Erwerbs-Obstbau
https://doi.org/10.1007/s10341-023-00875-y

ORIGINAL ARTICLE / ORIGINALBEITRAG

Variations of Chlorophyll, Proline, and Abscisic Acid (ABA) Contents in Grapevines (*Vitis Vinifera* L.) Under Water Deficit Conditions

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Received: 3 November 2022 / Accepted: 6 March 2023

Abstract
The aim of the research was to determine the impact of regulated deficit irrigations (RDIs) on the accumulation of chlorophyll, proline, and abscisic acid (ABA) in grapevines. Cuttings of *Vitis vinifera* L. cv 'Adakarası,' 'Papazkarası,' 'Karasakız,' 'Karalahana,' 'Yapıncak,' 'Vasilaki,' 'Cabernet Sauvignon,' and 'Sauvignon Blanc' were treated to different RDIs that were applied by reducing the maximum irrigation amount by 0%, 25%, 50%, and 75% according to reference evapotranspiration (ETo) under semi-controlled conditions over 2 years. 'Karalahana' and 'Sauvignon Blanc' cultivars reached the highest chlorophyl la (Chl_a) in the mean of 2 years. The highest total chlorophyll (Chl_{tot}) was determined in 'Karalahana' cultivar. The lowest Chl_{tot} was determined in 'Adakarası' cultivar. The proline with lower RDIs increased in 2019. In 2020, a relationship was discovered whereby, contrary to the previous year, RD_4 indicated the least content of proline. The highest ABA among RDIs was determined in the RD_4, but was not statistically significant. Leaf ABA was higher in 2019 in the mean of all cultivars and all RDIs. The impact of RDIs on ABA varied according to the cultivar and year. 'Adakarası' cultivar, like 'Cabernet Sauvignon' cultivar, may adjust its metabolic process to a decrease in water supply. 'Karasakız' cultivar also appears to be promising in terms of drought resilience.

Keywords Drought stress · Abiotic stress · Photosynthetic pigment · Organic solute · Phytohormone

Introduction

Climate change significantly affects both human life and natural systems by increasing temperatures through the release of greenhouse gases. This leads to reduced water availability and drought in semiarid and arid regions (Kizildeniz et al. 2021). Beyond its social and cultural importance, viticulture has become one of the most important economic and industrial professions in agriculture in past and present. But today, the sustainability of viticulture is being drastically challenged by the ongoing climate change (Santos et al. 2020). Viticulture, like agriculture, has to face changing environmental conditions. Drought is the most important factor affecting plant physiology among abiotic stressors. Understanding the plasticity of grape cultivars to water deficit is a major challenge for researchers and vignerons. This insight will be beneficial in realizing the potential of varietal diversity in a wine-growing region and also to adapt the decisions of vignerons and policymakers to the climate change. As in many important wine regions of Europe, consumers' interest in wines produced from mono grape cultivars has increased considerably in Turkey. Interest in autochthonous cultivars is increasing day by day (Capozzi et al. 2015). Therefore, there is an important tendency towards the utilization and consumption of old grape cultivars as well as the preservation of local biodiversity.

The response of grapevines to drought stress is a dynamic combination of molecular, biochemical, and physiological processes (Trenti et al. 2021). Drought affects woody plants by interrupting water flow and carbon assimilation (Vandegehuchte et al. 2015). In grapevines, reversible reactions to drought include a decrease in turgor pressure in the cell (Patakas and Noitsakis 1999), slower shoot growth (Kizildeniz et al. 2015, 2018a, 2021), reduced stomatal conductivity (gs) (Charrier et al. 2018), reduced photosynthesis

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(Chaves et al. 2010), and smaller berry size (Kizildeniz et al. 2018b). In contrast, irreversible effects such as chlorosis and leaf senescence (Kliewer and Weaver 1971), berry shriveling, delayed sugar accumulation (Kizildeniz et al. 2018b), berry color lightening, and shoot lignification (Bahar et al. 2011) can also occur in response to drought.

Thus, regulators have an important role to maintain cell homeostasis (Conde et al. 2015) and balance water intake in grapevines (Perrone et al. 2012). The genetic basis of drought response in grapevine rootstocks and cultivars still unclear (Chitarra et al. 2017). However, the drought response of some plant biochemicals is better studied. Although the relationship of biochemicals and fluid processes is as yet unclear, it is determined via genetic (Coupel-Ledru et al. 2017) and environmental (Lovisolo et al. 2016) determinats.

A well-known plant hormone is abscisic acid (ABA), which plays a role in many physiological processes and directly affects gs at the cellular level. ABA also modulates aquaporin gene expression and embolism healing during water shortage, as documented by several studies (Gomez-Cadenas et al. 2015; Dinis et al. 2018; Gambetta et al. 2020). Furthermore, studies are reporting that ABA accumulation is not related to gs in all cases exposed to abiotic stresses (Zandalinas et al. 2016; Balfagón et al. 2019). As a result, although it is varietal-dependent including grapevines (Soar et al. 2006; Niculcea et al. 2013; Pilati et al. 2017; Lehr et al. 2021), this hormone has a key function in grapevine drought stress adaption processes (Jia et al. 2017; Bernardo et al. 2021).

The grapevine is known for its high adaptability to drought conditions among woody species (de Ollas et al. 2019). This adaptability is due to its ability to adjust osmotic potential (Rodrigues et al. 1993; Patakas and Noitsakis, 1999) by accumulating osmotically active soluble substances that maintain turgor pressure and metabolic activity during water stress (Patakas et al. 2002). Under drought conditions, concentrations of amino acids such as valine, leucine, isoleucine, and proline increase (Munns and Tester 2008; Canoura et al. 2018). Although proline is primarily viewed as an osmolyte that accumulates under stress conditions, its contribution to osmotic potential is highly variable (Stines et al. 1999; Patakas et al. 2002; Tesfaye et al. 2014; Ulaş et al. 2014), with the highest accumulation observed under low water potential conditions and low concentrations in conditions such as salinity or cold damage (Kaplan et al. 2007; Sharma and Verslues 2010). Organic ions, such as calcium (Ca) and potassium (K), also play a role in osmotic regulation in grapevine leaves (Altintas and Candar 2012; Dreyer 2014; Degu et al. 2019; Villette et al. 2020).

