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Cabernet-Sauvignon Üzüm Çeşidinde Abiyotik Streslerin Primer / Sekonder Metabolitler ve Resveratrole Etkisi

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ÖZET

Araştırma; 41° 01' 11.15" N enlem ve 27° 40' 18.00" E boylamda ve denizden 60 m yüksekte ve 15 yaşındaki Cabernet-Sauvignon/110R omcaları kurulmuş ve iki yıl süreyle yürütülmüştür. Bağın dikim aralık ve mesafesi 2.6×0.9 m olup, asmalar çift kollu kordon Royat terbiye şekline sahiptir. Araştırma bağda, 3 farklı fenolojik dönemde (ben düşme, ben düşme-hasat ve hasat) 5 gün süre ile sabah ve akşam olmak üzere, Kontrol dahil 4 abiyotik stres uygulaması (Darbe, Yaprak Yaralama, UV-C) yapılmıştır. Yaprak Yaralama bir kez ve yapraklara çubuk ile vurularak gerçekleştirilmiştir. Darbe uygulaması plastik çekiç kullanılarak, UV-C uygulaması da günde iki kez 1 dakika süreyle yapılmıştır. Sonuçta abiyotik stres uygulamalarının primer metabolitlerden; SÇKM (23.69° Brix) ve TA (7.32 g L^{-1}) açısından önemli farklılık oluşturmadığı; sekonder metabolitlerde (toplam tanen, toplam antosiyanin, toplam fenolik madde, resveratrol) artış yönünde etkisi olduğu belirlenmiştir. Ayrıca toplam polifenol indeksini artırıcı etki gösterdikleri saptanmıştır. Resveratrol açısından, sırasıyla UV-C (0.35 mg kg^{-1}) ve Yaprak Yaralama (0.27 mg kg^{-1}) uygulamalarının etkileri diğerler iki uygulamadan (Darbe ve Kontrol) yüksek olduğu kaydedilmiştir.

Bahçe Bitkileri

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Anahtar Kelimeler
Stres
Fitoaleksin
Resveratrol
UV-C ışını
Şıra

Effect of abiotic stresses on primary / secondary metabolites and resveratrol in cv. Cabernet-Sauvignon

ABSTRACT

The research was located at latitude 41° 01' 11.15" N and longitude 27° 40' 18.00" E, at an altitude of 60 m above sea level, with 15-year-old Cabernet-Sauvignon/110R vines over two years. The vineyard has a planting distance of 2.6×0.9 m, and the vines are trellised to double cordon Royat. In the vineyard, 4 abiotic stress applications (Shock Action, Leaf Injury, UV-C) including the Control were applied twice a day (morning and evening) for 5 days during 3 different phenological stages (Veraison, Veraison-Harvest, and Harvest). The Leaf Injury was performed once by striking the leaves with a rod. The Shock Action was carried out using a plastic hammer, and the UV-C was applied twice a day for 1 minute. As a result, it was determined that the abiotic stress did not cause significant differences in primary metabolites such as Total Soluble Solids (23.69°Brix) and Total Acidity (7.32 g L-1) but had an increasing effect on secondary metabolites (total tannin, anthocyanin, TPC, resveratrol). Additionally, it was found that they had an enhancing effect on the TPI. In terms of resveratrol, the effects of UV-C (0.35 mg kg-1) and Leaf Injury (0.27 mg kg-1) were noted to be higher than the other two (Shock Action and Control).

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INTRODUCTION

Grape ripening, from veraison to harvest, involves significant changes in berry composition, including primary metabolites (sugars, organic acids) and secondary metabolites (phenolic compounds, taste-active molecules, aroma precursors, and aromas) (van Leeuwen et al., 2022). Traditionally, ripeness is determined by measuring Total Soluble Solids (TSS), Total Acidity (TA), or pH of the grape juice.

Temperature, water, light, and CO2 concentration are key abiotic factors that interact with vine and berry development in a manner dependent on the genotype (Keller, 2010; Ferrandino et al., 2023). Rienth et al. (2021) reported that abiotic factors control the synthesis and degradation of primary and secondary metabolites, either directly through biosynthetic pathways or indirectly through vine physiology and phenology.

Secondary metabolites are low molecular weight phenolic compounds that, while not essential for plant life, help defend against abiotic and biotic stress (Billet et al., 2018; Valletta et al., 2021). These include bioactive compounds like anthocyanins, organic acids, tannins, and flavonoids. Secondary metabolites categorized into phenolic compounds, terpenoids, and nitrogen compounds. Their levels vary based on factors such as variety, ripeness, climate, and post-harvest processing.

Phenolic compounds are important indicators of grape berry and wine quality (Candar, 2023a). Gindri et al. (2021) highlighted the importance of anthocyanins in grapes and wine. Moreover, red grape anthocyanins determine the final color of wine, which is a key factor in assessing its quality (Iland et al., 2004; Kennedy, 2010). Xavier Machado et al. (2021) found that grape remains (seeds, skins, etc.) contain about 70% of total phenolic compounds (TPC), including high levels of anthocyanin, gallic acid, catechin, epicatechin, and trans-resveratrol. Environmental factors, especially climatic extremes, can negatively impact the phenolic content of grape varieties. Luzio et al. (2021) speculated that some extent, the rise in secondary metabolites enhances the quality, aroma profiles, and antioxidant capacity of berries, must, and wine.

