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Drought Resilience in Wine Grapes: Genotypic Influence on Leaf Micronutrient Dynamics

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Abstract: This study aimed to examine variations in the micronutrient content of leaves in two-year-old indigenous and widely recognized grapevine cultivars under different levels of water stress. Eight wine grape cultivars (‘Adakarası,’ ‘Papazkarası,’ ‘Karasakız,’ ‘Karalahana,’ ‘Yapıncak,’ ‘Vasilaki,’ ‘Cabernet Sauvignon,’ and ‘Sauvignon Blanc’) were grown in pots as own-rooted plants and subjected to five distinct irrigation treatments—100%, 75%, 50%, and 25% of available water capacity (AWC), as well as the no-irrigation condition. The experiment was conducted over two consecutive years, from May to September, using a computer-controlled nutrition and irrigation system under semi-controlled conditions. The findings revealed that, except for sodium (Na), all micronutrient concentrations in leaf tissues increased in the second year of the study. The response of leaf micronutrient content to varying AWC levels was predominantly influenced by the cultivars' genotypic characteristics rather than the irrigation regime. Additionally, significant correlations were observed among micronutrient levels.

Keywords: Water shortage, indigenous cultivars, irrigation, micro elements

Şaraplık Üzümlerde Kuraklığa Dayanıklılık: Yaprak Mikro Besin Dinamikleri Üzerindeki Genotipik Etki

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Öz: Bu çalışmada, iki yaşındaki yerli ve yaygın olarak bilinen asma çeşitlerinin yaprak mikro besin element içeriklerinin farklı su stresi seviyeleri altında değişimlerinin incelenmesi amaçlanmıştır. Sekiz şaraplık üzüm çeşidi (‘Adakarası’, ‘Papazkarası’, ‘Karasakız’, ‘Karalahana’, ‘Yapıncak’, ‘Vasilaki’, ‘Cabernet Sauvignon’ ve ‘Sauvignon Blanc’) saksılarda kendi kökleri üzerinde yetiştirilmiş ve kullanılabilir su kapasitesinin (KSK) %100, %75, %50, %25’i ve hiç sulama yapılmayan koşul olmak üzere beş farklı sulama uygulamasına tabi tutulmuştur. Deneme, yarı kontrollü koşullarda, bilgisayar kontrollü bir besleme ve sulama sistemi kullanılarak Mayıs-Eylül ayları arasında, iki yıl üst üste yürütülmüştür. Bulgular, sodyum (Na) elementi hariç tüm mikro besin elementlerinin yaprak dokularındaki konsantrasyonlarının çalışmanın ikinci yılında arttığını ortaya koymuştur. Yapraklardaki mikro besin elementlerinin KSK seviyelerine tepkisi, sulama rejiminden ziyade çeşitlerin genetik özelliklerinden etkilenmiştir. Ayrıca, mikro besin elementleri düzeyleri arasında anlamlı korelasyonlar tespit edilmiştir.

Anahtar Kelimeler: Su kısıtı, yerli çeşitler, sulama, mikro elementler

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Introduction

The globe is experiencing growing water shortages, and the farming industry heavily relies on water supplies, rendering it susceptible to the effects of drought. Drought is a persistent issue that impacts numerous global regions and can significantly affect plant development and yield. Grapevines (*Vitis vinifera* L.) are among the most extensively cultivated crops worldwide, and their performance relies heavily on sufficient water availability (Jones, 2007; Tóth & Végvári, 2015; Santos et al., 2020). The ability of grapevines to withstand dry conditions is a vital element in their success, especially in areas where water is scarce.

The farming sector is encountering significant obstacles due to the rising occurrence and severity of droughts, resulting in water shortages on a global magnitude, as highlighted by the research of Gambetta et al. (2020), Santos et al. (2020) and Kizildeniz et al. (2021).

Key indirect climate crisis impacts that limit productivity and influence grape composition include drought, soil erosion and salinity. Areas characterized by a Mediterranean climate face the potential threat of compromised wine sustainability in forthcoming scenarios (Fraga et al., 2016; Santos et al., 2020).

Grapevine response to water deficit encompasses a multifaceted interplay between genetic factors, physiological processes, and biochemical pathways (Trenti et al., 2021). The impact of water deficit on woody plants manifests as a disruption in water transport and carbon assimilation (Vandegehuchte et al., 2015).

Water scarcity on grapevines can manifest as both temporary and permanent changes. Temporary responses include diminished cell turgidity (Patakas & Noitsakis, 1999), delayed vegetative growth (Kizildeniz et al., 2015; 2021), decreased stomatal aperture (Charrier et al., 2018), impaired photosynthesis (Chaves et al., 2010), and reduced berry size (Candar et al. 2019; Kizildeniz et al., 2018). Permanent responses include chlorosis and leaf degeneration, berry shrinkage, retarded sugar storage (Candar et al., 2019; Kizildeniz et al., 2018), lightening of berry color, and stem wood lignification (Bahar et al., 2011; Candar et al., 2022a). Grapevines are widely regarded as one of the most adaptable woody species capable of thriving in dry conditions (de Ollas et al., 2019). This flexibility stems from their ability to regulate osmotic potential (Patakas & Noitsakis, 1999; Rodrigues et al., 1993).

Patakas et al. (2002) propose that the buildup of osmotically active compounds help sustain cell turgor pressure and metabolic functions during times of water shortage. Furthermore, soil water content significantly impacts the complex interplay among grapevine functionality, soil properties, physiological responses, and berry quality (Candar et al., 2022a; Oertel et al., 2016; Paustian et al., 2016). Additionally, factors like temperature of soil, organic matter content, and porosity also significantly influence the speed of mineralization. Nevertheless, soil humidity and nutrient accessibility are the two main soil-related factors that interact in affecting crop production and quality.

Although the effects of fertilization, nutrient capacity, and different fertilizers on berry yield and quality have been thoroughly studied, their impact on grapevine physiology and performance has garnered minimal focus (Brataševec et al., 2013; Martínez et al., 2016; Zamudio et al., 2021).

Alterations in soil nutrition use can have a significant impact on grapevine productivity and berry quality. The potential for such changes is determined by factors such as climate, ambient CO_2 concentrations and varietal properties. However, yield and fruit ripening are often limited by the availability of resources in the root zone, including nutrients and water, as well as pest and disease pressure.

Understanding the interplay between the vine, water, and nutritional resources has become an increasingly pressing challenge for fruit production, particularly given the need to address the consequences of changing climate in severe conditions (Carvalho et al., 2019; Villette et al., 2020).

The presence of micronutrients is a vital element in regulating the vegetative development of grapevines and the qualities of the resulting fruit harvest. Essential microelements such as iron, manganese, and zinc are crucial for the normal functioning of the grapevine and its ability to produce high-quality fruit. However, water stress can disrupt the absorption and partitioning of these vital elements, resulting in suboptimal nutrition and reduced productivity. Microelements exhibit a profound impact that transcends the grapevine's growth, development, and yield, as they play role in shaping the quality of the produced wine. Proper concentrations of critical micronutrients facilitate the development of premium-grade berries, which enhance the flavor, fragrance, and overall excellence of wine.

The reality of global warming is now widely accepted. To mitigate the challenges associated with this occurrence, short-term interventions, including canopy management, application of sunscreens, supplemental drip irrigation, tillage, and preparedness for emerging vineyard pests and diseases, warrant consideration. Researchers have advocated for more long-term approaches, such as modifying the training system, selecting alternative clones or rootstocks, using different cultivars, or changing the growing location (Carbonneau & Bahar 2009; OIV 2014).