Chlorophyll (Chl) is the primary photoreceptor in photosynthesis (Quach et al. 2004). There are two kinds of chlorophyll in high plants. The major pigment is chlorophyll a (Chl_a), while the accessory pigments are chlorophyll b (Chl_b) and carotenoids. Chls are packed in protein complexes forming what is called the photosynthetic complexes (Rehman and Azhar 2020). The amount of Chl in grapevine leaves reacting to drought decreases drastically and triggers the reduction of photosynthesis (Haider et al. 2017). Chl_a and Chl_b ratios in the leaves are indicators of the plant's ability to use the light of different wavelengths (Dale and Causton 1992). Changes in the amount and ratio of chlorophyll are stress markers besides leaf senescence and affect the sustainability of metabolic processes in the plant (Filimon et al. 2016). The limits of Chl content can vary over a wide range, depending on the cultivar, climatic conditions, and phenophase of the plant (Bertamini and Nedunchezhian 2003; Gitelson et al. 2009; Rocchi et al. 2016). High temperatures can decrease the $Chl_a/_b$ ratios in C3 plant leaves (Aien et al. 2011), while water stress can significantly modify the content and distribution of Chl (Chl_a and Chl_b) in plant tissue (Rustioni and Bianchi 2021). Grapevines always show lower chlorophyll content under water deficit conditions, which can be used to diagnose plant water status and N availability (Meng et al. 2014; Wang et al. 2015; Verdenal et al. 2021). Chl content plays a key role in identifying the agronomic and commercial value of a cultivar, in addition to its fundamental role in photosynthesis (Luo et al. 2019). Imbalances in Chl amounts can affect grape appearance, indicating the importance of monitoring and managing Chl levels (Rustioni et al. 2013).

A better understanding of the efficacy of the phytochemicals mentioned in plants may enable the necessary manipulations to be more resistant under stress conditions due to climate change or to maintain agricultural production in more marginal areas (Boneh et al. 2012). Explaining the behavioral differences between grape cultivars can assist in optimizing cultivation strategies and preserving and enhancing wine quality in viticultural practices.

Further research is needed to understand how different grape cultivars are impacted by environmental factors, as current studies are limited (Duchêne 2016; Ollat et al. 2017). This is particularly important in Mediterranean-like climates, where extreme conditions during the summer months may exceed environmental thresholds (Bernardo et al. 2018). More information is necessary to understand the combined effects of local and regional environmental threats. Therefore, in this study, the effect of water deficit on the accumulation of Chl, proline, and ABA in eight wine grape cultivars cultivated in the Thrace region of Turkey was examined.

Table 1 Changes of Tekirdağ climate indicators between 1939–2019 and in experiment years

Tekirdağ	1939–2019	2019	2020	Reference
Annual precipitation (mm)	589.50	378.40	290.00	(MGM 2020)
Vegetation precipitation (mm)	196.70	129.80	83.60	
Growing degree days (day-degree)	1872.00	2157.00	2124.00	(Winkler et al. 1974)
Branas Hyl (°C.mm)	3595.20	2181.54	1328.10	(Branas et al. 1946)

Materials and Methods

Plant Material

The trial experiments were conducted under semi-controlled field conditions during the 2019 and 2020 growing seasons in Tekirdağ, Turkey (40°58′ N.- 27°28′ E). The autochthonous cultivars used on the trail were chosen for their wine quality and the increasing interest of wine producers in the region. International cultivars ‘Cabernet Sauvignon’ and ‘Sauvignon Blanc’ were chosen due to their different responses under deficit soil water conditions. The grapevine cuttings were pruned from the experimental vineyards of Tekirdağ Viticulture Research Institute (TVRI). The cuttings with seven to eight buds were taken from healthy vines that previously tested for important viruses and known as virus-free.

Cuttings (2 years old) of ‘Adakarası’ (Clone 153), ‘Papazkarası’ (Clone 289), ‘Karasakız,’ ‘Karalahana,’ ‘Yapıncak’ (Clone 175), ‘Vasilaki,’ ‘Cabernet Sauvignon,’ and ‘Sauvignon Blanc’ (*Vitis vinifera* L.) were cultivated in 14.0-l plastic pots in a perlite media until they reached 14–16 leaves, EL 29–31 (Lorenz et al. 1995). Before, at the stage of EL 15–17, all clusters and extra main shoots were removed, two to three shoots were left in each vine. Until the end of the experiment, the main shoot lengths of vines were kept at the level of 170–175 cm and the lateral shoots were removed to three or four leaves.

Growth Conditions and Experimental Design

Tekirdağ is located in the southern coastal region of Turkish Thrace where the Mediterranean climate is observed (Papp and Sabovljević 2003). According to the long-term climate data covering the years 1939–2019 in Tekirdağ; the annual average temperature is 14.00 °C, the coldest month is January with 4.70 °C, and the hottest month is August with 23.80 °C. The annual average rainfall is 589.50 mm. The period with the highest precipitation is between October and March, and the average precipitation in the vegetation period is 196.70 mm (Table 1).

The average temperature of the vegetation period in 2019 was 20.73 °C and in 2020 it was 20.20 °C. The average vegetation temperature between 1939 and 2019 was 19.53 °C.

Tekirdağ growing degree day (GDD) is calculated as 1872.00 day-degrees according to the average of 1939–2019 years. The value calculated in 2019 was 2157.00 day-degrees and the value calculated in 2020 was 2124.00 day-degrees. In recent years, it is seen that the climate class in terms of GDD has changed. In terms of the Hydrothermic Index (HyI), the long-term average is 3595.20 °C mm. It has been calculated as 2181.54 °C mm and 1328.10 °C mm in the last 2 years, respectively.