Valletta et al. (2021) found that stilbenes, including resveratrol (3, 4', 5-trihydroxystilbene), act as phytoalexins and are crucial for plant defense against phytopathogens (Del-Castillo-Alonso et al., 2016; Billet et al., 2018). Resveratrol is a natural phenolic compound produced by plants under stress. Grapes contain more resveratrol than any other natural source. Resveratrol is known to have two isomers: E-trans and Z-cis. The resveratrol found in plants is mostly the -trans isomer. While it is present in the highest amount in the berry skin, it is proportionally less in grape juice and wine (Hasan & Bae, 2017). The resveratrol concentrations in grapes vary by climate and vegetation period, with high levels found in Cabernet-Sauvignon. Such stresses enhance stilbene biosynthesis and accumulation (Valletta et al., 2021). Additionally, resveratrol is a phytoalexin linked to resistance against biotic stresses like Botrytis cinerea and Plasmopara viticola (Langcake & Pryce, 1977; Ferrandino et al., 2023).

Candar, (2023b) stated that wounding is one of the abiotic stress factors. At the same time, Candar (2023a) examined the impact of ten different human-made woundings on the leaves of Cabernet Sauvignon grapevines on the accumulation of grape berry metabolites. The research concluded that wounding treatments have the potential to diversify the phenolic compound profile and can be used for the management of these compounds compared to the control group. Climate change models provide unclear predictions about solar radiation of different wavelengths reaching Earth's surface. Ultraviolet (UV) radiation (100–400 nm) is crucial for the physiology of plants, mammals, humans, and ecosystems due to its high energy and impact (Ballaré et al., 2011). Ultraviolet (UV) rays impact plant morphology and physiology. UV-C light (100-280 nm), which does not reach the biosphere, stimulates the accumulation of phytoalexins in vine leaves and berries (Langcake & Pryce, 1977). Del-Castillo-Alonso et al. (2016) noted that UV temporarily affected phenolic components in berry skin during the growing season. Gindri et al. (2021) found that post-harvest UV-C application to Cabernet-Sauvignon grapes boosted secondary metabolite production and increased resveratrol in treated leaves. Post-harvest UV-C light treatment elevated phenolic compounds in organic grape juice, enhanced antioxidant capacity at low doses, and increased trans-resveratrol content in irradiated grapes. The cv. Cabernet-Sauvignon has high resveratrol production potential, concentrated in the skin for fungal resistance. Resveratrol production in the skin negatively correlates with berry development stages (Jeandet et al., 1991).

In this study, abiotic stress was applied to living vines. These stresses included shock action, leaf injury, and UV-C abiotic stress applications, which were applied under field conditions during three different phenological development stages (veraison, veraison-harvest, and harvest) for 5 days before harvest. The chemistry of grape berries, primary and secondary metabolites, including resveratrol, was examined.

MATERIAL and METHOD

Site selection and plant material

The research was conducted in the vineyards, located at 41° 01’ 11.15” N latitude and 27° 40’ 18” E longitude, at an altitude of 60 m above sea level. The study involved 15-year-old Cabernet-Sauvignon/110R vines. The vineyard's planting distance is 2.6×0.9 m, and the vines are trained in a double-cordon Royat system.

The research was set up using a Randomized Block Design. Four different stress applications (Control, Shock Action, UV-C, and Leaf Injury) were applied to the Cabernet-Sauvignon/110R graft combination vines during 3 different phenological development stages (Veraison, Veraison-Harvest, and Harvest). These applications were conducted with 3 replications, and each plot contained 3 vines. Homogeneity was ensured among the selected vines (Figure 1).

