). For STME, when pH values were examined, Stress 2 (3.34) and Control (3.31) were in the same significance group, while Stress 1 (3.26) was in another significance group. Regarding Location x Stress interactions, D x Stress 2 interaction (3.43) and D x Control interaction (3.38) were in the first significance group. On the other hand, B x Stress 1 interaction (3.31) formed the second significance group, and B x Stress 2 (3.25), B x Control (3.23), and D x Stress 1 (3.20) values constituted the last significance group. When pH values were examined concerning LOME, D had a pH value of 3.34, and B had a pH value of 3.27. The findings are consistent with the study of Munitz et al. (2016), where they reported that different vine water statuses did not have a significant effect on grape must pH. Similarly, the results align with the study by Bahar et al.

(2017), where they observed that although the pH values of groups separated based on stress levels were not statistically significant, vines under stress levels above -0.7 MPa had the lowest pH values in the must. However, the results contradict the findings of Gil et al. (2015), who reported that small-sized berries contained high pH, and Caruso et al. (2023), who indicated that irrigation affects pH. It is thought that this discrepancy may have arisen from differences in field and soil types.

Table 1. pH values in different berry size groups based on location-soil type
Çizelge 1. Arazi ve toprak tipine bağlı olarak farklı tane boyut gruplarında pH

Location and Stress		Berry Size			LOME
		10mm-12mm	12mm-14mm	14mm-16mm	
Dryland-shallow soil (D)		3.34±0.05	3.32±0.04	3.34±0.03	3.34±0.02 a
Baseland-deep soil (B)		3.24±0.02	3.26±0.02	3.29±0.02	3.27±0.01 b
					STME
Control		3.32±0.06	3.29±0.03	3.32±0.03	3.31±0.02 **a**
Stress 1		3.23±0.03	3.25±0.03	3.29±0.03	3.26±0.02 **b**
Stress 2		3.33±0.05	3.34±0.04	3.35±0.04	3.34±0.02 **a**
					LocationxStress int
	Control	3.42±0.07	3.35±0.03	3.37±0.03	3.38±0.03 **A**
D	Stress 1	3.17±0.02	3.19±0.02	3.24±0.02	3.20±0.01 **C**
	Stress 2	3.43±0.03	3.42±0.02	3.43±0.04	3.43± 0.02 **A**
	Control	3.21±0.04	3.23±0.03	3.26±0.02	3.23± 0.02 **C**
B	Stress 1	3.29±0.01	3.31±0.01	3.35±0.04	3.32± 0.01 **B**
	Stress 2	3.23±0.02	3.25±0.02	3.26±0.01	3.25± 0.01 **C**
	BSME	3.29±0.03	3.29±0.02	3.31±0.02	

STME %5 LSD = 3.710355E-02 (Small bold letters); Location x Stress int. LSD %5 = 5.247234E-02 (Big bold letters), LOME %5 = 1.029411 (Small letters)

Total phenolic content (mg kg^{-1})

Table 2 revealed statistically significant differences among the grape berry size groups concerning total phenolic content, Berry Size Main Effect (BSME), STME, Location x Stress, Location x Stress x Berry size interaction, and LOME. In terms of total phenolic content, BSME analysis revealed that the 14mm-16mm berry size group had the highest value of 1615.68 mg kg^{-1}. In terms of total phenolic content, D had a value of 1627.67 mg kg^{-1} and B had a value of 1461.49 mg kg^{-1} for LOME. For the total phenolic content in terms of STME, Control had a value of 1639.00 mg kg$^{-1,}$ and Stress 1 had a value of 1361.73 mg kg^{-1}, forming the first significant group. The stress 1 x 14mm-16mm group had the highest value of 1811.63 mg kg^{-1}. B x Stress 1 x 14-16mm group had a value of 2087.58 mg kg^{-1}, having the highest total phenolic content.