Healthy and morphologically uniformed (by size and the number of leaves) plants were irrigated and fertilized with computer-controlled irrigation and fertilization system provided by Teori Yazılım Ltd. (İstanbul, Turkey) until the end of the experiment. For fertilization slightly modified versions of Hoagland and Arnon (1950) were applied during 2 vegetation years (Candar et al. 2021). Integrated pest management practices have been applied as in local standards in both years. Throughout the experiment, five regulated deficit irrigation (RDI) treatments were applied to vines. RDI treatments were created by reducing the maximum irrigation amount by 0%, 25%, 50%, and 75% according to reference evapotranspiration (ETo). The maximum irrigation amount (8 L day^{-1}, RDI$_1$) as control was calculated according to Ilahi and Ahmad (2017) and observations of the growth power and phenological periods. Drippers that irrigate 4 L h^{-1} were controlled by computer system to adjust the irrigation time and amount. Total daily water amount per treatment also controlled in software and it was applied in five equal amounts at five different times during the day. RDI treatments were applied 49 days between 28.07–16.09 in 2019 and 74 days between 18.07–02.10 in 2020 (Table 2). During the period of limited irrigation practices, the pots were closed to prevent water entry to the growing medium from outside. The data of 0% application were not presented in this study because all plant leaves had fallen.

The experimental design was a completely randomized blocks, with a total of 960 vines, consisting of three replications and eight vines for each replication and five treat-

Table 2 The amount of regulated deficit irrigation (RDI) treatments reduced over the maximum irrigation amount

Treatment	Irrigation time (min day^{-1})	Irrigation amount (L day^{-1})
RDI$_1$	120	8.00
RDI$_2$	90	6.00
RDI$_3$	60	4.00
RDI$_4$	30	2.00
RDI$_5$	0	0.00

ments, according to the randomized blocks trial pattern. Since ‘Karasakız’ cultivar had missing plants in the first year of the experiment, data were collected only in 2020.

Measurements and Analyses

Sample Collection

All leaf samples were collected 1 week after the end of the deficit irrigation treatments. Concentrations of Chl, proline, and ABA were defined in healthy and fully expanded leaves, by the random selection of 10 leaves per plant (5th-7th leaf on the shoot) and 10 leaves total per related parameter. Manually harvested leaves, in the morning were transported to the laboratory immediately and frozen. All of the leaf samples were maintained at –20 °C until analysis. They were kept in the freezer at –80 °C for 1 h before analysis, to ensure more homogeneous disintegration. They were crushed without waiting as they were taken from the freezer and treated with extraction solutions according to the analysis to be made.

Determination of Chlorophyll Content

The following formulations of Porra (2002) modified from Arnon (1949) were used to determine the amounts of Chl_a and Chl_b. A total of 1 g of each leaf sample was diluted 1/10 with 80% acetone and mixed at rotary shaker for 1 h at 20 °C. The solution was centrifuged at 4500 rpm for 10 min and the spectrophotometer (Shimadzu, UVmini-1240, Japan) readings were made at wavelengths of 663.6 nm and 646.6 nm. Chl_a and Chl_b results were expressed as $\mu g\,g^{-1}$ and total chlorophyll (Chl_{tot}) was expressed as $\mu g\,g^{-1}$.

$$\mathrm{Chlorophyll_a}\,(\mu g\,g^{-1}) = 12.25(\mathrm{A}_{663.6}) - 2.55(\mathrm{A}_{646.6})$$

$$\mathrm{Chlorophyll}_b\,(\mu g\,g^{-1}) = 20.31(A_{646.6}) - 4.91(A_{663.6})$$

$$\mathrm{Total\ chlorophyll}(\mu g\,g^{-1}) = 17.76(A_{646.6}) + 7.34(A_{663.6})$$

Determination of Proline Content

The amount of proline was analyzed spectrophotometrically by the acid ninhydrin method according to Bates et al. (1973). A 0.5-g leaf sample was crushed and 3% sulfosalicylic acid was added. The solution was homogenized at rotator (Dragon Lab, MX-RD-Pro, China) for 1 h, then centrifuged at 4500 rpm for 10 min. At the end of the time, the filtrate, which was separated from the residue remaining in the upper part, was separated for analysis. 2 ml of acid ninhydrin and 2 ml of glacial acetic acid were added to 2 ml of filtrate and kept in a water bath at 100 °C for 1 h. The tubes were kept in an ice bath for 5 min to stop the reaction. After adding 2 ml of cold toluene to the solution and standards, they were vortexed for a short time. After waiting for 30 min at room temperature, toluene phase was aspirated from the samples with a pipette, and absorbance values were obtained at 520 nm wavelength in the spectrophotometer (Shimadzu, UVmini-1240, Japan). The absorbance values were calculated as µg proline via the proline standard graph by using 10, 20, 40, 80, and 100 $\mu g\ ml^{-1}$ proline.

Determination of Abscisic Acid (ABA) Content

ABA extractions of leaves were made according to Baydar and Ülger (1998) and by modifying to Kelen et al. (2004) 10 ml of 70% methanol was included in 1 g of leaf sample, after stirring in a mixer for 1 h, the solution was centrifuged (Hettich Universal, 320R, Germany) at 4500 rpm for 10 min and the upper part with methanol was stored at –18 °C until analysis. A total of 2 ml was taken from the samples, methanol was evaporated in an oven at 35 °C under vacuum, and the remaining residues were dissolved in 0.1 M KH_2PO_4 solution. The solution pH was adjusted to 2.5 using 0.1 N HCL. It was then treated three times with ethyl acetate. After the ethyl acetate parts were removed, the remaining part was dried in a vacuum oven at 35 °C, the last remaining residues were dissolved in methanol and passed through 0.45-µm PTFE filters and given to the high-performance liquid chromatography (HPLC) (Shimadzu, LC-20A, Japan) system. HPLC readings were made according to Lei et al. (2016).

Statistical Analysis

SPSS 15.0 was used to do statistical data analysis. (IBM® SPSS® Statistics). The significance of the differences between treatments was determined using one-way analysis of variance (ANOVA) and Duncan’s multiple range test. Results were taken into consideration statistically significant at 5% levels of significance.

Results and Discussion

Chlorophyll Content

During the experiment, Chl_a and Chl_b values were significantly different among cultivars. In 2019, ‘Karalahana’ had the highest Chl_a content at 446.97 $\mu g\,g^{-1}$, while the lowest content was found in the ‘Adakarası’ cultivar at 294.59 $\mu g\,g^{-1}$. In 2020, ‘Cabernet Sauvignon’ and ‘Sauvignon Blanc’ cultivars had higher Chl_a content, with values of 235.74 $\mu g\,g^{-1}$ and 227.65 $\mu g\,g^{-1}$, respectively, while ‘Adakarası’ had the lowest value of 180.35 $\mu g\,g^{-1}$, forming the lowest statistical group (Fig. 1).