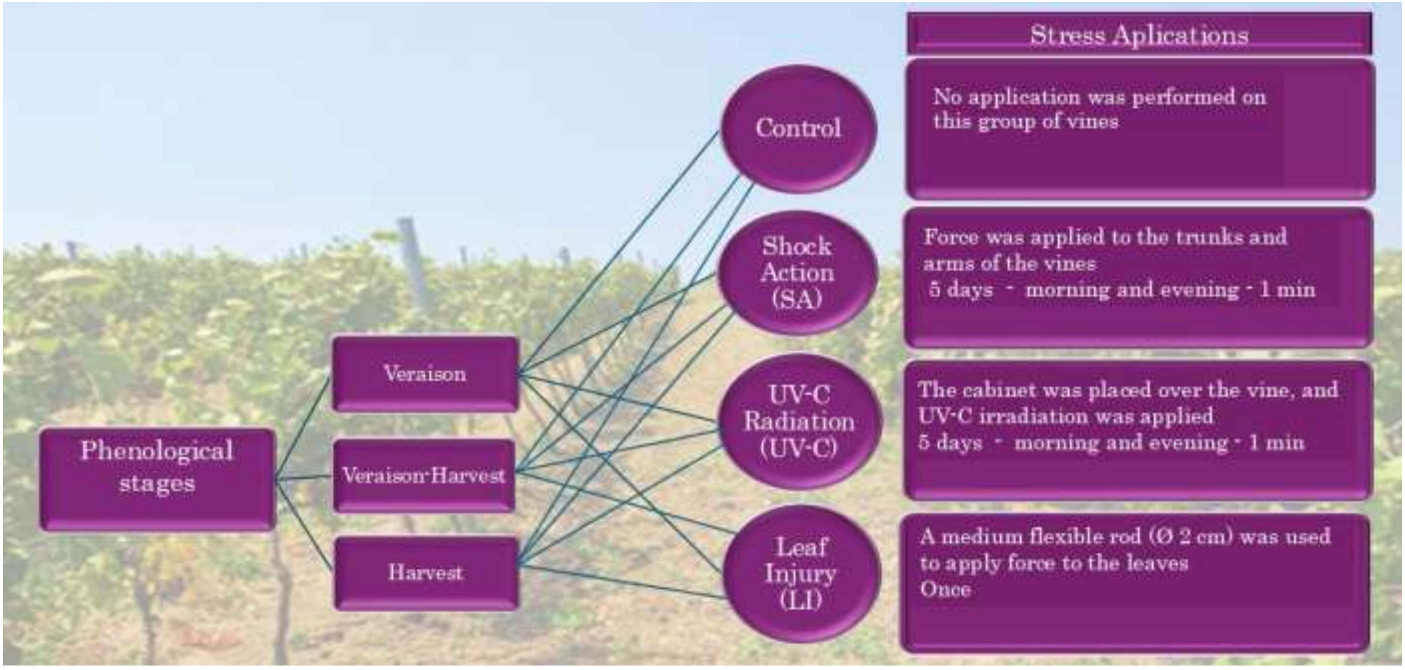

Figure 1. Experimental plan
Şekil 1. Deneme planı

Phenological stages

The stress applications were carried out during the following phenological stages (Coombe, 1995):

Veraison (V) stage: The onset of color change in the clusters and berry softening (EL35),

Veraison-Harvest (V-H) stage: Continued berry softening and color change, approaching harvest (EL35-EL38),

Harvest (H) stage: Berry ripening (EL38).

Harvest was manually conducted on 24.09.2017 and 27.09.2019.

Stress applications

The Shock Action and UV-C light abiotic stress applications were performed twice daily (morning and evening) for 5 days, while the leaf injury application was done once. In this study, shock action and leaf injury, selected as abiotic stress factors, were chosen because they are among the physiological interactions used to enhance grape quality (especially resveratrol accumulation) (Del-Castillo-Alonso et al., 2021; Bahar et al., 2024b).

Control (C): No application was performed on this group of vines.

Shock Action (SA): Force was applied to the trunks and arms of the vines in the vineyard using a plastic-covered hammer. However, the force was not strong enough to damage the vines, only to shake them. In this way, it was applied for 5 days during the veraison, veraison-harvest, and harvest periods. The Shock Action application was performed twice a day, in the morning and evening.

UV-C Radiation (UV-C): A rectangular cabinet with a 254 nm, 30-watt UV-C lamp was used. The cabinet had five sides covered with a light-impermeable membrane. The cabinet was placed over the vine, and UV-C irradiation was applied. The UV-C application was performed twice a day, in the morning and evening. The UV-C cabinet was held over the vine for 1 minute. In this way, it was applied for 5 days during the veraison, veraison-harvest, and harvest periods.

Leaf Injury (LI): A medium flexible rod (Ø 2 cm) was used to apply force to the leaves on both sides of the vine once, aiming to shred some leaves. This application, aimed at breaking into pieces irregularly, was performed once during the veraison, veraison-harvest, and harvest periods.

Grape chemistry and maturity indexes

To determine grape composition, standard measurements of TSS, TA, and pH were performed (Cemeroğlu, 2007). Sugar concentration and maturity indices like TSS/TA and pH^2x°Brix were calculated (Blouin & Guimberteau, 2000).

Secondary metabolites

After removing the seeds, the grapes were crushed, centrifuged, and filtered. Total anthocyanin content was measured using the pH differential method (Cemeroğlu, 2007), total tannin content at 760 nm with the Folin-Denis reagent, and total phenolic content (TPC) at 765 nm with the Folin-Ciocalteu reagent (Merck, Darmstadt, Germany), converted to gallic acid equivalent (Waterhouse, 2002; Kurt et al., 2023). TPI was read at 280 nm (INRA, 2007). Resveratrol was detected using HPLC with a fluorescence detector, and concentration was calculated using LC Solutions software, with a calibration graph (R^2=0.999).

Trial design and statistical analysis

Statistical data analysis was conducted using JMP 17. Analysis of variance (ANOVA) was employed to assess the significance of differences between treatments, and significant differences were further categorized using the LSD test. All results are expressed as the mean of three replications with ± standard error (SE).

RESULTS and DISCUSSION

Total Soluble Solids (TSS) (°Brix)

It has been determined that the TSS value of 2017 (23.97±0.13°Brix) is greater than that of 2019 (23.40±0.20°Brix). When examined in terms of Applications Main Effect (AE), the values are ranked from highest to lowest as C (24.00±0.24°Brix), UV-C (23.69±0.22°Brix), SA (23.64±0.22°Brix), and LI (23.42±0.32°Brix). According to the Phenologic Stage Main Effect (SE), this ranking is V-H (23.58±0.21°Brix), V (23.71±0.23°Brix), and H (23.77±0.21°Brix). The results are in line with the findings of Bahar et al. (2024c) (23.50°Brix to 25.25°Brix), Bahar et al. (2018) (23.13°Brix), Cebrián-Tarancón et al. (2024) (23.60°Brix), and Bindon et al. (2013) (23.01°Brix). However, the research findings contradict those of Bahar & Yaşasin (2010) (21.16°Brix), Antalick et al. (2015) (22.70°Brix), Jiang et al. (2013) (19.86-22.41°Brix), and Chapman et al. (2005) (25.30°Brix); it is thought that this difference may be due to location, soil, etc. On the other hand, Bramley (2005) and Tisseyre et al. (2008) reported that year-to-year variations in TSS values are not an effective parameter for determining grape quality in the following season. The results of this study are consistent with these findings.