Biodiversity found in viticultural nations provides substantial prospects for modifications in clones, rootstocks, and grape varieties within the wine industry. The potential advantages offered by this diversity, as well as the native *Vitis vinifera* L. cultivars, have been thoroughly investigated. To foster sustainable winemaking in emerging vineyard regions and guarantee the sustained feasibility of Mediterranean vineyards, it is crucial to capitalize on the adaptation and genetic diversification of indigenous gene resources (Vouillamoz et al., 2006; Ergül et al., 2011; Hizarci et al., 2012; Balda et al., 2014; Yılmaz et al., 2020). To promote sustainable winemaking in new vineyard zones and secure the viability of Mediterranean vineyards in the future, it is necessary to take advantage of the adaptation and genetic diversification of indigenous gene resources (Bernardo et al., 2021; Candar et al. 2021). Adopting eco-friendly winemaking in novel vineyard regions and ensuring the long-term viability of Mediterranean vineyards necessitates leveraging the adaptive capacity and genetic diversity of native germplasm. The selection of appropriate rootstocks and cultivars can enhance sustainability by influencing input expenditures and waste management procedures within the vineyard, such as labor allocation, water usage, nutrient removal, soil stewardship, and minimizing vehicular movement between rows.

In recent years, there has been increasing scholarly attention on utilizing indigenous grape varieties as a method for adjusting to changing climate conditions (Capozzi et al., 2015). These regionally adapted cultivars have developed over time in response to the distinct ecological conditions of their areas, including the occurrence of drought. They have developed strategies to cope with water stress, such as deep root systems, efficient water use, and increased drought tolerance.

When it comes to plant nutrition and creating sustainable global food systems, it's crucial for everyone involved to approach nutrient management in a holistic manner. This means considering the entire life cycle of nutrients and tackling the various challenges associated with them in the food system. To achieve these goals, it's essential to come up with integrated and targeted strategies and practices for plant nutrition that strike a balance between productivity and environmental concerns. According to Dobermann et al. (2023), these solutions must be appropriate for diverse agriculture and economic sectors in different areas, countries and communities. One of the key factors in accomplishing these objectives is to identify the nutritional requirements of local plant varieties.

This paper seeks to address a gap in the current knowledge regarding the influence of varying degrees of drought stress on the micronutrient status of young grapevines. The research employs a controlled experimental approach, exposing local grapevine cultivars to varying levels of water shortage and measuring the impacts on the concentrations of essential microelements in the leaves of local grapevine. Understanding the relationship between water availability and the microelement status of local grapevines will provide information for vineyard managers, helping them to optimize their water management practices to improve the microelement status of their crops. Thus, increasing productivity and quality, as well as assisting policymakers in guiding the development of more ecologically sustainable and adaptable grapevine cropping systems.

Materials and Methods

Experimental Location and Cultivars

The research was carried out throughout the 2019–2020 growing seasons in the trial plots of the Viticulture

Research Institute, located at the specified geographical coordinates 40.96°N - 40.97°N latitude and 27.46°E - 27.47°E longitude, at an elevation of 30-35 meters in Tekirdağ, Türkiye.

In this trial, two years old cuttings of ‘Cabernet Sauvignon,’ ‘Sauvignon Blanc’ ‘Adakarası’ (clone 153), ‘Karalahana’, ‘Papazkarası’ (clone 289), ‘Yapıncak, ‘Karasakız’ (clone 175), and ‘Vasilaki’, (*Vitis vinifera* L.) cultivars were used. Grapevines were cultured on their own roots, without grafting. The local grapevine cultivars used in the experiment were preferred because of their remarkable wine quality, which they have been adapted to the region for many years, and the rising interest of vignerons and consumers in the recent period. The grapevine cultivars ‘Cabernet Sauvignon’ and ‘Sauvignon Blanc’ were chosen because of their contrasting reactions to drought stress (Simonneau et al., 2017).

Trial Conditions and Experimental Design

An automated irrigation and fertilization system was installed in the outdoor plots. The cuttings, comprising 7-8 buds, were sourced from virus-free mother plants that had been rigorously screened for major viruses from the vineyards at the Tekirdağ Viticulture Research Institute (TVRI).

Grapevines were planted and cultivated in 14-liter plastic pots filled with perlite medium (Kale Perlit, Türkiye) until they reached 14 to 16 leaves with a length of 170-175 cm, approximately corresponding to the phase EL 29-31 (Lorenz et al., 1995).

Prior to reaching the expected shoot length, at approximately EL 15-17, all clusters and unwanted stems were detached, and only 2 to 3 stems were retained on each single grapevine. During the experiment, the primary shoots of the grapevine were maintained at a length between 170 and 175 cm, while the lateral stems were pruned down to 3 to 4 leaves. Upon completion of the first experimental year, the grapevines were trimmed back to 2 to 3 buds. This cultivation strategy was then replicated in the second year.

After the vines attained a consistent shoot length (EL 29-31) following cluster removal, after determining the perlite’s water retention capacity, plants were watered with a standardized amount corresponding to that limit. The irrigation volume was then forecasted, and a daily irrigation regimen was established per pot to induce water stress in the plants.

A daily maximum irrigation limit of 8 L was established based on Ilahi and Ahmad (2017), and lower water volumes of 6 L, 4 L, and 2 L were implemented according to the reference evapotranspiration (ETo). In addition, another application was made without irrigation. Thus, 8L of total water per day equals 100% daily available water capacity (AWC) AWC_{100}, 6L total water amount AWC_{75}, 4L total water amount AWC_{50}, 2L total water AWC_{25} and 0 L total water AWC_0 treatments were formed. The computerized system determined the amount of water to be applied per application by dividing the daily total into five portions across the day. This method allowed for the simulation of water-constrained conditions in a controlled environment. The irrigation schedule, including the daily amount, daily total time and application dates during the experimental years was thoroughly documented in Candar et al. (2021; 2022b; 2023). At the beginning of the water restriction period, the pots were closed with plastic bags to expose grapevine stems and to keep the growing medium in the pots free from undesired rainfall, although there was no precipitation during the water restriction period in the two years.

Experimental trials employed slightly adapted versions of the Hoagland and Arnon (1950) methodology. Fertilization was customized based on the developmental stage of the plants, their phenology, and the specific aims of the research. The solution was modified in accordance with the growing rate of the grapevines, the phenological period, and the research objectives. Analysis of the solutions performed three times in both years revealed no deficient nutrient elements (Candar et al., 2021). The field was managed for weed control, and the grapevines were protected against diseases and pests in accordance with local standards in both experimental years.

The study followed a completely randomized block structure, incorporating three replicates, each containing eight vines, resulting in a total of 960 vines. There were five treatments applied in compliance with the randomized block trial pattern. In the year 2019 there were only ‘Adakarası’, ‘Papazkarası’, ‘Karalahana’, ‘Yapıncak’, ‘Vasilaki’, ‘Cabernet Sauvignon’ and ‘Sauvignon Blanc’ cultivars due to missing plants in the ‘Karasakız’ cultivar during the first experiment year; data of ‘Karasakız’ were only collected in 2020.

Sample Collection, Analysis of Micronutrients in the Leaves

Ten healthy, fully developed leaves per plant were randomly selected among the 5th to 7th leaves from the tip of the shoot to determine microelements as described by Dami and Smith (2019). The foliage was collected during the last week of September 2019 and the first week of October 2020, one week following the termination of the deficit irrigation regimes. Samples were obtained from the complete leaves rather than the petioles of the young plants, as this allowed for the collection of a sufficient quantity for analysis. According to Cancela et al. (2018), leaf blades are more suitable for nutrient analysis than petioles. Therefore, a dry matter blend of both blades and petioles was used for the examination.