Although not statistically significant, the RDI_1 vines had the highest Chl_a content in 2019, while RDI_4 vines had the lowest content. Results from 2020 exhibited a statistically significant but different trend from the previous year, with RDI_1 vines having the maximum daily water amount observed but the lowest Chl_a contents.

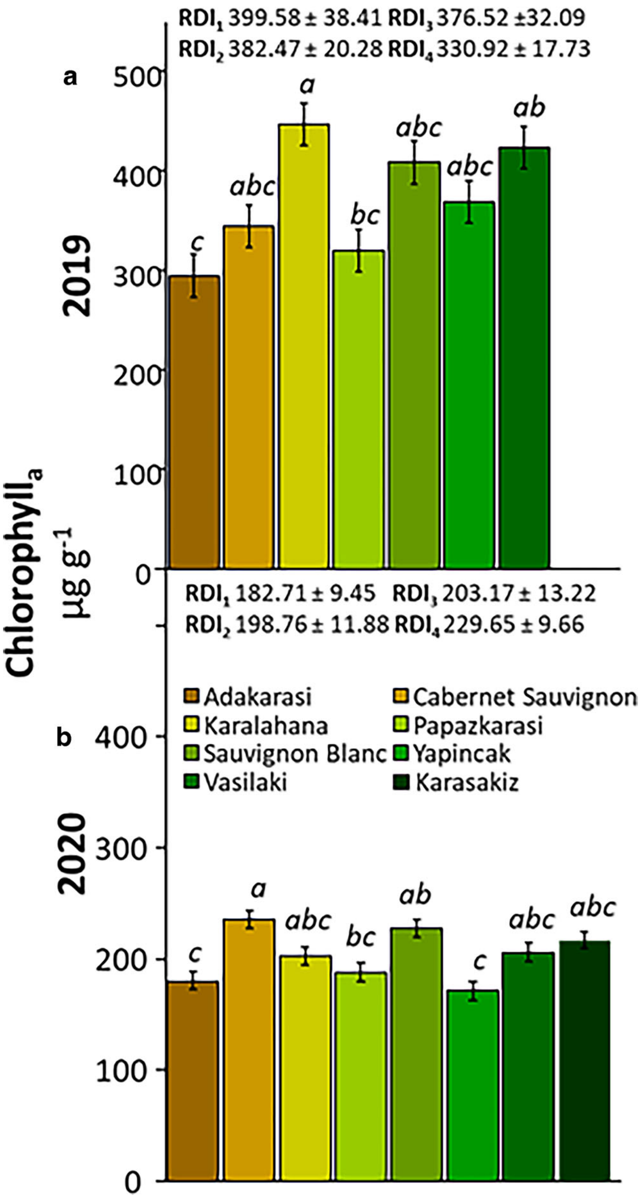

Fig. 1 The impact of cultivars and treatments on the amount of leaf $chlorophyll_a$ in 2 consecutive years. Different letters indicate significant differences among treatments ($P < 0.05$) based on Duncan's test ($n = 5$). Data are presented as means ± standard error ($n = 5$). Treatment and cultivar interactions are not presented in the figures because they are not statistically significant. RDI_1 refers to the 100%, maximum 8-L irrigation amount per day; RDI_2 refers to the 75%, daily 6-L irrigation amount; RDI_3 refers to the 50%, daily 4-L irrigation amount; and RDI_4 refers to the 25%, daily 2-L irrigation amount. **a** Trial year 2019, **b** trial year 2020. *RDI* regulated deficit irrigation

Significant changes in Chl_b content were found among cultivars in both years. In 2019, the 'Karalahana' cultivar had the highest Chl_b content at 182.57 $\mu g\,g^{-1}$, while in 2020, the 'Cabernet Sauvignon' cultivar had the highest Chl_b content at 56.03 $\mu g\,g^{-1}$. The 'Adakarası' cultivar had the lowest Chl_b content in both years, with values of 115.82 $\mu g\,g^{-1}$ in 2019 and 68.68 $\mu g\,g^{-1}$ in 2020. The difference between Chl_b amounts between RDI treatments is statistically insignificant in 2019 but significant in 2020. In 2020, RDI_4 constituted the highest statistically significant class (Fig. 2).

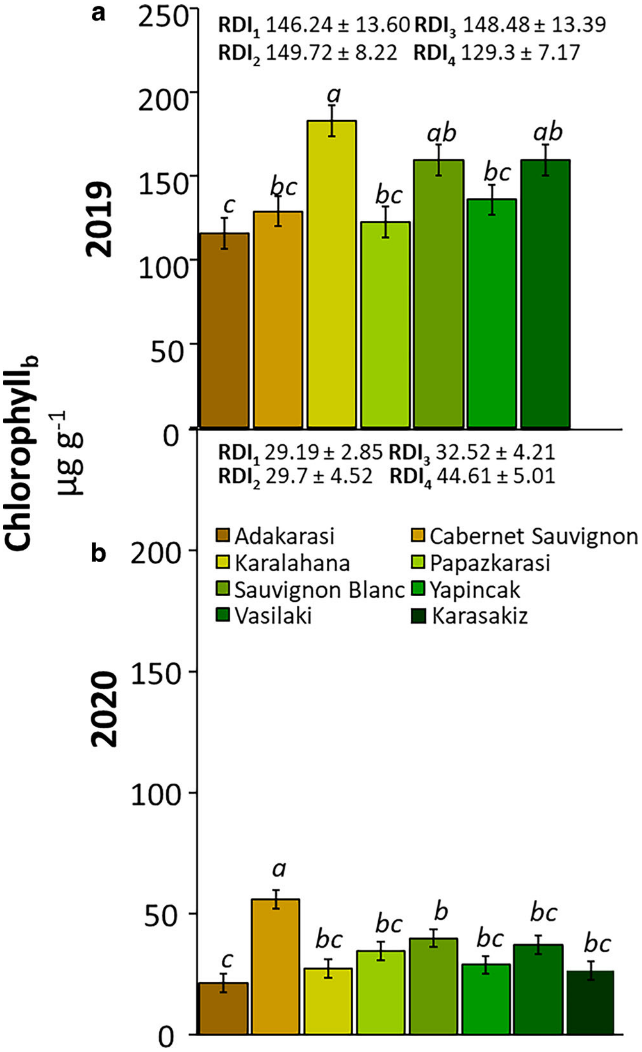

Fig. 2 The impact of cultivars and treatments on the amount of leaf $chlorophyll_b$ in 2 consecutive years. Different letters indicate significant differences among treatments ($P < 0.05$) based on Duncan's test ($n = 5$). Data are presented as means ± standard error ($n = 5$). Treatment and cultivar interactions are not presented in the figures because they are not statistically significant. RDI_1 refers to the 100%, maximum 8-L irrigation amount per day; RDI_2 refers to the 75%, daily 6-L irrigation amount; RDI_3 refers to the 50%, daily 4-L irrigation amount; and RDI_4 refers to the 25%, daily 2-L irrigation amount. **a** Trial year 2019, **b** trial year 2020. *RDI* regulated deficit irrigation