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

It has been determined that the total acidity was 7.62±0.09 g L^{-1} in 2017 and 7.02±0.14 g L^{-1} in 2019. According to the SE, the TA values are ranked in descending order as V (7.43±0.16 g L^{-1}), V-H (7.36±0.17 g L^{-1}), and H (7.16±0.19 g L^{-1}). According to the AE, the values are ranked in ascending order as LI (7.19±0.21 g L^{-1}), C (7.25±0.16 g L^{-1}), UV-C (7.31±0.18 g L^{-1}), and SA (7.53±0.18 g L^{-1}). Bahar et al. (2024c) reported that the highest TA value was obtained from LI (8.10 g L^{-1}), similar to the study. Similarly, Chapman et al. (2005) found a value of 6.93 g L^{-1}. On the other hand, Antalick et al. (2015) recorded this value as 4.40 g L^{-1}, and Cebrián-Tarancón et al. (2024) as 5.80 g L^{-1}, which are considerably lower than the findings of this study. Additionally, Bindon et al. (2013) found it to be between 8.30-5.30 g L^{-1}, Bahar & Yaşasin (2010) found it to be 8.64 g L^{-1}, and Jiang et al. (2013) found it to be between 6.3-11.9 g L^{-1}. It is thought that this difference may be due to location, climate, year, etc. However, as noted by Tisseyre et al. (2008), the TA value was also not found to be an effective parameter for determining grape quality in the following season.

pH

In terms of AE, it was found that the C had a pH value of 3.28±0.02, while the others had a value of 3.27±0.01. Regarding SE, it was observed that the V and H periods had a value of 3.26±0.01, while the V-H period had a value of 3.30±0.01. The research findings are consistent with those of Bahar et al. (2018) 3.33; Bahar et al. (2024c) 3.31; Bahar & Yaşasin (2010) 3.39; Bindon et al. (2013) 3.18-3.48; and Jiang et al. (2013) 3.10-3.40. However, the results are not consistent with those researchers who found a pH value of 3.58 (Cebrián-Tarancón et al., 2024), 3.41-3.53 (Candar, 2023a), 3.69 (Antalick et al., 2015), and 3.69 (Chapman et al., 2005). This discrepancy may be due to climate, location, training system, etc. The changes in TSS, TA, and pH values observed in the research were noted to be consistent with the findings of Trought & Bramley (2011) and Baluja et al. (2012), who reported that these parameters are influenced by phenological development stages (V, V-H).

Sugar Concentration (g L^{-1})

The Year Main Effect (YE) was found to be significant for sugar concentration, with the sugar concentration in 2017 determined to be 237.78±1.60 g L^{-1} and the value in 2019 to be 231.62±2.52 g L^{-1}. In similar studies conducted on the Cabernet Sauvignon variety, the sugar concentration was determined to be 205.70 g L^{-1} by Bahar & Yaşasin (2010), 231.10 g L^{-1} by Bahar et al. (2018), and 251.58 g L^{-1} by Bahar et al. (2024c). The differences between the findings of the researchers and the results of this study can be attributed to variations across the years.

Sugar Per Berry (mg $berry^{-1}$)

There was also a difference in the sugar per berry between the years, with a higher value in 2017 (88.73±2.06 mg $berry^{-1}$) compared to 2019 (81.35±2.41 mg $berry^{-1}$). In line with the research findings, Bahar et al. (2024c) reported that the average amount of sugar per gram of berry in the Cabernet Sauvignon variety ranged between 88.22 mg $berry^{-1}$ and 103.00 mg $berry^{-1}$.

Sugar Per Gram of Berry (mg 1 g $berry^{-1}$)

In terms of AE, the values were ranked as LI 77.21±1.27 mg 1 g $berry^{-1}$, SA 77.97±0.85 mg 1 g $berry^{-1}$, UV-C 78.84±0.98 mg 1 g $berry^{-1}$, and Control 79.47±0.98 mg 1 g $berry^{-1}$. Similarly, Bahar et al. (2024c) reported that the average amount of sugar per gram of berry ranged between 75.73 mg 1 g $berry^{-1}$ and 83.84 mg 1 g $berry^{-1}$. Korkutal et al. (2019) found values of V 86.50 mg $berry^{-1}$, Half-Maturity 78.75 mg $berry^{-1}$, and Before Maturity 85.91 mg $berry^{-1}$, which are in line with the research findings.