The leaf samples were processed in the laboratory. They were rinsed with tap water, followed by a 0.1 N HCl solution to eliminate surface contaminants, finally rinsed with distilled water. After shade drying, the samples were placed in a laboratory oven at 70°C until they reached a stable weight. The dried samples were then ground and processed for analysis, following the methodology described by Benito et al. (2013). The leaf samples were analyzed using ICP-OES equipment (Inductively Coupled Plasma, Spectro, Spectroblue FMX36, Germany) at the Namık Kemal University Central Research Laboratory to detect the presence of Iron (Fe), Copper (Cu), Zinc (Zn), Manganese (Mn), Boron (B), Molybdenum (Mo), and Sodium (Na) elements.

Climate Data

Descriptive mesoclimate weather data, such as temperature, relative humidity, light intensity, wind speed, and total precipitation, were monitored for two consecutive years using a weather station located within the experimental area. The data was collected at a height of two meters above ground.

Statistical Analysis

The ANOVA was performed using R statistical environment (R Core Team, 2016) and the packages agricolae, ggplot2, and pls. The data was normalized before analysis. Two-way ANOVA was used to identify differences in related parameters, taking into consideration the factors of treatment (AWC100, AWC75, AWC50, AWC25, and AWC0) and cultivar for consecutive years followed by Tukey multiple range tests at 5%. Moreover, the bivariate relationships of the data were analyzed. A hierarchical clustering analysis (HCA) was also performed to compare the quantitative data using Ward's method with squared Euclidean distance. Additionally, a Principal Component Analysis (PCA) was conducted utilizing the JMP 17.0 statistical software.

Results and Discussion

Climate Data

Tekirdağ experiences a Mediterranean climate typified by sweltering and arid summers as well as temperate winters, which corresponds to the Csa classification within the Köppen-Geiger climate system based on long-term data (Öztürk et al., 2017; Yılmaz & Çiçek, 2018). Precipitation is concentrated primarily during the winter and spring months. Inland regions exhibit a continental climate pattern, which leads to cooler winter temperatures relative to coastal areas (Papp & Sabovljević, 2003).

Based on long-term climate data spanning from 1939 to 2019 in Tekirdağ, the annual mean temperature is 14.0°C. The coldest month is January, with an average temperature of 4.7°C, while the hottest month is August, with an average temperature of 23.8°C. The region experiences an annual average precipitation of 589.5 mm. The period with the highest rainfall extends from October to March, whereas the mean precipitation during the vegetation period amounts to 196.70 mm.

In 2019, the average temperature during the vegetation period was 20.73°C, while in 2020 it was 20.2°C. In comparison, the long-term average temperature for the vegetation period between 1939 and 2019 was 19.5°C. The typical growing degree day (GDD) in Tekirdağ is calculated as 1872 day-degrees based on the average from 1939 to 2019. However, in 2019, this value was 2157 day-degrees, while in 2020, it was 2124 day-degrees. In recent years, a noticeable shift in climate classification based on GDD has been observed. During the periods of restricted irrigation in both trial years, no precipitation was recorded. When examining the Hydrothermic Index (HyI), the long-term average is 3595.20°C mm. Nevertheless, in the last two years, it has been calculated as 2181.54°C mm and 1328.10°C mm, respectively, indicating a significant decrease in the HyI in the recent past.

Micronutritional Changes

Iron (Fe) is a vital micronutrient that is essential for plant growth and physiological processes. In Mediterranean vineyards, high active lime content and alkalinity can cause chlorosis, which is a common problem associated with iron deficiency. This nutritional issue can negatively impact plant health, shorten their lifespan in the long run, and cause significant problems in the short term such as stunted root and shoot growth, decreased productivity, and reduced fruit quality (Abadia et al., 2011; Covarrubias & Rombola, 2013). Iron (Fe) does not directly form part of the chlorophyll structure, but it plays a significant role in its formation (Yağmur et al., 2005). The levels of chlorophyll and ferredoxin in plant leaves are dependent on the Fe content, with a decrease in Fe resulting in a corresponding decrease in these pigments. Certain enzymes that rely on Fe for their activity, such as Fe-superoxide dismutase, may become less active when Fe is deficient, leading to necrosis in the areas between the veins, particularly in young leaves. Iron also plays a role in the regulation of stomatal conductance, which affects the uptake of CO_2 and water by the plant. Deficiencies in iron can lead to chlorosis, reduced growth and reduced yield.

The 'Karalahana' and 'Sauvignon Blanc' cultivars tend to have the highest Fe content in both years, the 'Karasakız' cultivar was introduced alongside the other two in 2020. While the AWC response of cultivars generally exhibited a decreasing trend, the 'Adakarası' cultivar was identified as an exception in 2019. In the first year of the experiment, the 'Adakarası' cultivar exhibited the highest Fe value in the AWC_{25} treatment, while in other cultivars, the highest Fe concentrations were found in the AWC_{75} and AWC_{100} treatments. In 2020, higher Fe content was also detected in all cultivars with the AWC_{100} treatments. The cultivars 'Yapıncak' and 'Vasilaki' had the lowest Fe content (Figure 1). AWC_{100} means statistically significantly differed from other AWCs in 2020.

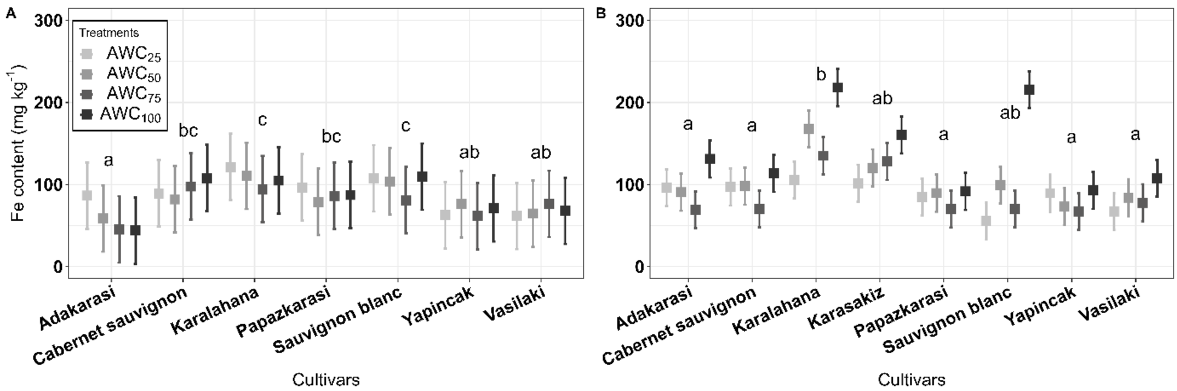

Figure 1. Fe content of the cultivars is presented as the mean ± standard error based on replicate measurements. Year 2019 is labeled as 'A' and 2020 as 'B'. Statistical differences between cultivar means ($p < 0.05$) were assessed using the Tukey test (n = 15), as treatment and interaction effects were not significant. The lowest mean value is listed first in alphabetical order.

Studies have examined the interaction of leaf iron content with grapevine plants, high active-limestone resistance of rootstocks (Bavaresco et al., 1991; Covarrubias & Rombola, 2013; Vannozzi et al., 2017), fertilizer (Nikolic & Kastori, 2000; Reyes et al., 2006), and the impacts of iron deficiency on development and physiological reactions (Bertamini & Nedunchezian, 2003). However, there is limited research on the genotypic reactions of varieties (Özdemir, 2018). According to Bertamini and Nedunchezian (2003), Fe deficiency diminishes the vegetative growth of the 'Pinot Noir' cultivar. It also impacts membrane permeability, decreases both the exchange of CO_2 and photosynthesis levels, and results in reduced leaf area and dry matter accumulation. In our study, since all the plant nutritional values given are within threshold values, it is not possible to make evaluations regarding the lack or toxicity of Fe.

However, the interactions of cultivars and changing AWCs are not statistically significant similarly to Oliveira et al. (2015), which reported that there was no difference in the iron content of Syrah grape leaves when different irrigation regimes were applied, they have been found to differ.