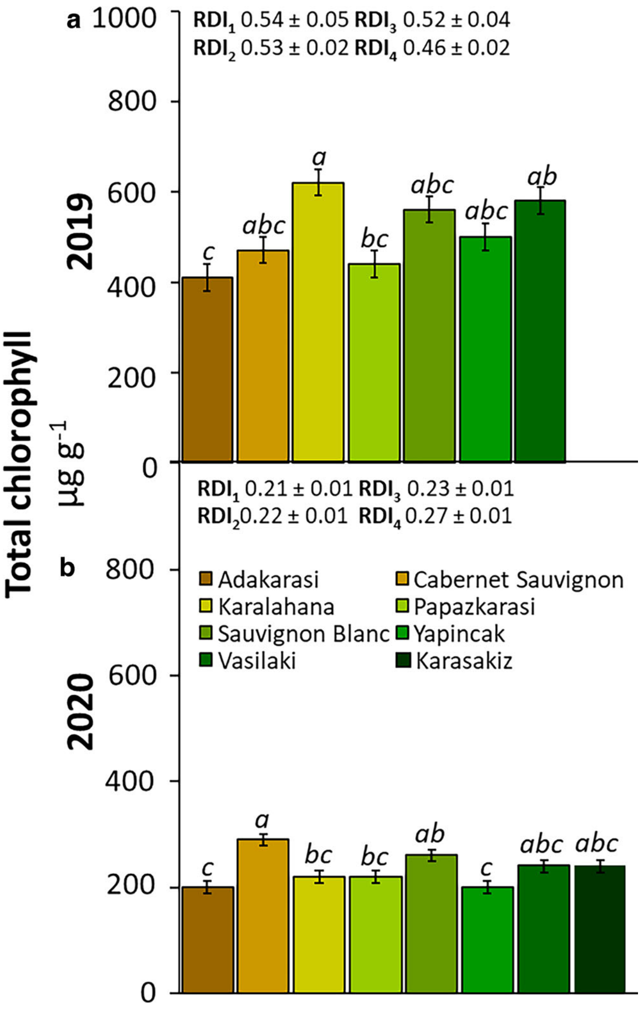

Fig. 3 The impact of cultivars and treatments on the amount of leaf total chlorophyll in 2 consecutive years. Different letters indicate significant differences among treatments ($P < 0.05$) based on Duncan's test ($n = 5$). Data are presented as means ± standard error ($n = 5$). Treatment and cultivar interactions are not presented in the figures because they are not statistically significant. *RDI$_1$* refers to the 100%, maximum 8-L irrigation amount per day; *RDI$_2$* refers to the 75%, daily 6-L irrigation amount; *RDI$_3$* refers to the 50%, daily 4-L irrigation amount; and *RDI$_4$* refers to the 25%, daily 2-L irrigation amount. **a** Trial year 2019, **b** trial year 2020

Figure 3 shows the effects of cultivars and treatments on Chl$_{tot}$. The 'Karalahana' cultivar had the highest Chl$_{tot}$ amount, with a value of 620 µg g^{-1} in 2019 and 420 µg g^{-1} in 2020. The 'Adakarası' cultivar had the lowest Chl$_{tot}$ amount for both years.

The differences in irrigation treatments were found to be statistically significant in 2020; the highest Chl$_{tot}$ amount was determined in RDI$_4$, but this difference was not statistically significant in 2019 (Fig. 3).

Chl$_a$/Chl$_b$ ratios varied greatly among cultivars, but no statistically significant changes were found. The high Chl$_a$/Chl$_b$ ratios observed in the 'Adakarası' cultivar, which is known for its tolerance to drought and high temperatures, may be a result of stress conditions, as suggested by previous studies (Aien et al. 2011; Rustioni and Bianchi 2021). Similarly, 'Cabernet Sauvignon,' another drought-resistant cultivar, did not show any changes in its Chl$_a$/Chl$_b$ ratio under water scarcity conditions, as reported in previous research by Martim et al. (2009) and Zulini et al. (2007). Leaf pigment concentrations (Chl$_a$, Chl$_b$, and carotenoids) were not impacted by lack of water. A possible explanation for the lack of correlation between water restriction and leaf pigment concentration may be due to differences in nutrient intakes of cultivars (Casanova-Gascón et al. 2018).

The measures for chlorophylls vary between 2 years and cultivars, as per the criteria. However, the values correspond to the limits set in previous literature (Luo et al. 2019; Martín-Tornero et al. 2020). In 2020, the main effects of irrigation treatments were found to be statistically significant. The RDI$_4$ treatment, which had the highest drought effect, showed the highest values for Chl$_a$, Chl$_b$, and Chl$_{tot}$. Although there are contrasting views (Haider et al. 2017), the available data is compatible with Rustioni and Bianchi's (2021) findings, which suggest that plants respond to drought stress by increasing the concentration of Chls in woody tissues. However, in 2019, a different case was observed regarding RDI treatments, and it can be speculated that this situation was caused by variability among genotypes (Rustioni and Bianchi 2021). When the Chl contents were evaluated for the years 2019 and 2020, it was found that in 2020, Chl$_a$ was 1.86 times lower than the previous year, Chl$_b$ was four times lower, and Chl$_{tot}$ was half the previous year's value. This evaluation is in line with the calculated Growing Degree Days (GDD) index, which shows that the GDD value for 2019 was slightly higher than that of 2020 (2157.00 day-degrees). The increase in GDD in 2019 resulted in higher Chl contents in the tissues of the cultivars. Genotypes play a significant role in determining the concentration of photosynthetic pigments in woody tissues, suggesting that Chl content is primarily controlled by genetic factors. Conversely, the pigment proportion's variability is mainly attributed to environmental conditions (Rustioni and Bianchi 2021). Additionally, changes in the amount and ratio of Chl are not only indicative of leaf senescence but also act as stress markers that can have an impact on the plant's metabolic processes (Filimon et al. 2016).