Maturity Indexes

TSS/TA (g L^{-1})

The TSS/TA values for stress applications were numerically ranked in ascending order as SA 3.15±0.07 g L^{-1}, UV-C 3.25±0.08 g L^{-1}, LI 3.28±0.12 g L^{-1}, and Control 3.32±0.09 g L^{-1}. Regarding the application periods x application interaction, the highest value was obtained from the H x LI interaction at 3.42±0.09 g L^{-1}, and the lowest value from the V x LI interaction at 3.12±0.08 g L^{-1}. The combination with the highest value in the S x A x Y interaction was H x LI x 2019 (3.77±0.11 g L^{-1}). Bahar et al. (2024c) found that this value ranged between 2.97-3.44 g L^{-1}, which is within a similar range to the study.

pH^2x°Brix (g L^{-1})

When considering a pH^2x°Brix value above 260 g L^{-1} as full maturity (Blouin & Guimberteau, 2000), C (257.88±4.80 g L^{-1}) is the closest value. This result is parallel with Candar (2023a) as 259.86 g L^{-1}. This is followed by the UV-C (253.62±2.58 g L^{-1}), SA (252.25±4.13 g L^{-1}), and LI (250.78±4.13 g L^{-1}) applications. Bahar et al. (2018) reported this value to be 255.93 g L^{-1}, and Bahar et al. (2024c) found it to range between 247.97 g L^{-1} and 265.84 g L^{-1}, which aligns with the research findings.

Seconder Metabolites

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

Abiotic stress applications at different stages for Cabernet Sauvignon cv. had statistically significant effects on the total anthocyanin content, considering YE, SE, and S x A, S x A x Y, and S x Y interactions (Table 1). The difference between the trial years was found to be statistically significant. In 2019, the total anthocyanin content (1479±75.33 mg kg^{-1}) was found to be higher than in 2017 (1306±56.19 mg kg^{-1}). The research findings are in line with the observation of Moreno-Olivares et al. (2024) that the experimental years influenced total anthocyanin. In terms of Stage Main Effect (SE), significant differences in total anthocyanin content were observed among the phenological development stages where abiotic stress applications were performed. In H stage (1576±102.44 mg kg^{-1}) has the highest anthocyanin content same as Baluja et al. (2012) findings. The research results align with those of other researchers; in general, anthocyanin content increases rapidly during the first 3-4 weeks following veraison, then stabilizes or undergoes slight changes around harvest (Holt et al., 2010). For the S x A interaction, the highest value was found in the H x SA interaction (1821±303.20 mg kg^{-1}). V x LI (1056±79.89 mg kg^{-1}) and V x SA (1071±68.53 mg kg^{-1}) had the lowest total anthocyanin values. The highest value obtained from the S x A x Y interaction was 2489±109.39 mg kg^{-1} (H x SA x 2019). The lowest value was obtained from V x LI x 2017 (978±54.96

mg kg^{-1}) interaction. In the S x Y interaction, the highest value was obtained in the H x 2019 combination (1844±149.01mg kg^{-1}). The other interactions were in the same significance group. These values align with the findings of Bahar et al. (2024a) 1094 mg kg^{-1}, Bahar et al. (2024c) 1043.841 mg kg^{-1}, and Bindon et al. (2013) 1.37-1.87 mg g^{-1}.

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

The effect of abiotic stress applications applied at different growth stages was found to be statistically significant in terms of YE, AE, SE, S x A, and A x Y interactions (Table 2). The total tannins in 2019 (7.00±0.20 g kg^{-1}) was found to be significantly higher than in year 2017 (4.53±0.14 g kg^{-1}). The findings of Ortega-Regules et al. (2008) that tannin concentration varies across years is consistent with our research. It was determined that the application (AE) that increased the total tannin the most was SA (6.35±0.51 g kg^{-1}). While the UV-C application followed this, the other two applications, LI and C, were in the same significance group. When SE was examined, the H (6.06±0.34 g kg^{-1}) was recorded as the period with the highest total tannin value. The V (5.53±0.32 g kg^{-1}) had the lowest total tannin value. In terms of the S x A interaction, H x UV-C had the highest value at 6.80±0.63 g kg^{-1}, while V x LI had the lowest value at 4.55±0.78 g kg^{-1}. On the other hand, for the A x Y interaction, the SA x 2019 interaction had the highest (8.38±0.19 g kg^{-1}), all applications in 2017 recorded the lowest values. The obtained results are consistent with the findings of Bahar et al. (2024c) 3.23 g kg^{-1}-4.26 g kg^{-1}, Korkutal et al. (2019) 8475.20 mg kg^{-1} in the V period, and Bindon et al. (2013) 3.26-4.15 mg g^{-1}. However, it conflicts with the findings of Jiang et al. (2013) (2.3-5.3 g L^{-1}), which may be due to the research location (China).

Total Phenolic Index (TPI)

In terms of TPI, the effects of YE, AE, SE, and S x Y are statistically significant. Accordingly, it was observed that the TPI value for 2019 (7.95±0.32) was higher than the value for 2017 (7.13±0.45). In terms of AE, abiotic stress applications were grouped together (SA 8.24±0.35; LI 7.98±0.32; and UV-C 7.74±0.30). C (6.21±0.22) has the lowest TPI value. According to SE, H had the highest TPI value at 8.23±0.34. V-H and V stages followed this stage. When the S x Y interaction was examined, it was determined that the V x 2017 interaction (5.32±0.12) had the lowest TPI. For the Cabernet Sauvignon cv., Blouin & Guimberteau (2000) reported a TPI value of 13.30; Bahar et al. (2024a) 6.00; Bahar et al. (2024c) 9.76; and Bahar et al. (2018) between 5.31-6.87. The obtained TPI values align with the findings of researchers other than Blouin & Guimberteau (2000).