Copper (Cu) is a heavy metal that serves as a catalyst for various chemical reactions in plants. It is responsible for the synthesis of enzymes such as polyphenol oxidase, monoamine oxidase, and other phenolases during the photosynthesis cycle (Kovacic et al., 2013). Higher plants need small quantities of Cu for normal functioning of the plant (Rehman et al., 2019). Insufficient Cu can lead to a reduction in biological functions and ultimately plant death, whereas high amounts of Cu can be poisonous to living organisms. Elevated copper concentrations can adversely impact a range of physiological processes, including photosynthesis, root growth, enzyme function, pigment and protein production, as well as cell division (Cambrolle et al., 2015; Castro et al., 2021; Lai et al., 2010; Juang et al., 2012). Studies investigating the effects of copper compounds used for *Plasmopara viticola* (Berk.et. Curt.) and *Botrytis cinerea* Pers. management have found that excess soil Cu can be an oxidative stress-enhancing factor (Miotto et al., 2014). Elevated copper concentrations have been observed in various components of the wine production system, including grape berries, must, winemaking equipment, and the final wine product, which may be attributable to environmental copper contamination of water and soil resources (Sun et al., 2018). This trend has negative effects on both the performance of yeast during fermentation and the safety of wine consumers, as noted by Sun et al. (2016). However, it has been shown that sufficient P can limit the negative impact of extreme Cu by regulating physiological processes (Baldi et al., 2018). The level of copper concentration that leads to toxicity varies depending on the grapevine cultivar and the rooting conditions, as noted in studies by Cambrolle et al. (2015), Kopittke et al. (2010) and Tiecher et al. (2018).

Since the content of Cu in all applications and varieties falls within the limit values, it is not possible to discuss the effects of deficiency or excess (Jones et al., 1991). The Cu content of cultivars showed no statistically significant difference in both years. Among the cultivars, 'Karalahana' exhibited the highest Cu content, while 'Sauvignon Blanc' showed the lowest. In 2020, the mean Cu content was higher than in the first year. 'Adakarası' had the highest Cu content, while 'Cabernet Sauvignon' had the lowest. Notably, 'Papazkarası' was an exception in 2020, while other varieties tended to have increased Cu content with increasing levels of irrigation (Figure 2).

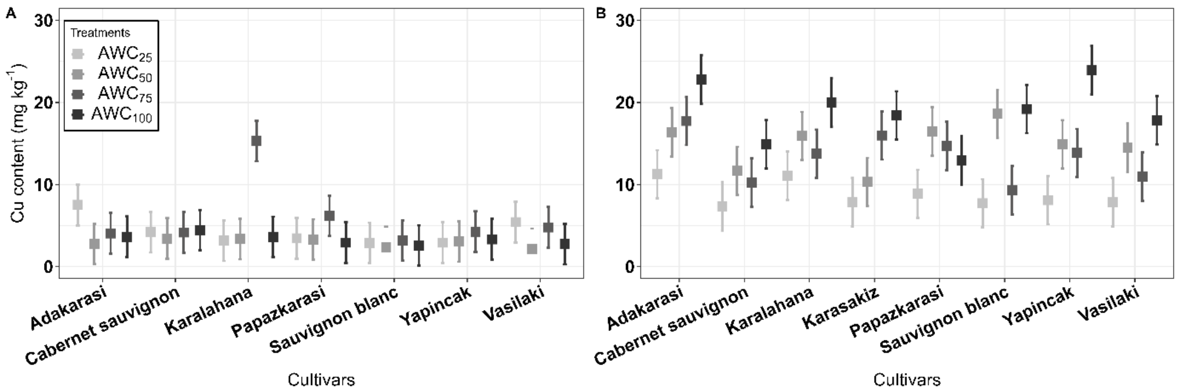

Figure 2. Cu content of the cultivars is presented as the mean ± standard error based on replicate measurements. Year 2019 is labeled as 'A' and 2020 as 'B'.

It is possible that the storage of copper elements in perennial vegetative organs, rather than in one year old shoots of grapevine species and the increased root amount in the second year may be related (Juang et al., 2012). Statistically significant differences were found between the AWCs in 2020. AWC_{25} had the lowest Cu content, while AWC_{100} had the highest Cu content. The AWC_{100} treatments resulted in the highest Cu contents in 2020, while AWC_{75} was found in higher Cu content 2019. This result contradicts that of Oliveira et al. (2015), who showed that the Cu content of leaves did not significantly vary under different irrigation regimes in five-year-old soil grown grapevines. On the other hand, Cambrolle et al. (2015) discovered that wild species exhibited greater tolerance to Cu in their research conducted with 41B rootstock - a hybrid of two *Vitis vinifera* L. ssp. sylvestris subspecies *Vitis vinifera* L. cv. Chasselas and *Vitis berlandieri* Planch. Additionally, cultivars may display genotypic differences in terms of the uptake, transport, and utilization of this nutrient element.

Zinc (Zn) is a micronutrient that is more commonly deficient in rapidly growing young tissues and in arid conditions due to its slow transport in soil and plants as a divalent ion. Zn deficiency is a frequently encountered problem in dry and semi-dry climates where viticulture is intense, including Türkiye (Cakmak et al., 1996; White & Zasoski, 1999). Availability of a sufficient Zn source is one of the requirements for plants to cope with drought during the vegetation period (Grewal & Williams, 2000). Zn plays an important role in the formation and operation of some growing hormones and many enzymes, including fruit set stage, cell division, strong attachment of berries to clusters, integrity of cell membranes, and photosynthesis cycles (Ramos & Romero, 2016). Its deficiency causes irregular growth, low berry set, growth retardation, and yield losses in young leaves. It has also been notified that Zn shortage decreases water-use efficiency in plants, and sufficient Zn levels make plants more resistant to water deficit in the soil. Zn enhances resistance to damage caused by biotic and abiotic stress (Cakmak et al., 1996; Zhao & Wu, 2017; Sabir & Sari, 2019; Cakmak, 2000).

During the initial year of the trial, it was observed that the Zn contents of all cultivars tended to decrease as the water content increased. The 'Papazkarası' cultivar showed the maximum Zn amount, while the 'Yapıncak' cultivar resulted the lowest content in 2019. In the second year, Zn content increased with increasing irrigation, except for 'Karalahana' and 'Yapıncak' cultivars. In 2020, the 'Adakarası' cultivar produced the richest Zn level, while the Karasakız and 'Cabernet Sauvignon' cultivars represented the lowest concentration. The AWC_{25} plants reached the highest Zn content in 2019, whereas the AWC_{50} and AWC_{100} plants had the highest Zn content in 2020 (Figure 3). Unlike Oliviera et al. (2014) results were statistically significant.

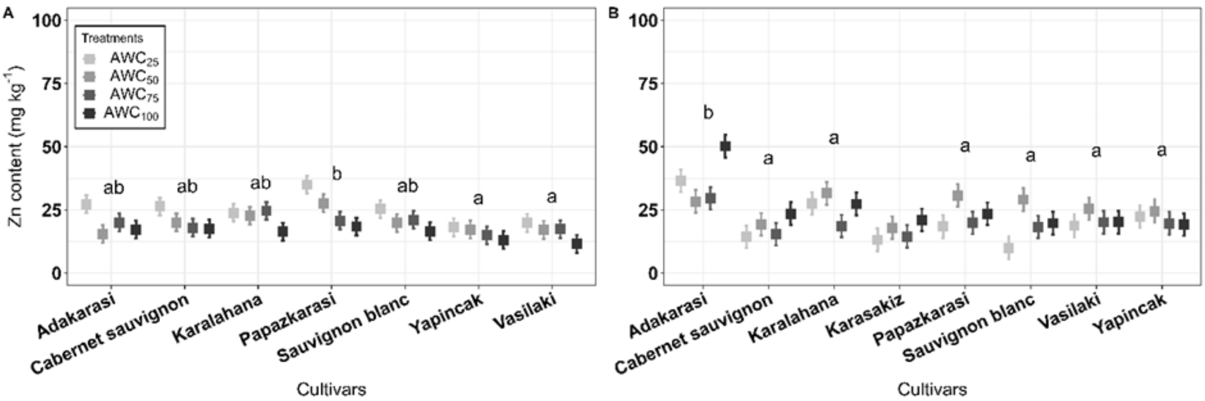

Figure 3. Zn content of the cultivars is presented as the mean ± standard error based on replicate measurements. Year 2019 is labeled as 'A' and 2020 as 'B'. Statistical differences between cultivar means ($p < 0.05$) were assessed using the Tukey test ($n = 15$), as treatment and interaction effects were not significant. The lowest mean value is listed first in alphabetical order.