Table 2. Total phenolic content in different stress levels according to land-soil type
Çizelge 2. Arazi ve toprak tipine bağlı olarak farklı stres seviyelerinde toplam fenolik madde miktarı

Location and Stress		Berry Size			LOME
		10mm-12mm	12mm-14mm	14mm-16mm	
Dryland-shallow soil (D)		1719.66±62.53 *a*	1601.01±47.49 *a*	1562.35±53.83 *a*	1627.67±33.20 a
Baseland-deep soil (B)		1367.73±100.42 *b*	1347.73±103.01 *b*	1669.00±106.30 *a*	1461.49±64.13 b
					STME
Control		1713.66±26.90 ab	1625.67±169.30 abc	1577.68±81.94 bcd	1639.00±61.04 **a**
Stress 1		1683.66±100.17 ab	1403.72±59.02 de	1811.63±123.41a	1633.01±67.27 **a**
Stress 2		1233.75±111.80 e	1393.72±38.49 de	1457.71±29.51 cd	1361.73±44.5 **b**
					LocationxStress int
	Control	1767.64±16.00 *BCD*	1787.64±12.00 *ABC*	1759.64±20.00 *BCD*	1771.65±9.16
D	Stress 1	1907.62±0.00 *AB*	1535.69±0.00 *CDEF*	1535.69±0.00 *CDEF*	1659.67±61.99
	Stress 2	1483.70±4.00 *CDEF*	1479.70±4.00 *CDEF*	1391.72±0.00 *EF*	1451.71±15.10
	Control	1659.67±21.16 *BCDE*	1463.71±341.95 *DEF*	1395.72±8.00 *EF*	1506.37±106.55
B	Stress 1	1459.71±4.00 *DEF*	1271.75±0.00 *FG*	2087.58±0.00 *A*	1606.35±123.34
	Stress 2	983.80±0.00 *G*	1307.74±0.00 *F*	1523.70±0.00 *CDEF*	1271.75±78.45
	BSME	1543.69±71.51 AB	1474.37±63.02 B	1615.68±59.22 A	

STME LSD %1 = 126.9938 (Small-bold letters); LOME LSD %1 = 130.9765 (Small letters); BSME LSD %5 = 94.59129 (Big letters); Location x Berry size int. LSD %1 = 179.5963 (Small-italic letters); Stress x Berry size int. LSD %1 = 219.9596 (Small-underline letters); Location x Stress x Berry size int. LSD %1 = 311.0699 (Big-italic letters)

The findings are not in line with Mulero et al. (2010) and Provost & Pedneault (2016) studies, which reported the same phenolic content values for organic and conventional grapes during harvest time. The total phenolic values in the conventional (=Control) vineyard were lower than those in the organic vineyard's Stress 1 and Stress 2, which is believed to be due to location differences. The results are consistent with Martin & Rasmusen (2011) who reported that organic vineyards had higher total

phenolic content compared to conventional vineyards. However, it is not consistent for D, which is believed to be due to differences in water accessibility and soil structure. On the other hand, Melo et al. (2015) reported that the phenolic content in the small berry group was higher than that in the medium and large berry groups, which is not consistent with the results. It was observed that D in the 10mm-12mm berry size group had a higher total phenolic content, while B had a higher phenolic content in the 14mm-16mm berry size group, which is believed to be due to differences in soil and land structure.

Total anthocyanin content (mg kg^{-1})

When total anthocyanin content is considered, Location, stress groups, different berry size groups, and their interactions were found to be statistically significant (Table 3). When the total anthocyanin content was examined for BSME, it was observed that the 10mm-12mm berry size group had the highest value (1245.07 mg kg^{-1}). The fact that the highest anthocyanin content is found in the smallest berry size is consistent with the findings of Zouid et al. (2013), Lafontaine et al. (2013), and Gil et al. (2015). However, it contradicts the results of Chen et al. (2018), who found an increase in anthocyanin with an increase in berry size. It is believed that this discrepancy may be due to differences in terrain-soil type and climate.

The high anthocyanin content was obtained from Location D (1406.47 mg kg^{-1}), and the low value was from Location B (879.78 mg kg^{-1}). Regarding STME, the Stress 1 level (1247.91 mg kg^{-1}) had the highest, and Control (1025.69 mg kg^{-1}) had the lowest anthocyanin content among stress levels. The findings of Koundouras et al. (2006), Romero et al. (2010), Zarrouk et al. (2012), Cheng et al. (2014), Öner (2014), and Munitz et al. (2016) that anthocyanin content can increase under water deficit conditions align with the findings of this study in Dryland-shallow soil.