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

In cv. Cabernet Sauvignon, statistical differences in TPC were found between YE, AE, SE, A x Y, and S x Y interactions (Table 3). The TPC for 2017 (3414±86.99 mg kg^{-1}) is lower than that for 2019 (3889±136.47 mg kg^{-1}). Ramos et al. (2024) reported that changes in temperature and rainfall can also affect grape phenolic content, and therefore grape quality. It has been suggested that the difference in TPC between the years may have resulted from this. In terms of AE, SA (3939±213.19 mg kg^{-1}) and UV-C (3771±152.36 mg kg^{-1}) are in the same importance group. The LI (3578±184.05 mg kg^{-1}) application is in the second importance group, while C is in the last importance group (3318±77.43 mg kg^{-1}). In terms of SE, H had the highest value (3937±171.43 mg kg^{-1}), and V had the lowest value (3301±135,43 mg kg^{-1}). The findings of Bahar et al., (2024c) at 3268.99 mg kg^{-1} for TPC are in line with the research. In terms of the S x Y interaction, SA x 2019 (4577±288.35 mg kg^{-1}) had the highest TPC value, while C x 2017 (3235±116.44 mg kg^{-1}) had the lowest TPC value. It should not be overlooked that the H x 2019 interaction (4418±263.84 mg kg^{-1}) also had the highest TPC value.

Resveratrol (mg kg^{-1})

It was found that only the AE has a statistically significant effect on resveratrol concentration (Table 4). The UV-C abiotic stress application (0.35±0.06 mg kg^{-1}) was determined to be the most effective in increasing the resveratrol value in the Cabernet Sauvignon variety. The research findings are consistent with the finding that UV-C and leaf wounding treatments were effective in enhancing *trans*-resveratrol levels in *Cabernet Sauvignon* at harvest time (Bahar et al., 2024c). This was followed by LI (0.27±0.05 mg kg^{-1}), while C (0.07±0.02 mg kg^{-1}) and SA (0.05±0.02 mg kg^{-1}) were in the third importance group. The findings of Romero-Pérez et al. (1999), which reported 0.50 mg L^{-1} *trans*-resveratrol and 0.06 mg L^{-1} *cis*-resveratrol in red grape juice, are consistent with the research. Çaylak et al. (2009) recorded the resveratrol content in Marmara Region wines as 0.252 mg L^{-1}. In 2017, resveratrol values ranged between 0.08-0.28 mg kg^{-1} among the applications, while in 2019, resveratrol values ranged between 0-0.42 mg kg^{-1}. Candar (2023a) reported that *trans*-resveratrol ranged between 0.36-3.59 mg kg^{-1}. Specifically, it was determined that leaf wounding applied 15 days before harvest increased the *trans*-resveratrol content by 35.78% compared to the Control group. Numerically, the high value in SE was recorded for V-H (0.22±0.04 mg kg^{-1}).

Table 1. The effects of abiotic stresses applied during different growth stages on the total anthocyanin content in the cv. Cabernet-Sauvignon

Çizelge 1. Farklı gelişme dönemlerinde uygulanan abiyotik streslerin Cabernet-Sauvignon üzüm çeşidinde toplam antosiyanin miktarı üzerine etkileri

Stage	Apps	S x A x Y int.			A x Y int.				S x Y int.			
		2017	2019	S x A		2017	2019	AE		2017	2019	SE
V	C	1539±391.76 BCDE	1208±129.04 CDE	1373±198.78 **BCD**								
	SA	1142±127.72 CDE	1000±45.54 DE	1071±68.53**D**	C	1294±160.49	1291±82.58	1293±87.55	V	1225±109.83 B	1168±58.59 B	1197±61.16 **b**
	UV-C	1242±95.55 CDE	1332±71.54 CDE	1287±57.04 **CD**								
	LI	978±54.96 E	1135±150.81 CDE	1056±79.89 **D**								
V-H	C	1274±291.91 CDE	1423±163.03 BCDE	1348±153.19 **BCD**	SA	1222±72.67	1559±237.24	1391±127.08				
	SA	1371±168.60 CDE	1187±59.04 CDE	1279±89.79 **CD**					V-H	1385±91.42 B	1426±85.89 B	1405±61.48 **ab**
	UV-C	1576±209.90 BCDE	1369±177.23 CDE	1473±130.33 **ABCD**								
	LI	1319±26.48 CDE	1723±162.98 BC	1521±116.76 **ABC**	UV-C	1446±96.21	1576±126.28	1511±78.61				
H	C	1070±123.04 DE	1244±159.21 CDE	1157±98.00 **CD**								
	SA	1154±45.79 CDE	2489±109.39 A	1821±303.20 **A**					H	1307±92.54 B	1844±149.01 A	1576±102.44 **a**
	UV-C	1520±164.86 BCDE	2028±37.83 AB	1774±136.56 **AB**	LI	1261±106.62	1491±117.79	1376±82.00				
	LI	1485±256.43 BCDE	1617±137.20 BCD	1551±133.33 **ABC**								
YE		1306±56.19 b	1306±75.33 b									

YE *p*<0.1=134.1977; S x A x Y intr. *p*<0.1=620.1465; S x A intr. *p*<0.1=438.5098; S x Y intr. *p*<0.01=310.0732; SE *p*<0.01=286.5193

V (Veraison), V-H (Veraison-Harvest), H (Harvest), UV-C (UV-C Light), LI (Leaf Injury), C (Control), SA (Shock Action), AE (Application Main Effect), SE (Phenologic Stage Main Effect), YE (Year Main Effect), S x A x Y intr. (Stage X Application X Year interaction), A x Y intr. (Application X Year interaction), S x Y intr. (Stage X Year interaction). Results expressed as mean of three replications with ± SE.