According to Sabır and Sarı (2019), Zn fertilization promotes vegetative and generative growth in Italia and Alphonse Lavallée cultivars grafted on 99R. Therefore, the high Zn content in 'Adakarası', especially in 2020, could be related to the variety's strong vegetative development and its tendency to form continuous clusters even with AWC_{25} and AWC_{50} treatments until the end of the study. Sabır and Sarı (2019) also found that foliar application of Zn increases berry weight, length, and diameter in plants under limited irrigation. In the current research, these two cultivars exhibited distinct patterns in terms of Zn nutrient accumulation

Manganese (Mn) is a bivalent micronutrient that is commonly deficient in both younger and older leaves, presenting as interveinal and mosaic forms of chlorosis. Mn is critical for photosynthetic processes and the structural integrity of numerous enzymes. The symptoms of Mn deficiency are more severe on leaves exposed to the sun in advanced phenological periods, and it may also delay the maturity of berries (Salisbury & Ross, 1992).

In 2019, the 'Cabernet Sauvignon' cultivar recorded the maximum Mn levels, while the 'Vasilaki' cultivar represented the lowest Mn content. The responses of changing AWC treatments among cultivars did not show any trend. However, genotypic differences in response to various AWCs were not determined to be statistically significant. In 2020, Mn contents were detected at higher levels than in the previous year. Most cultivars, except for 'Karalahana' and 'Papazkarası', responded to increased irrigation by increasing the leaf Mn content. 'Karalahana' and 'Papazkarası' cultivars had the highest Mn contents in the AWC_{50} application (Figure 4). Leibar et al. (2017) also reported that restricted irrigation caused an increase in Mn content during the harvest period in the Tempranillo cultivar. However, according to Oliveira et al. (2015), full, regulated, and deficit irrigation regimes did not have a statistically significant effect on the Mg content of the leaves. In terms of the main effect of the cultivar, the cultivar 'Karalahana' had the maximum Mn concentration, whereas the cultivar 'Vasilaki' contained the least Mn.

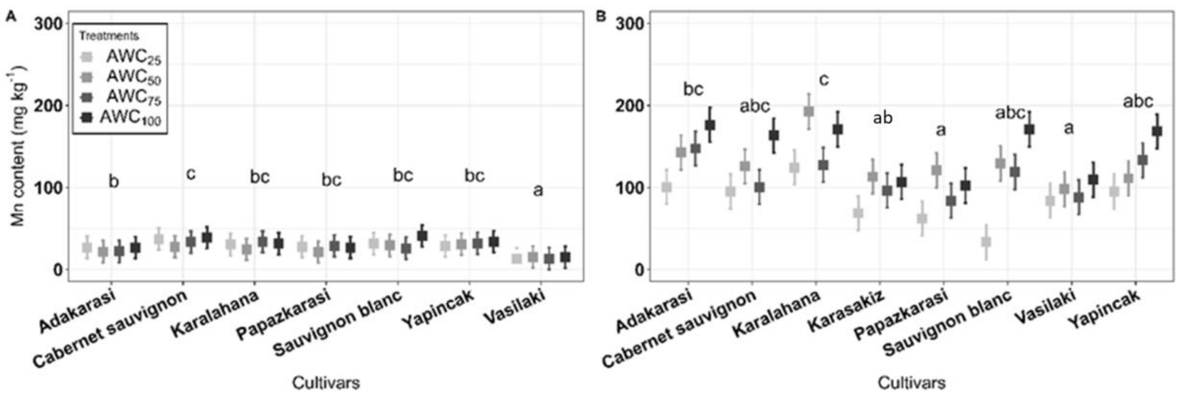

Figure 4. Mn content of the cultivars is presented as the mean ± standard error based on replicate measurements. Year 2019 is labeled as 'A' and 2020 as 'B'. Statistical differences between cultivar means ($p < 0.05$) were assessed using the Tukey test ($n = 15$), as treatment and interaction effects were not significant. The lowest mean value is listed first in alphabetical order.

Boron (B) is absorbed by plants as boric acid and is slowly distributed throughout the tissues. It lack is known to limit root growth and hinder cell division in shoot and leaf tips. Since it participates in the elongation of the pollen tube, its insufficiency results in a reduction in berry set. The uptake of B is reduced in arid conditions. Nevertheless, it is a nutrient element that exhibits high mobility in the environment particularly in cases of excessive irrigation or precipitation (Salisbury & Ross, 1992; Pearson & Goheen, 1998). B fertilization is proven to boost berry set, growth and yield (Christensen et al., 2005; Güneş et al., 2015).

The ‘Cabernet Sauvignon’ and ‘Sauvignon Blanc’ cultivars had the maximum B contents in 2019, while the ‘Karalahana’ cultivar had the lowest. With statistically significant differences, increasing AWC treatments appeared to increase B content in the ‘Cabernet Sauvignon’, ‘Papazkarası’, and ‘Vasilaki’ cultivars. ‘Adakarası’, ‘Karalahana’, and ‘Yapıncak’ cultivars had the highest content in AWC_{75} treatments. In 2020, the ‘Sauvignon Blanc’ cultivar found the richest B amount, and the ‘Karalahana’ cultivar had the lowest, as in the previous year. ‘Adakarası’, ‘Cabernet Sauvignon’, ‘Sauvignon Blanc’, ‘Yapıncak’, and ‘Karasakız’ cultivars showed the maximum B content in AWC_{100} treatment, while ‘Karalahana’, ‘Papazkarası’, and ‘Vasilaki’ cultivars had the highest B content in AWC_{50}. Nevertheless, no statistically significant differences were found (Figure 5). In both years, the B content tended to increase with increasing irrigation. However, it was determined that the levels of B content did not increase to a toxic level and did not exhibit any toxicity symptoms throughout the experimental years.

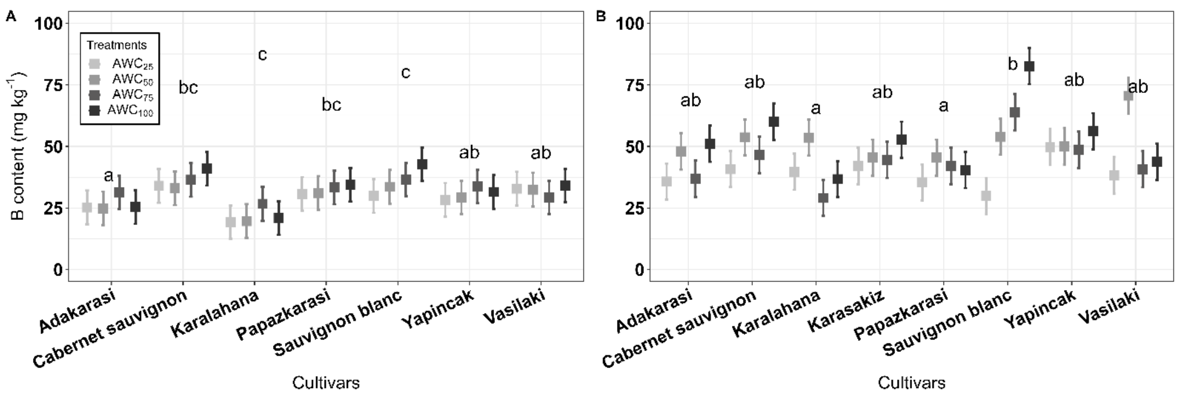

Figure 5. Content B of the cultivars is presented as the mean ± standard error based on replicate measurements. Year 2019 is labeled as 'A' and 2020 as 'B'. Statistical differences between cultivar means ($p < 0.05$) were assessed using the Tukey test ($n = 15$), as treatment and interaction effects were not significant. The lowest mean value is listed first in alphabetical order.