Table 3. Total anthocyanin content in different stress levels depending on land-soil type
Çizelge 3. Arazi ve toprak tipine bağlı olarak farklı stres seviyelerinde toplam antosiyanin miktarı

Location and Stress		Berry Size			LOME
		10mm-12mm	12mm-14mm	14mm-16mm	
Dryland-shallow soil (D)		1625.00±61.83 *a*	1351.41±17.80*b*	1243.01±21.72 *c*	1406.47±38.30 a
Baseland-deep soil (B)		865.15±36.58 *e*	821.79±52.59 *f*	952.39±94.08 *d*	879.78±37.98 b
					STME
Control		1094.08±131.93 e	1026.72±157.90 g	956.26±157.59 h	1025.69±82.30 **c**
Stress 1		1409.23±177.99 a	1080.15±140.24 f	1254.36±2.08 b	1247.91±78.10 **a**
Stress 2		1231.91±199.80 c	1152.93±57.14 d	1082.47±35.32 e	1155.77±67.64 **b**
					LocationxStress int
D	Control	1389.09±0.00 *C*	1379.80±0.00*D*	1308.56±7.74 *E*	1359.15±12.92 **C**
	Stress 1	1807.22±0.00 *A*	1393.74±0.00*C*	1259.01±0.00 *G*	1486.66±82.47 **A**
	Stress 2	1678.68±1.55 *B*	1280.69±1.55 *F*	1161.45±0.00 *I*	1373.61±78.19 **B**
B	Control	799.08±0.00 *M*	673.64±0.00 *P*	603.95±0.00 *Q*	692.22±28.55 **F**
	Stress 1	1011.23±1.55 *K*	766.56±0.00 *O*	1249.72±0.00 *H*	1009.17±69.74 **D**
	Stress 2	785.14±0.00 *N*	1025.17±1.55 *J*	1003.49±0.00 *L*	937.934±38.33 **E**
	BSME	1245.07±98.51 A	1086.60±69.64 C	1097.93±58.61B	

STME LSD %1= 3.007017 (Small-bold letters); Location x Stress int. LSD %1 = 4.252564 (Big-bold letters); LOME LSD %1 = 5.208306 (Small letters); BSME LSD %1 = 3.007017 (Big letters); Location x Berry size int. LSD %1 = 4.252564 (Small-italic letters); Stress x Berry size int. LSD %1 = 5.208306 (Small-underline letters); Location x Stress x Berry size int. LSD %1 = 7.365656 (Big-italic letters)

Total monomeric anthocyanin content (pH differential method) (mg kg^{-1})

The effects of berry size, stress, and location, as well as their interactions, on the total monomeric anthocyanin content were found to be significant (Figure 2). When the total monomeric anthocyanin content was examined for BSME, it was observed that the lowest value was obtained from the 14mm-16mm berry size group (133.66 mg kg^{-1}), and the highest value was obtained from the 10mm-12mm group (160.30 mg kg^{-1}). These findings are consistent with the results of Zouid et al. (2013) and Lafontaine et al. (2013), which also reported a negative relationship between berry size and anthocyanin content.

Total tannin content (mg kg^{-1})

STME, Location x Stress, BSME, Location x Berry size, Stress x Berry size, Location x Stress x Berry size, and LOME were found to be significant (Table 4). It has been observed that there is a negative correlation between the total tannin content and berry size. This finding is parallel to the studies of van Leeuwen et al. (2009).

LOME total tannin values were recorded as D (5165.76 mg kg^{-1}) and B (4565.83 mg kg^{-1}). However, the research findings contradict the study of Lafontaine et al. (2013), who reported that tannin content increases with an increase in berry size. This discrepancy can be attributed to differences in location and vineyard soil characteristics.

Total phenolic content determination with antioxidant PCR method (g kg^{-1})

STME, BSME, Location x Stress interactions, Location x Berry size, Stress x Berry size, Location x Stress x Berry size, and LOME were found to be statistically significant (Table 5). The highest value of 121.95 g kg^{-1} was obtained from the 14mm-16mm berry size group. Regarding LOME, D (123.81 g kg^{-1}) had the highest value. In terms of the Location x Berry size interaction, the total antioxidant values of the berries in the D x 10mm-12mm size group were the highest (132.00 g kg^{-1}). These findings are not consistent with the results reported by Buchner et al. (2014), Mulero et al. (2010), and Provost & Pedneault (2016), who found no significant difference in total antioxidant content between conventional and organic vineyards. For STME, Control (128.71 g kg^{-1}) and Stress 1 (124.17 g kg^{-1}) were in the same group. In the Stress x Berry size interactions, the Stress 1 x 14mm-16mm group (137.60 g kg^{-1}) had the highest value. Looking at the Location x Stress interaction, D x Control (132.20 g kg^{-1}) had the highest value, and B x Stress 2 (98.36 g kg^{-1}) had the lowest value. In the Location x Stress x Berry size interactions, the highest value was obtained from the B x 14mm-16mm x Stress 1 interaction (158.36 g kg^{-1}). On the other hand, the results are not consistent with the findings of Chen et al. (2018), who reported a linear relationship between berry size and anthocyanin concentration, as well as with the result that water stress increases anthocyanin concentration (Koundouras et al., 2006; Romero et al., 2010). It is thought that these discrepancies may be due to differences in grape variety, climate, vineyard, and soil type.