Table 2. The effects of abiotic stresses applied during different growth stages on the total tannin content in the cv. Cabernet-Sauvignon
Çizelge 2. Farklı gelişme dönemlerinde uygulanan abiyotik streslerin Cabernet-Sauvignon üzüm çeşidinde toplam tanen miktarı üzerine etkileri

Stage	Apps	S x A x Y int. 2017	S x A x Y int. 2019	S x A		A x Y int. 2017	A x Y int. 2019	AE		S x Y int. 2017	S x Y int. 2019	SE
V	C	4.90±0.50	6.52±0.15	5.71±0.43 *ABCD*	C				V			
	SA	4.61±0.28	8.19±0.51	6.40±0.84 *AB*		4.76±0.27 C	6.09±0.20 B	5.43±0.23 **B**		4.41±0.26	6.66±0.38	5.53±0.32 b
	UV-C	4.92±0.43	6.00±0.43	5.46±0.36 *ABCD*								
	LI	3.19±0.09	5.91±1.08	4.55±0.78 *D*	SA							
V-H	C	4.03±0.44	5.75±0.49	4.89±0.48 *CD*		4.31±0.19 C	8.38±0.19 A	6.35±0.51 **A**	V-H			
	SA	4.04±0.22	8.35±0.05	6.20±0.96 *ABC*						4.44±0.25	6.98±0.34	5.71±0.33 ab
	UV-C	4.18±0.21	6.22±0.23	5.20±0.47 *BCD*	UV-C							
	LI	5.50±0.60	7.62±0.38	6.56±0.57 *AB*		4.84±0.23 C	6.8±0.38 B	5.81±0.32 **AB**				
H	C	5.36±0.12	6.03±0.30	5.69±0.20 *ABCD*					H			
	SA	4.30±0.48	8.62±0.38	6.46±1.00 *AB*	LI					4.76±0.24	7.37±0.34	6.06±0.34 a
	UV-C	5.42±0.12	8.18±0.31	6.80±0.63 *A*		4.21±0.40 C	6.73±0.41 B	5.47±0.41 **B**				
	LI	3.95±0.42	6.64±0.15	5.30±0.63 *BCD*								
YE		4.53±0.14 **b**	7.00±0.20 **a**									

YE $p<0.1=0.5135$; S x A x Y intr. $p<0.1=1.4502$; A x Y intr. $p<0.1=1.1840$; AE $p<0.1=0.8372$; SE $p<0.5=0.4159$
V (Veraison), V-H (Veraison-Harvest), H (Harvest), UV-C (UV-C Light), LI (Leaf Injury), C (Control), SA (Shock Action), AE (Application Main Effect), SE (Phenologic Stage Main Effect), YE (Year Main Effect), S x A x Y intr. (Stage X Application X Year interaction), A x Y intr. (Application X Year interaction), S x Y intr. (Stage X Year interaction). Results expressed as mean of three replications with ± SE.

Table 3. The effects of abiotic stresses applied during different growth stages on the TPC in the cv. Cabernet-Sauvignon

Çizelge 3. Farklı gelişme dönemlerinde uygulanan abiyotik streslerin Cabernet-Sauvignon üzüm çeşidinde toplam fenolik madde miktarı üzerine etkileri

Stage	Apps	S x A x Y int. 2017	S x A x Y int. 2019	S x A
V	C	3194±336.40	3277±125.04	3236±161.57
	SA	3259±96.93	3738±453.33	3499±233.33
	UV-C	3144±165.79	3430±57.19	3287±101.25
	LI	3543±889.70	2820±503.46	3181±484.89
V-H	C	3127±136.47	3299±142.10	3213±96.06
	SA	3474±200.21	4494±79.03	3984±247.49
	UV-C	3538±215.80	3843±210.29	3690±150.98
	LI	3862±237.10	4099±129.71	3980±131.91
H	C	3383±115.26	3626±220.20	3505±123.66
	SA	3171±163.21	5499±77.31	4335±526.62
	UV-C	3680±60.35	4993±156.06	4336±302.79
	LI	3594±359.24	3553±126.70	3574±170.60
YE		3414±86.99 **b**	3889±136.47 **a**	

A x Y int.	2017	2019	AE
C	3235±116.44 C	3401±101.03 BC	3318±77.43 *b*
SA	3302±91.48 BC	4577±288.35 A	3939±213.19 *a*
UV-C	3454±113.61 BC	4088±246.18 AB	3771±152.36 *a*
LI	3666±2859.58 BC	3491±241.19 BC	3578±184.05 *ab*