Molybdenum (Mo) plays a crucial role in the nitrate reductase enzyme, which converts nitrate to nitrite, thereby supporting plant metabolism. Additionally, it is responsible for the functionality of enzymes responsible for abscisic acid production (Masi & Boselli, 2011). Mo also provides yield increase by increasing cluster weight and reducing millerandage in clusters by increasing berry set (Longbottom et al., 2005; Williams et al., 2005).

In 2019, the 'Adakarası' cultivar exhibited the maximum Mo concentration, while 'Cabernet Sauvignon' displayed the lowest. The highest contents were observed in 'Adakarası', 'Papazkarası', 'Sauvignon Blanc', and 'Yapıncak' cultivars in AWC_{25} treatments, and in 'Cabernet Sauvignon', 'Karalahana', and 'Vasilaki' cultivars in AWC_{75} treatments. However, these changes were not statistically significant. AWCs that decreased in terms of AWC main effect tended to contain higher Mo, and the mean differences were statistically significant. In 2020, unlike the previous year, 'Cabernet Sauvignon' showed the greatest Mo concentration, while 'Sauvignon Blanc' exhibited the lowest. Although the Cultivar x AWC interaction was not statistically significant, 'Adakarası', 'Cabernet Sauvignon', 'Papazkarası', 'Vasilaki', and 'Karasakız' cultivars exhibited the highest contents in AWC_{100}. The highest content was determined in AWC_{50} in 'Karalahana' and 'Sauvignon Blanc' cultivars, and in AWC_{25} in 'Yapıncak' cultivars. This time, AWC main effects responded with higher contents to increased water capacity (Figure 6).

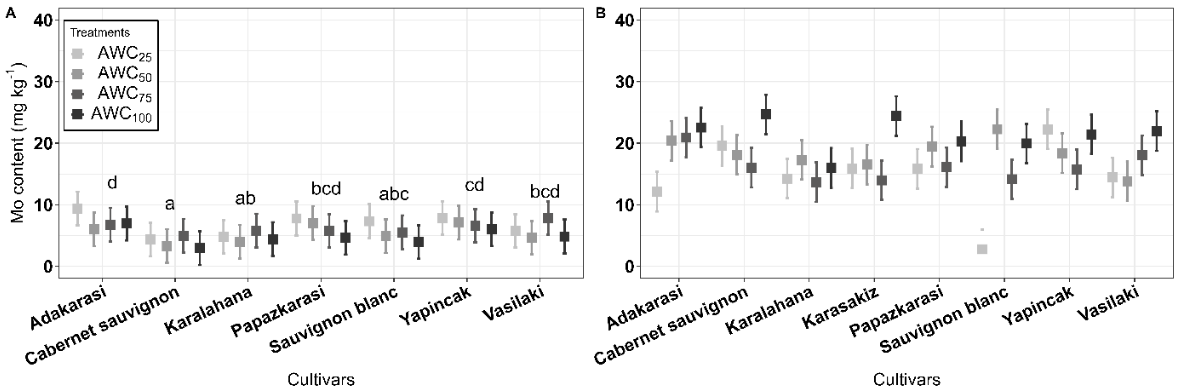

Figure 6. Mo content of the cultivars is presented as the mean ± standard error based on replicate measurements. Year 2019 is labeled as 'A' and 2020 as 'B'. Statistical differences between cultivar means ($p < 0.05$) were assessed using the Tukey test ($n = 15$), as treatment and interaction effects were not significant. The lowest mean value is listed first in alphabetical order.

In both years, it was observed that the Mo contents significantly exceeded the optimal range of 0.15-0.35 mg kg^{-1} (Jones et al., 1991). Excessive Mo in plants does not have a toxic effect; rather, it is the high amount of soluble Mo in the growing medium that causes excessive intake by the plants. Under suitable conditions, this amount can increase up to 100 times, but it does not cause poisoning (Turan and Horuz, 2012). Mo is mobile in phloem bundles and can be found in high amounts in plant tissues when applied externally and during the appropriate phenological period (Gupta, 1997). Williams et al. (2007) applied Mo nutrient element to the Merlot cultivar and some rootstocks by spraying it on leaves. They reported that the petiole content of the vines without Mo application before flowering was 0.07-0.13 mg kg^{-1}, whereas during the flowering period, it was found to be at the content of 4.20-10.30 mg kg^{-1} in the Mo-applied vines. In the current study, although no extra dose of Mo was applied, high amounts of Mo content were detected in all cultivars. However, it was determined that it had no effect on the general physiology of the plant.

Sodium (Na) is a micronutrient that can cause physiological disruptions, such as limiting nutrient uptake from roots and distribution to shoots, leading to problems in yield and vegetative growth when it accumulates excessively with chlorine (Walker et al., 2010). Although the use of rootstocks in viticulture is a strategy to combat salinity, the use of salinity-tolerant genotypes may also be another approach (Fisarakis et al., 2001). However, it is reported that Na can be beneficial for plants in cases of potassium-deficient growing media (Maathuis, 2014).

In 2019, the 'Karalahana' cultivar showed the maximum Na concentration, whereas the 'Cabernet Sauvignon' cultivar showed the minimum. 'Adakarası', 'Papazkarası', 'Sauvignon Blanc', and 'Yapıncak' cultivars showed higher Na contents in response to AWC_{25} treatment. The highest contents of 'Cabernet Sauvignon', Karalahana, and 'Vasilaki' cultivars were observed in AWC_{75} treatment. In 2020, the cultivar 'Yapıncak' exhibited the maximum Na concentration, while the cultivar 'Cabernet Sauvignon' exhibited the lowest Na concentration among the cultivars. 'Adakarası', 'Papazkarası', and 'Karasakız' cultivars had their highest Na content in AWC_{100} treatment, whereas 'Yapıncak' cultivar had its highest Na content in AWC_{75} treatment. 'Karalahana', 'Sauvignon Blanc', and Vasilaki cultivars showed higher Na contents in AWC_{50} treatment. AWC_{25}

treatment caused a higher Na content only in 'Cabernet Sauvignon' cultivar (Figure 7). In terms of AWC main effects, AWC_{50} resulted in a statistically significant higher content. Lebiar et al. (2017) reported that Na deficiency was observed only during the veraison period in vines exposed to water stress.