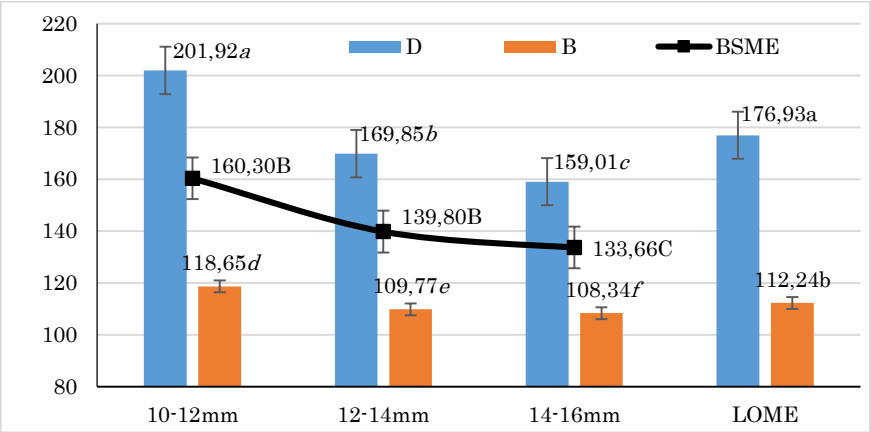

Figure 2. Total monomeric anthocyanins (pH differential method) in different stress levels depending on land-soil type

Şekil 2. Farklı tane boyut gruplarına göre toplam monometrik antosiyanin (pH differansiyel metodu) miktarları

BSME LSD %1 = 1.182305 (Big letters); Location x Berry size LSD %1 = 1.672031 (Small-italic letters)

Table 4. Total tannin content at different stress levels based on land-soil type

Çizelge 4. Arazi ve toprak tipine bağlı olarak farklı stres seviyelerinde toplam tanen miktarı

Location and Stress		Berry Size			LOME
		10mm-12mm	12mm-14mm	14mm-16mm	
Dryland-shallow soil (D)		5559.75±137.34 *a*	4865.97±217.54 *c*	5072.12±373.22 *b*	5165.76±155.96 a
Baseland-deep soil (B)		4730.31±311.31 *d*	4646.82±348.04 *e*	4560.36±275.97 *f*	4645.83±174.24 b
					STME
Control		5489.87±161.28 b	5013.56±454.28 d	4197.98±270.78 h	4900.47±216.13 **b**
Stress 1		5060.52±187.41 c	4577.50±343.08 g	5675.47±136.04 a	5104.50±169.33 **a**
Stress 2		4883.86±530.04 e	4678.13±258.03 f	4575.27±478.35 g	4712.42±239.73 **c**
					LocationxStress int
D	Control	5129.84±19.49 *H*	3998.33±33.77 *L*	3592.60±8.99 *O*	4240.26±230.27 **E**
	Stress 1	5478.69±23.66 *E*	5344.52±11.83 *F*	5979.60±4.47 *A*	5600.94±96.94 **B**
	Stress 2	6069.04±4.47 *A*	5255.07±4.47 *G*	5644.17±38.99 *D*	5656.09±118.08 **A**
B	Control	5849.90±7.75 *C*	6028.79±4.47 *AB*	4803.36±7.74 *I*	5560.68±191.11 **C**
	Stress 1	4642.35±13.42 *J*	3810.48±7.75 *M*	5371.35±4.47 *F*	4608.06±225.50 **D**
	Stress 2	3698.68±4.47 *N*	4101.19±4.47 *K*	3506.36±4.47 *P*	3768.74±87.65 **F**
	BSME	5144.75±193.25 A	4756.40±200.86 C	4816.24±233.55 B	