Based on the previous determination, the change in the pigment proportion seen in the Chl$_{a/b}$ ratio in 2020 compared to the previous year is reasonable. Especially in 2020, the four-fold decrease in Chl$_b$ increased the Chl$_{a/b}$ values by an average of 3.65 times. Therefore, it can be speculated that the change in Chl$_b$ ratio is more significant in explaining the drought effect. Based on the earlier findings, the observed change in pigment proportion, as evidenced by the Chl$_{a/b}$ ratio, between 2020 and the previous year appears justifiable. In particular, the four-fold decrease in Chl$_b$ in 2020 led to an average increase of 3.65 times in Chl$_{a/b}$ values. Therefore, it is reasonable to speculate that the reduction in Chl$_b$ ratio is a more significant factor in explaining the drought effect. It has been found that both the relatively drier year of 2019 and the fact that the plants were in their first year resulted in higher Chl contents in response to the drought, which was felt more intensely by the plants in 2019. In 2020, the significant decrease in Chl$_b$ caused an increase in Chl$_{a/b}$ ratios. Moreover, since this response was not solely regulated by Chl content, the increase in the levels of macro and micro nutrients, particularly Ca (Candar et al. 2021) and Fe (data not disclosed), respectively, in perennial tissues in the second year also contributed to the drought resistance response (Fig. 4).

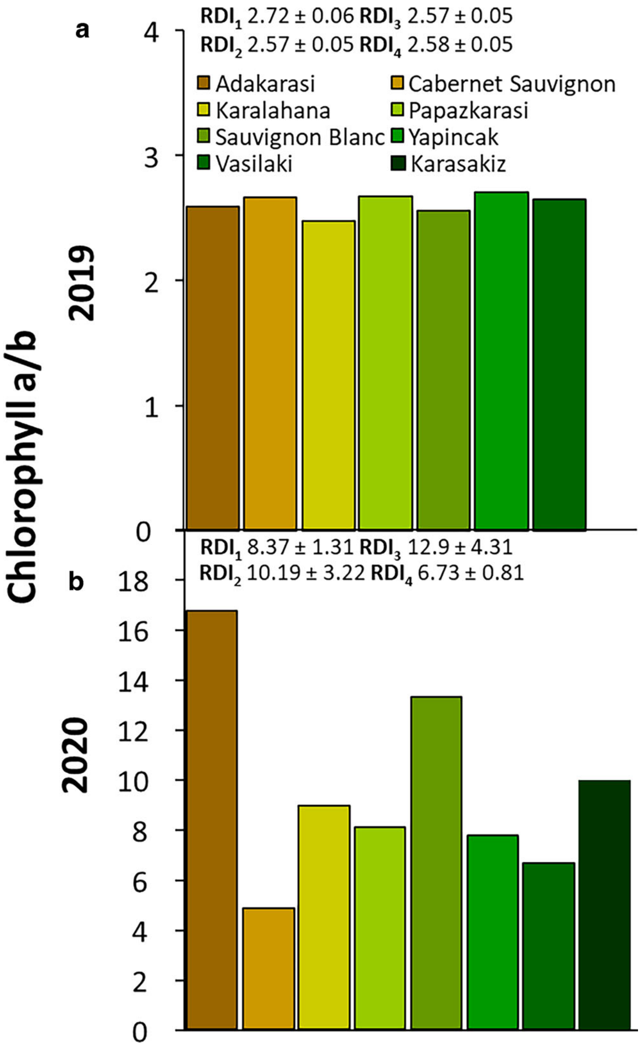

Fig. 4 The impact of cultivars and treatments on the amount of leaf total chlorophyll$_{a/b}$ in 2 consecutive years. Different letters indicate significant differences among treatments ($P<0.05$) based on Duncan's test ($n=5$). Data are presented as means ± standard error ($n=5$). Treatment and cultivar interactions are not presented in the figures because they are not statistically significant. RDI_1 refers to the 100%, maximum 8-L irrigation amount per day; RDI_2 refers to the 75%, daily 6-L irrigation amount; RDI_3 refers to the 50%, daily 4-L irrigation amount; and RDI_4 refers to the 25%, daily 2-L irrigation amount. **a** Trial year 2019, **b** trial year 2020. *RDI* regulated deficit irrigation

These results suggest that the Chl$_{tot}$ amounts in leaves may not be directly related to photosynthesis amount and vigor. While 'Adakarası,' 'Cabernet Sauvignon,' and 'Sauvignon Blanc' cultivars had the highest vigor parameters and 'Adakarası' and 'Cabernet Sauvignon' cultivars had the highest average photosynthesis amounts during the experiment years, although not shared in this study, this is not in agreement with the findings of Villar et al. (2012) and Chavarria et al. (2012), who showed that high chlorophyll levels in 'Merlot,' 'Cabernet Sauvignon,' and 'Sauvignon Blanc' cultivars correspond to high photosynthesis amounts. One possible explanation for the discrepancy is that the leaf samples in this study were taken towards the end of the vegetative cycle, and therefore the chlorophyll content in the leaves may have varied due to leaf senescence (Canton et al. 2017). However, this phenomenon can be clearly explained by the Chl$_{a/b}$ ratio. The Chl$_{a/b}$ ratio shows a distinct pattern that is related to the amount of light that the tissues are exposed to and their potential contribution to overall photosynthesis. Drought conditions cause this ratio to increase in woody tissues, bringing it closer to the values seen in tissues with higher levels of photosynthetic activity (Rustioni and Bianchi 2021). In particular, the higher Chl$_{a/b}$ ratios observed in the 'Adakarası' and 'Sauvignon Blanc' cultivars in 2020 can be directly linked to the levels of photosynthesis during the experimental years. Furthermore, it is worth noting that the 'Adakarası' cultivar is traditionally known for its drought resistance.