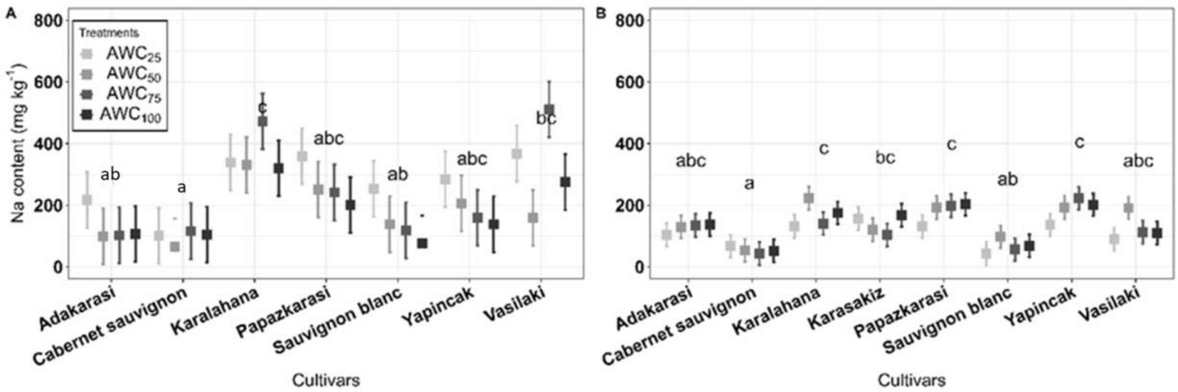

Figure 7. Na content of the cultivars is presented as the mean ± standard error based on replicate measurements. Year 2019 is labeled as 'A' and 2020 as 'B'. Statistical differences between cultivar means ($p < 0.05$) were assessed using the Tukey test ($n = 15$), as treatment and interaction effects were not significant. The lowest mean value is listed first in alphabetical order.

Bivariate Analysis

Figure 8 shows a correlation matrix of the micronutrients examined. Fe has a positive correlation with Mn, Cu, and Zn, with values of 0.50, 0.42, and 0.33, respectively. This indicates that as the levels of Fe increase, the levels of Mn, Cu, and Zn also tend to increase. Cu has a positive correlation with Zn (0.42) and a higher correlation with Mn (0.80), which suggests that increasing Cu levels tend to elevate the concentrations of Zn and Mn as well. The highest positive correlation in the entire matrix is between Mn and Mo, with a value of 0.82. Mn was also found to be positively correlated with B, with a value of 0.65. In contrast, Na has a negatively correlated effect on Mn (-0.24) and B (-0.25). This suggests that as the levels of Na increase, the levels of Mn and B tend to decrease.

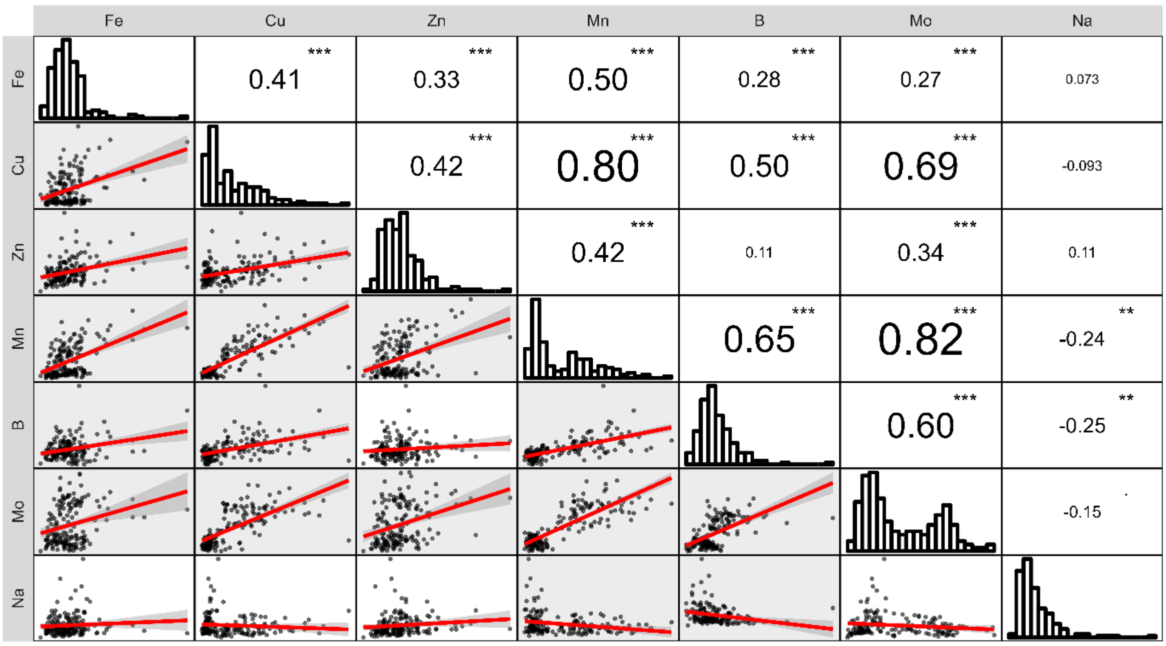

Figure 8. The scatterplot matrix displays selected variables, presenting bivariate scatterplots with fitted linear regression distributions. The red line represents the trend in the relationships between the variables. Frequency histogram overlays are also provided. The Pearson's coefficients of determination (R2) and the statistical significance of the coefficients are indicated by the text size and * symbol above the diagonal with absent representing p-values greater than 0.05, * indicating p-values less than 0.05, ** indicating p-values less than 0.01, and *** indicating p-values less than 0.001. Fe; Iron content (mg kg^{-1}), Cu; Copper content (mg kg^{-1}), Zn; Zinc content (mg kg^{-1}), Mn; Manganese content (mg kg^{-1}), B; Boron content (mg kg^{-1}), Mo; Molybdenum content (mg kg^{-1}), Na; Sodium content (mg kg^{-1}).

Hierarchical Clustering Analyses (HCA)

Due to the distinct variations in micronutrient variables, a Hierarchical Clustering Analysis (HCA) was conducted. This analysis resulted in the differentiation of six clusters among both the seven cultivars and variables, each distinguished by varying Euclidean distances (Figure 9).

In the sixth cluster, the 'Cabernet Sauvignon' cluster exhibited a relatively low distance from the 'Sauvignon Blanc' cluster, implying a strong level of likeness between both cultivars. Moving to the fifth cluster, the 'Papazkarası' cluster assimilated the 'Vasilaki' cluster, albeit with a slightly greater distance compared to the previous step, suggesting a somewhat diminished similarity between these two cultivars. In the fourth cluster, 'Papazkarası' again took the lead by incorporating the 'Yapıncak' cluster. This step displayed a higher distance compared to previous stages, indicating a more pronounced dissimilarity in terms of micronutrient content between these clusters. The third cluster emerged when the cultivar 'Adakarası' absorbed the 'Papazkarası' cluster at a relatively greater distance. In the subsequent second cluster, the 'Adakarası' cluster assimilated the 'Cabernet Sauvignon' cluster, with a relatively high distance, thereby underlining a significant dissimilarity between these two clusters. Finally, in the last step, the 'Adakarası' cluster incorporated the 'Karalahana' cluster, resulting in the amalgamation of all data points into a single cluster.

The initial clustering brought together 'Cabernet Sauvignon' and 'Sauvignon Blanc', indicating their close relationship due to remarkably low dissimilarity. As clustering progressed, 'Papazkarası' emerged as a central variety, effectively assimilating 'Vasilaki' and 'Yapıncak'. Despite its divergence from 'Papazkarası', 'Adakarası' absorbed it before encompassing 'Cabernet Sauvignon'. This ascendancy of 'Adakarası' as a central variety underscored its pivotal role in shaping the overall clustering structure. Ultimately, the clustering culminated in a single cluster, amalgamating 'Adakarası' with 'Karalahana' (Figure 9).