STME LSD %1 = 22.68181 (Small-bold letters); Location x Stress int. LSD %1 = 32.07692 (Big-italic letters); LOME LSD %1 = 22,68181 (Small letters); BSME LSD %1 =22.68181 (Big-bold letters); Location x Berry size int. %1 =32.07692 (Small-italic letters); Stress x Berry size int. %1 = 39.28605 (Small-underline letters); Location x Stress x Berry size int. LSD %1 = 55.55886 (Big-italic letters)

The total antioxidant content (H_2O_2 method g kg^{-1}) values were not found to be statistically significant (Data not shown). The STME antioxidant values were not found to be statistically significant, but they were

ranked from highest to lowest as follows: Control (91.60 g kg^{-1}), Stress 1 (98.84 g kg^{-1}), and Stress 2 (112.11 g kg^{-1}). D obtained a value of 88.72 g kg^{-1}, and B obtained a value of 112.98 g kg^{-1}. These results are in line with Mulero et al. (2010), Bunea et al. (2012), Buchner et al. (2014), and Provost & Pedneault (2016), who reported no difference in antioxidant values between organic and conventional vineyards.

Total polyphenol index (TPI)

Table 6 presents the TPI values. Regarding TPI, BSME values were not found to be statistically significant; however, they were ranked from highest to lowest as follows: 12mm-14mm group (7.72), 10mm-12mm group (6.84), and 14mm-16mm group (5.56).

The TPI values, in ascending order, for LOME are D (6.15) and B (7.26). When examined for STME, the numerical values are Control (6.00), Stress 2 (6.68), and Stress 1 (7.43). Blouin & Guimberteau (2000) reported an average TPI of 13.3 for Cabernet-Sauvignon grape berries. However, the highest TPI value (10.23) obtained in this study was significantly lower, measuring, which is considerably below the value reported by other researchers (Bahar et al., 2017). Furthermore, it has been observed that the total phenolic content in organic vineyards is higher than in conventional vineyards due to their increased exposure to biotic stresses (Mulero et al., 2010; Martin & Rasmussen, 2011; Bunea et al., 2012).

Table 5. Antioxidant PCR method at different stress levels depending on the land-soil type
Çizelge 5. Arazi ve toprak tipine bağlı olarak farklı stres seviyelerinde antioksidan PCR metodu

Location and Stress		Berry Size			LOME
		10mm-12mm	12mm-14mm	14mm-16mm	
Dryland-shallow soil (D)		132.00±4.86 *a*	121.60±2.64 *c*	116.55±2.86 *d*	123.38±2.36 a
Baseland-deep soil (B)		106.24±6.93 *f*	112.00±7.06 *e*	127.35±7.83 *b*	115.20±4.40 b
					STME
Control		132.90±2.44 c	136.09±1.83 b	117.15±4.10 e	128.71±2.57 **a**
Stress 1		127.91±7.52 d	107.00±4.67 g	137.60±9.28 a	124.17±5.07 **b**
Stress 2		96.54±7.32 h	107.30±3.63 g	111.09±2.03 f	104.98±3.03 **c**
					LocationxStress int
D	Control	138.36±0.00 *D*	132.00±0.00 *E*	126.24±1.09 *F*	132.20±1.78 **A**
	Stress 1	144.73±0.00 *B*	117.45±0.00 *G*	116.85±0.30 *GH*	126.34±4.60 **B**
	Stress 2	112.91±0.00 *J*	115.34±1.21 *I*	106.55±0.00 *L*	111.60±1.36 **E**
B	Control	127.45±0.00 *F*	140.18±0.00 *C*	108.06±0.30 *L*	125.23±4.67 **C**
	Stress 1	111.09±0.00 *K*	96.55±0.00 *N*	158.36±0.00 *A*	122.00±9.33 **D**
	Stress 2	80.18±0.00 *O*	99.27±0.00 *M*	115.64±0.00 *HI*	98.36±5.12 **F**
	BSME	119.12±5.16 B	116.80±3.83 C	121.95±4.25 A	

STME LSD %1 = 0.6154878 (Small-bold letters); Location x Stress int. LSD %1 = 0.8704298 (Big-bold letters); LOME LSD %1 = 0,8704298 (Small letters); BSME LSD %1 = 0.6154868 (Big letters); Location x Berry size int. LSD %1= 0.8704298 (Small-italic letters); Stress x Berry size int. LSD %1 = 1.066054 (Small-underline letters); Location x Stress x Berry size LSD %1 = 1.507629 (Big-italic letters)