Proline Content

Proline contents in cultivars showed no significant variations in 2019, but in 2020, significant differences were observed. The highest proline content was found in 'Yapıncak' cultivar (4.87 µmol g FW^{-1}), and the lowest was in 'Cabernet Sauvignon' (1.74 µmol g FW^{-1}) during the second year of the study (Fig. 5).

The research showed low proline levels in the drought-tolerant 'Adakarası' cultivar, similar to 'Cabernet Sauvignon'. Meanwhile, the traditionally water-stress sensitive cultivars, 'Sauvignon Blanc,' 'Yapıncak,' and 'Vasilaki,' had relatively high proline levels. This suggests that water-stress sensitive cultivars increase proline levels as a coping mechanism, while resistant cultivars adapt through other means. In 2019, proline levels increased as water amount decreased, as expected. However, in 2020, the opposite trend was observed, with the RD$_4$ application resulting in the lowest amount of proline (Fig. 5).

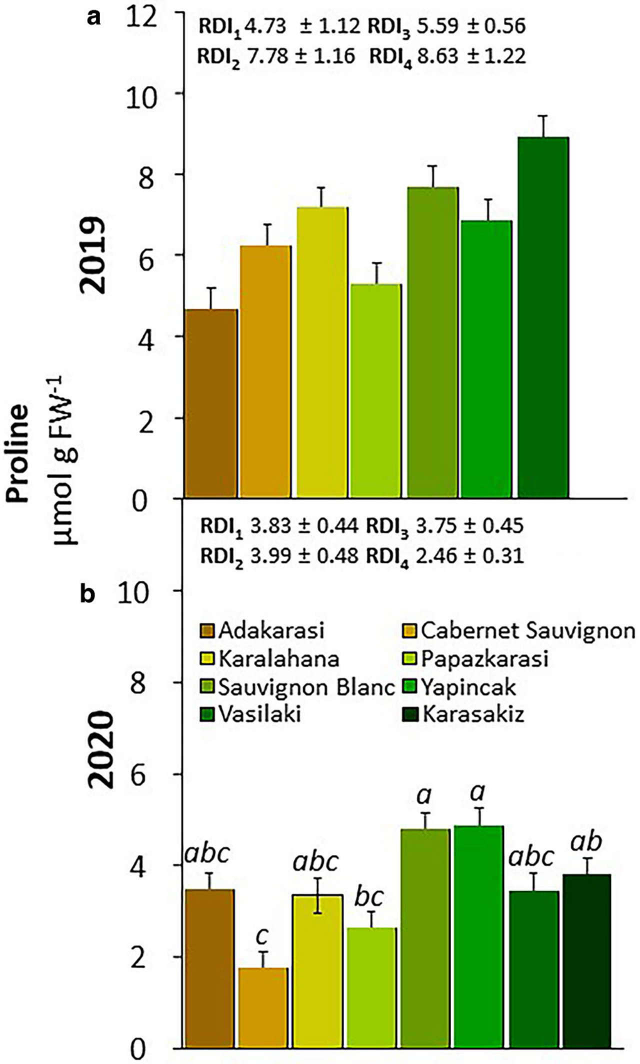

Fig. 5 The impact of cultivars and treatments on the amount of leaf proline in 2 consecutive years. Different letters indicate significant differences among treatments ($P < 0.05$) based on Duncan's test ($n = 5$). Data are presented as means ± standard error ($n = 5$). Treatment and cultivar interactions are not presented in the figures because they are not statistically significant. *RDI$_1$* refers to the 100%, maximum 8-L irrigation amount per day; *RDI$_2$* refers to the 75%, daily 6-L irrigation amount; *RDI$_3$* refers to the 50%, daily 4-L irrigation amount; and *RDI$_4$* refers to the 25%, daily 2-L irrigation amount. **a** Trial year 2019, **b** trial year 2020. *RDI* regulated deficit irrigation

The study's results are in agreement with previous research on both cultivars and irrigation treatments. Hernández-Orte et al. (1999) found that the amino acid (AA) profile of a cultivar may remain consistent over time, but the AA concentration can vary significantly. Additionally, it has been reported that total AAs can remain stable in grapevine leaves under water stress conditions (Patakas et al. 2002). The accumulation of osmolytes is also regulated by ABA (Ju et al. 2020).

Proline is a small, soluble and non-toxic amino acid (Ashraf and Foolad 2007) that plays an important role in regulating osmotic pressure in the cell by maintaining a balance between the cytoplasm and vacuoles. In addition, proline helps to mitigate the damaging effects of reactive oxygen species (ROS), maintains membrane integrity, and enhances the activity of antioxidant enzymes. These protective properties against oxidative stress are also observed in grapevine plants (Ozden et al. 2009; Min et al. 2019).

However, it has also been reported that this osmotic regulation in the vine plant is carried out by the accumulation of various ions during drought instead of organic solutes (Patakas et al. 2002). Adaptation with the accumulation of Ca^{++} and K^{+} in water drought conditions is a sustainable method that requires less energy via solubilization of nutrients by microbes (Greenway and Munns 2003; Munns and Tester 2008; Ma et al. 2020; Villette et al. 2020). In a previous study with the same plants and experimental method, 'Adakarası' cultivar had the highest Ca^{++} content, while 'Cabernet Sauvignon' cultivar had the highest K^{+} content (Candar et al. 2021). In this study, it can be said that the accumulation of organic ions in grapevine leaves is more prominent in adaptation to drought.

Abscisic Acid (ABA) Content

In both years, there was a statistically significant difference in the total amount of ABA based on the cultivar. 'Adakarası' had the highest amount of ABA in 2019, with a value of 1.88 µg g FW^{-1}, while 'Karasakız' had the highest amount in 2020, with a value of 0.77 µg g FW^{-1}. In contrast, the lowest ABA values were found in 'Papazkarası' cultivar, with values of 0.87 µg g FW^{-1} in 2019 and 0.45 µg g FW^{-1} in 2020 (Fig. 6).

ABA amount showed statistically significant differences for irrigation treatments in 2020 but not in 2019. The highest ABA concentration was found in the RD$_4$ treatment. Overall, leaf ABA concentration was higher in 2019 than in 2020. The impact of irrigation on hormone accumulation varied depending on the cultivar and year.