S x Y int.	2017	2019	SE
V	3285±212.04 **C**	3316±178.03 **C**	3301±135.43 *b*
V-H	3500±116.03 **BC**	3933±145.21 **AB**	3717±101.56 *ab*
H	3457±106.70 **BC**	4418±263.84 **A**	3937±171.43 *a*

YE p<0.01=406.4150; A x Y intr. p<0.01=812.8301; SE p<0.01=497.7547; AE p<0.5=439.8252; S x Y intr. p<0.5=538.6740

V (Veraison), V-H (Veraison-Harvest), H (Harvest), UV-C (UV-C Light), LI (Leaf Injury), C (Control), SA (Shock Action), AE (Application Main Effect), SE (Phenologic Stage Main Effect), YE (Year Main Effect), S x A x Y intr. (Stage X Application X Year interaction), A x Y intr. (Application X Year interaction), S x Y intr. (Stage X Year interaction). Results expressed as mean of three replications with ± SE.

Table 4. The effects of abiotic stresses applied during different growth stages on the *trans*-resveratrol levels in the cv. Cabernet-Sauvignon
Çizelge 4. Farklı gelişme dönemlerinde uygulanan abiyotik streslerin Cabernet-Sauvignon üzüm çeşidinde resveratrol miktarı üzerine etkileri

Stage	Applications	S x A x Y int.		
		2017	2019	S x A
V	C	0.05±0.05	0.00±0.00	0.03±0.02
	SA	0.05±0.05	0.00±0.00	0.03±0.02
	UV-C	0.26±0.25	0.52±0.26	0.39±0.17
	LI	0.26±0.14	0.27±0.01	0.26±0.06
V-H	C	0.15±0.02	0.14±0.07	0.15±0.03
	SA	0.08±0.07	0.00±0.00	0.04±0.03
	UV-C	0.37±0.08	0.46±0.09	0.42±0.05
	LI	0.20±0.08	0.39±0.21	0.29±0.11
H	C	0.05±0.04	0.05±0.04	0.05±0.03
	SA	0.18±0.11	0.00±0.00	0.09±0.06
	UV-C	0.20±0.02	0.28±0.13	0.24±0.06
	LI	0.18±0.17	0.29±0.24	0.24±0.13
YE		0.17±0.032	0.20±0.04	

	A x Y int.		
	2017	2019	AE
C	0.08±0.02	0.06±0.03	0.07±0.02 B
SA	0.10±0.04	0.00±0.00	0.05±0.02 B
UV-C	0.28±0.08	0.42±0.09	0.35±0.06 A
LI	0.21±0.07	0.32±0.09	0.27±0.05 AB

	S x Y int.		
	2017	2019	SE
V	0.16±0.07	0.20±0.08	0.18±0.05
V-H	0.20±0.04	0.25±0.07	0.22±0.04
H	0.15±0.05	0.15±0.07	0.15±0.04

YE $p<0.01=0.2521$
V (Veraison), V-H (Veraison-Harvest), H (Harvest), UV-C (UV-C Light), LI (Leaf Injury), C (Control), SA (Shock Action), AE (Application Main Effect), SE (Phenologic Stage Main Effect), YE (Year Main Effect), S x A x Y intr. (Stage X Application X Year interaction), A x Y intr. (Application X Year interaction), S x Y intr. (Stage X Year interaction). Results expressed as mean of three replications with ± SE.

CONCLUSION

-Abiotic stresses have been observed to have a greater effect on increasing secondary metabolites than on primary metabolites.

-For total tannins: SA (6.35±0.51 mg kg^{-1}), UV-C (5.81±0.32 mg kg^{-1}), and LI (5.47±0.41 mg kg^{-1}) provided the highest values, while C (5.43±0.23 mg kg^{-1}) gave the lowest value. If an increase in total tannins is desired, these three applications can be used.

-In terms of total anthocyanins: UV-C (1511.64±78.61 mg kg^{-1}), SA (1391.05±127.08 mg kg^{-1}), and LI (1376.51±82.00 mg kg^{-1}) were found to be more effective than C (1293.38±87.55 mg kg^{-1}).

-For increasing TPC: SA (3939.75±213.19 mg kg^{-1}) and UV-C (3771.82±152.36 mg kg^{-1}) were found to be more effective than the other.

-Regarding *trans*-resveratrol: The UV-C (0.35±0.06 mg kg^{-1}) application was found to have higher values compared to LI (0.27±0.05 mg kg^{-1}) and the other applications.

When evaluated by phenological stages:

-H stood out with the highest values for total tannins (6.06±0.34 mg kg^{-1}), total anthocyanins (1576.34±102.44 mg kg^{-1}), TPC (3937.92±171.43 mg kg^{-1}), and TPI (8.32±0.39).

-For resveratrol, the V-H (0.22±0.04) showed high values.

As a result, in Tekirdağ conditions, Shock Action application is recommended 5 days before the Harvest to increase total tannins, TPC, and TPI. Additionally, UV-C and Leaf Injury applications are also considered viable. To increase total anthocyanins, UV-C application is recommended 5 days before harvest. For resveratrol increase, it is suggested to perform UV-C and Leaf Injury treatments during the Veraison-Harvest period. These research results are considered useful in determining the applications for increasing the important bioactive compound *trans*-resveratrol.

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Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest

The authors declare no conflict of interest.

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