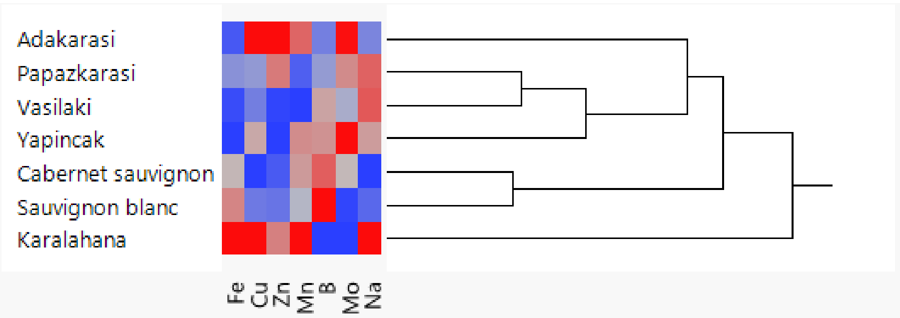

Figure 9. Hierarchical clustering analyses of cultivars in terms of micronutrient variables, where the blue boxes represent low content and red boxes have high content of the variables. Fe; Iron content (mg kg^{-1}), Cu; Copper content (mg kg^{-1}), Zn; Zinc content (mg kg^{-1}), Mn; Manganese content (mg kg^{-1}), B; Boron content (mg kg^{-1}), Mo; Molybdenum content (mg kg^{-1}), Na; Sodium content (mg kg^{-1}).

Principal Components Analysis (PCA)

The data included seven grapevine cultivars and seven micronutrient variables, which were analyzed using Principal Component Analysis (PCA) based on the covariance matrix. Two separate biplots were generated to individually evaluate the responses of the cultivars and variables. The arrangement of the results within the PCA quadrants demonstrated significant diversity among the cultivars.

The initial four principal components comprise 88.56% of the total variance monitored in the dataset, with PC1 contributing 50.41% and PC2 explaining 18.04% of the variation (Figure 10).

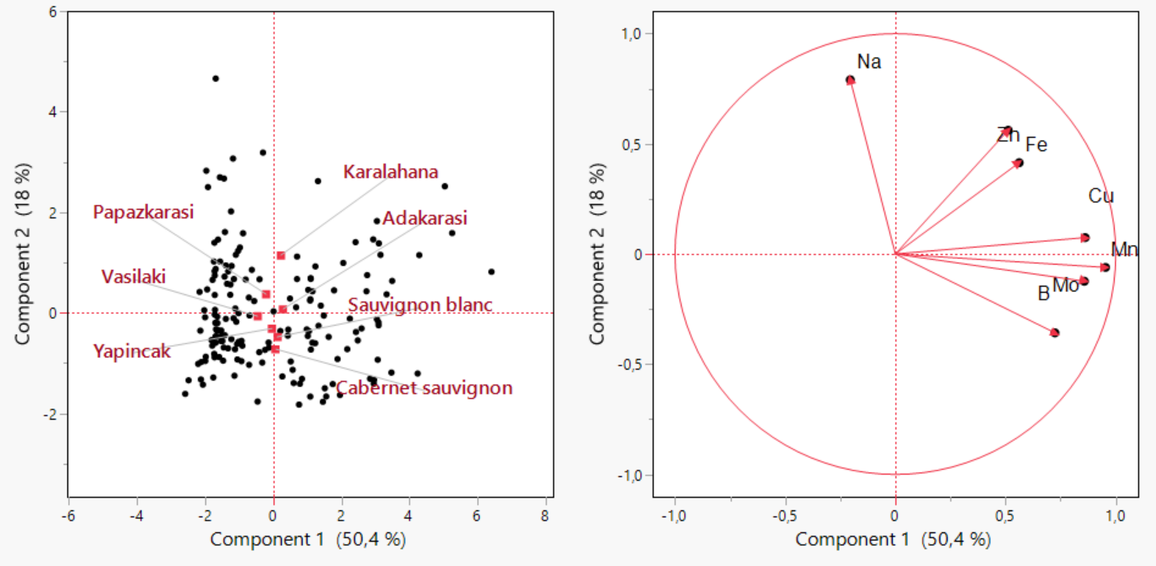

Figure 10. Principal component analysis using mean variable values. Left; PCA biplot of cultivars, right; PCA biplot of micronutrients. All variables are displayed. The variable's contribution level is represented by the size of the arrows. Fe; Iron content (mg kg^{-1}), Cu; Copper content (mg kg^{-1}), Zn; Zinc content (mg kg^{-1}), Mn; Manganese content (mg kg^{-1}), B; Boron content (mg kg^{-1}), Mo; Molybdenum content (mg kg^{-1}), Na; Sodium content (mg kg^{-1}).

The first principal component (PC1) exhibited relatively higher positive correlations with ‘Adakarası’, ‘Karalahana’ and ‘Sauvignon blanc’. They share some common characteristics or contribute to the pattern represented by PC1. This pattern suggests that PC1 is associated with the presence of the analyzed micronutrients inherent in these specific grape cultivars. ‘Cabernet Sauvignon’ also found itself on the positive side of PC1 with a very small difference. PC1 showed negative correlations with ‘Papazkarası’ and ‘Vasilaki’. These cultivars have negative loadings on PC1, suggesting that they oppose the pattern represented by PC1. On the other hand, the second principal component (PC2) displayed higher positive correlations with ‘Karalahana’, and relatively lower positive correlations ‘Papazkarası’ and ‘Adakarası’. Negative correlations in PC2 were ‘Cabernet Sauvignon’ and ‘Sauvignon Blanc’. ‘Vasilaki’ and ‘Yapıncak’ have relatively low loadings on both PC1 and PC2, suggesting they don’t strongly align with either of these components.

Regarding micronutrient contents, PC1 exhibited positive correlations with Fe, Cu, Zn, Mn, B, and Mo, while showing a negative correlation with Na. This implies that PC1 is indicative of the abundance of these micronutrients within the grape cultivars. Conversely, PC2 displayed a singular positive correlation with Na, underscoring its association with the presence of sodium in the grape cultivars. Zn, Fe and Cu were also taking place in the positive axis of PC2.

Conclusion

Except for copper in both years and molybdenum in 2020, significant variances in micronutrient levels were observed across all cultivars' leaves. The micronutrient composition in leaves exhibited variations among cultivars for all elements, with each micronutrient being notably influenced by the year. Micronutrient levels, excluding sodium, showed elevated levels in the second year of the trial, likely due to increased root mass and nutrient accumulation in perennial structures. Alterations in leaf nutrient content could be linked to arid conditions and, in certain instances, genetic traits. Enhanced water availability in the root zone may lead to increased dry matter content in leaves, consequently resulting in elevated levels of specific nutrients.

Hierarchical Clustering Analysis (HCA) unveiled distinct clusters among cultivars and variables, including the close 'Cabernet Sauvignon'-'Sauvignon Blanc' relationship and the pivotal role of 'Papazkarası' in amalgamating 'Vasilaki' and 'Yapıncak'. In Principal Component Analysis (PCA), PC1 indicated diverse micronutrients across cultivars, while PC2 highlighted unique compounds in 'Sauvignon Blanc' and 'Yapıncak', including sodium content in cultivars.

Yet, delineating the precise reaction of grapevine varieties to abiotic climatic factors remains intricate due to a myriad of factors, encompassing genetic constituents, root dynamics, and fluctuations in transmission mechanisms across diverse circumstances. Forecasts regarding the crisis project that temperature escalation and disruptions in the atmospheric water balance will impact water and nutrient circulation, as well as the efficacy and accessibility of soil moisture for grapevines. Further investigation is necessary to explore the relationship between crop load and the interaction between rootstock and scion. Additionally, it is vital to scrutinize how distinct cultivars assimilate, convey, utilize, and retain plant micronutrients across various experimental frameworks.

Additional Information and Declarations

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Authors' Contributions: The trial was planned and designed by SC and EB. Field experiments and measurements were performed by SC, BA, TA. ME. SC and EŞ made formal analysis, data curation, conceptualization and visualization. SC and EŞ wrote the manuscript. EŞ, İK and EB made critical revisions to the manuscript for intellectual content. Final draft of manuscript was read and approved by all authors.

Conflict of Interests: The authors have no conflicts of interest to declare.

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