ABA, ethylene, and auxins are natural plant growth regulators that play a significant role in regulating plant stress responses and are present in high concentrations in plants (Pieterse et al. 2012). Particularly under water stress conditions, the concentration of ABA in the xylem tissues increases, regulating hydraulic conductivity (Schachtman and Goodger 2008). This regulation involves non-transcriptional metabolic pathways that control immediate responses such as gs, while slower responses, such as shoot growth control, are regulated by gene activities (Hubbard et al. 2010). In some cases, external application of ABA can trigger protein transport and carbon metabolism (Conde et al. 2011; Sah et al. 2016). Low ABA levels are often associ-

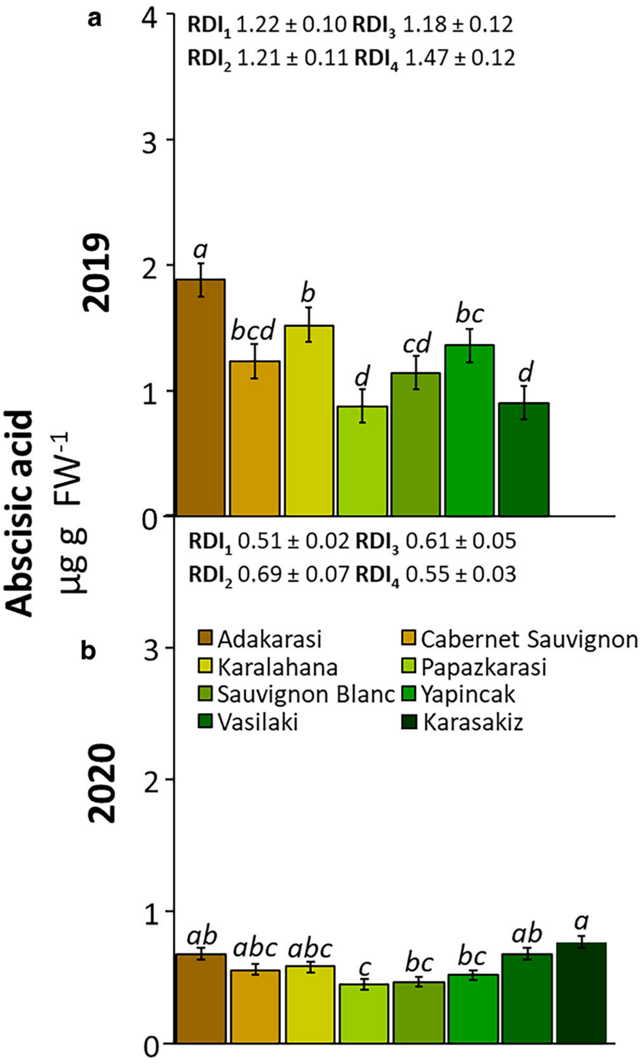

Fig. 6 The impact of cultivars and treatments on the amount of leaf abscisic acid in 2 consecutive years. Different letters indicate significant differences among treatments ($P < 0.05$) based on Duncan's test ($n = 5$). Data are presented as means ± standard error ($n = 5$). Treatment and cultivar interactions are not presented in the figures because they are not statistically significant. *RDI₁* refers to the 100%, maximum 8-L irrigation amount per day; *RDI₂* refers to the 75%, daily 6-L irrigation amount; *RDI₃* refers to the 50%, daily 4-L irrigation amount; and *RDI₄* refers to the 25%, daily 2-L irrigation amount. **a** Trial year 2019, **b** trial year 2020. *RDI* regulated deficit irrigation

ated with non-water-stress conditions or cultivars that cannot cope with water stress (Dinis et al. 2016a, b; Brito et al. 2019). As soil dries out, the concentration of ABA in roots, shoots, and leaves increases rapidly (Beis and Patakas 2015). Lower ABA levels in leaves are strongly correlated with lower gs and improved water status (Dinis et al. 2018; Bernardo et al. 2021).

The study's results on ABA align with existing literature. Although the mean of both years showed a numerical increase in ABA concentration under the RDI_4 treatment, statistical significance was not observed. This could be attributed to the experimental method, which was not conducted under fully controlled conditions. Furthermore, ABA may not play a significant role in grapevine's defensive mechanisms under high UV exposure. Moreover, the responses of cultivars and irrigation treatments to various climatic factors may not have been fully determined. Since different climatic factors can have a combined effect, it is difficult to explain their impact by a single parameter.

Conclusion

Based on the results presented, it is evident that cultivar responses can vary significantly under different climatic conditions. Apart from water scarcity treatments, the precise responses of cultivars to other abiotic climatic factors remain difficult to determine due to various factors such as genetic components, root behavior, transport system variability, crop load, and rootstock/scion relationships, which require further study.

In conclusion, this study confirms that the 'Adakarası' cultivar of *Vitis vinifera* L. is capable of adapting its metabolic processes to reduced water availability similar to 'Cabernet Sauvignon'. The 'Karasakız' cultivar shows promise in terms of drought resistance, based on 1-year data. The other autochthonous cultivars, 'Papazkarası,' 'Karalahana,' 'Yapıncak,' and 'Vasilaki', have adapted to specific regions based on their origin.

These research findings can improve our understanding of Chl, proline, and ABA dynamics and help define the behavior of local wine grape cultivars under the mentioned conditions.

Acknowledgements The authors would like to thank all editors, reviewers, and the agronomists Jannat Khan, Ecem Kübra Demirkapı, and Şevval Vural for their contribution to the field and laboratory works. Authors would also like to thank Sigfrido Romeo for corrections in the text.

Funding This study was carried out under the project TAGEM/BBAD/B/19/A1/P6/06, which was supported and funded by the General Directorate of Agricultural Research and Policies of the Republic of Turkey Ministry of Agriculture and Forestry.

Author Contribution Authors S. Candar and E. Bahar planned and designed the trial. S. Candar performed the field experiments and made statistical analysis. G.U. Seçkin performed laboratory analyzes. T. Kizildeniz, İ. Korkutal and E. Bahar made critical revisions of the manuscript for intellectual content. All authors read and approved the final manuscript.

Conflict of interest S. Candar, G.U. Seçkin, T. Kizildeniz, İ. Korkutal, and E. Bahar declare that they have no competing interests.

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