Table 6. TPI values at different stress levels based on land-soil type
Çizelge 6. Arazi ve toprak tipine bağlı olarak farklı stres seviyelerinde TPI değerleri

Location and Stress		Berry Size			LOME
		10mm-12mm	12mm-14mm	14mm-16mm	
Dryland-shallow soil (D)		6.72±1.23	6.56±1.26	5.16±1.30	6.15±0.71
Baseland-deep soil (B)		6.96±1.35	8.88±0.76	5.95±0.54	7.26±0.57
					STME
Control		5.82±1.39	7.17±1.45	5.02±0.98	6.00±0.73
Stress 1		7.43±1.86	9.37±1.29	5.49±0.84	7.43±0.85
Stress 2		7.26±1.52	6.62±1.17	6.16±1.75	6.68±0.82
					LocationxStress int
D	Control	5.73±2.78	4.90±2.12	3.92±1.44	4.85±1.12
	Stress 1	7.98±2.51	8.00±2.33	5.45±1.50	7.14±1.16
	Stress 2	6.45±1.68	6.77±2.59	6.12±3.84	6.44±1.43
B	Control	5.92±1.37	9.43±0.96	6.12±1.23	7.16±0.83
	Stress 1	6.88±3.27	10.73±1.05	5.53±1.14	7.72±1.30
	Stress 2	8.08±2.83	6.48±0.36	6.20±0.71	6.92±0.90
	BSME	6.84±0.89	7.72±0.77	5.56±0.69	

N.S. (Not Significant)

CONCLUSION

In the same vineyard, variations were observed in total phenolic compounds, anthocyanins, and tannins accumulation depending on stress levels under Dryland-shallow soil and Baseland-deep soil conditions. Additionally, the phytochemical parameters of the berries were influenced by berry size. The 10mm-12mm group consistently showed desired values in all criteria. Therefore, it is suggested to develop cultural practices aimed at

reducing berry size in this vineyard. Regarding stress levels (Ψ_{pd}) and TSS, vineyards experiencing extreme water scarcity (Stress 2 < -0.8 MPa) generally exhibited lower TSS values. Baseland-deep soil vines showed moderate TSS levels (22.81 ºBrix) due to relatively lower stress levels. However, under Dryland-shallow soil conditions, even with extreme water scarcity, vineyards showed lower sugar accumulation (TSS: 20.94 ºBrix). In conclusion, TSS varied depending on soil type and stress level, while berry size had a relatively minor impact on TSS.

Higher total phenolic compound levels were measured in berries between 14mm-16mm in size, considering all stress levels and locations. However, vines under moderate stress conditions showed higher total phenolic compound levels regardless of berry size. Total anthocyanin and total tannin levels were higher in berries between 10mm-12mm under Stress 1 conditions, regardless of location. Among the different conditions, Baseland-deep soil under conventional (Control) conditions exhibited the lowest total anthocyanin levels (799.08 mg kg^{-1}), while Dryland-shallow soil under conventional conditions showed the highest levels (1389.09 mg kg^{-1}). Total tannin levels were also higher under Dryland-shallow soil conditions compared to Baseland-deep soil. Total antioxidant levels were highest in Dryland-shallow soil vines under moderate stress conditions (Stress 1) and lower in vines under high-stress conditions (Stress 2), showing no correlation with berry size. The total Polyphenol Index did not significantly differ with berry size, stress, or location, but it was found to be higher in organic vineyards compared to conventional vineyards. Overall, vines under Stress 1 (> -0.8 MPa) conditions showed the best results in terms of total phenolic compounds, total anthocyanins, total tannins, and total antioxidants.

In conclusion, to achieve high-quality must and wine from Cabernet-Sauvignon grapes in Tekirdağ province, it is advisable to cultivate under Dryland-shallow soil conditions, where leaf water potential (Ψ_{pd}) can drop to -0.8 MPa during the pre-dawn period, and to prefer berries between 10mm and 12mm in diameter by making a selection based on berry size.

CONTRIBUTIONS

İ.K. contributed to the investigation, writing, review, and editing; E.B. contributed to the investigation and review; M.U. contributed to the investigation and writing.

CONFLICT of INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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