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Morphophysiological and antioxidant responses of different grape cultivars from the Caspian Basin under varying water deficit conditions

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ABSTRACT

The study investigated the effects of water deficit on the morphophysiological and antioxidant responses of seven grape cultivars from the Caspian Basin. Three-bud cuttings from grapevine cultivars were prepared, rooted in sawdust, and transplanted into pots with soil, sand, and peat. A factorial experiment design, considering three water deficit levels (100 % as control, 70 %, 40 % field capacity [FC]) and seven cultivars, was performed. Morphophysiological and antioxidant responses were measured at the onset of visible drought symptoms, enabling a comparative evaluation of cultivar-specific adaptations to water stress. As water deficit increased, shoot length, leaf fresh and dry weights decreased, with the lowest values observed under the 40 % FC treatment. Interestingly, the Muscat Yamtazini cultivar maintained the highest total chlorophyll content in the 70 % FC treatment. Cultivar and water deficit also had significant main effects on internode length and lateral shoot length. The Bidane Sefid cultivar exhibited the highest internode length but the lowest lateral shoot length. Water deficit treatments reduced internode length and drastically decreased lateral shoot length, with the 40 % FC treatment having the lowest lateral shoot length. Water deficit treatments significantly affected stem diameter, lateral shoot number, and canopy temperature. The 40 % FC treatment resulted in the lowest stem diameter and lateral shoot number, while displaying the highest canopy temperature. Relative water content (RWC) of leaves and roots and membrane stability index (MSI) decreased with increasing water deficit. The Qazagiski Ramphi and Chefte cultivars maintained the highest leaf and root RWC under water deficit, while the Chefte cultivar had the lowest MSI. Leaf and root proline content, total protein, and hydrogen peroxide showed significant interaction effects between cultivar and water deficit. Antioxidant enzyme activities increased with drought stress, and the Muscat Yamtazini, Kishmish Hisrao, and Qazagiski Ramphi cultivars exhibited distinct responses. Based on their performance under drought stress, the studied cultivars were ranked, with Muscat Yamtazini being the most tolerant, followed by Kishmish Hisrao and Qazagiski Ramphi.

KEYWORDS: drought stress tolerance, hydrogen peroxide, proline, relative water content, *Vitis vinifera*

INTRODUCTION

The cultivated grapevine (*Vitis vinifera*) is shown to have originated partly in the Caucasus region, which encompasses parts of modern-day Georgia, Armenia, and Azerbaijan (Khan *et al.,* 2020; Dong *et al.,* 2023). This region, situated between the Black and Caspian Seas, is considered the primary center of origin for the species. The wild ancestor of the cultivated grapevine, known as *Vitis vinifera* subsp. *sylvestris*, is native to the Caucasus and surrounding areas, as well as parts of Central Asia and the Mediterranean basin. Archeological evidence suggests that the domestication of the grapevine likely began in the Caucasus region, with the earliest known evidence dating back to around 6,000-8,000 years ago (McGovern *et al.,* 2013). The rich diversity of grapevine varieties and the long history of viticulture in the Caucasus and Caspian Basin regions attest to the importance of these areas as the birthplace of the cultivated grapevine.

The Caspian Basin is facing significant challenges due to the impacts of climate change. This area is particularly vulnerable to the increasing frequency and severity of drought events (Lioubimtseva & Henebry, 2009). Situated between the Caucasus and Central Asian regions, the Caspian Basin experiences a continental climate, characterised by hot, dry summers and relatively cold winters (Kostianoy & Kosarev, 2005). However, in recent decades, this delicate balance has been disrupted, leading to a concerning trend of rising temperatures and declining precipitation levels (Lioubimtseva & Henebry, 2009). The effects of climate change on the Caspian Basin are multifaceted. Prolonged drought periods have led to the depletion of freshwater resources, affecting both agriculture and the region's fragile ecosystems (Kattsov *et al.,* 2020).

A major environmental challenge, drought severely impairs the growth and productivity of grapevines (Vahdati & Lotfi, 2013; Wang *et al*., 2021). Grapevines minimise drought effects through adaptations like stomatal closure and solute accumulation leading to conservation of water-use but reducing photosynthesis activity and chlorophyll content. This limits carbohydrate production, affecting growth and development (Haider *et al.,* 2016; Ghorbani *et al.,* 2019). Drought stress has also been shown to promote the production of reactive oxygen species in grapevines, which can damage cellular membranes, proteins, and other important biomolecules (Gill & Tuteja, 2010). In response to oxidative stress, grapevines mitigate its effects by activating antioxidant defense mechanisms, including the upregulation of enzymes such as peroxidase (POD) and catalase (CAT) (Lin *et al.,* 2023). However, if the drought stress is prolonged, the plant's antioxidant system may become overwhelmed, leading to further cellular damage and impaired growth. The vegetative growth, reproductive development, and ultimately, the yield and quality of grapes are significantly impacted by the combined physiological, biochemical, and metabolic responses due to drought stress. Understanding these complex mechanisms is crucial for developing effective strategies to mitigate the adverse effects of drought and ensure sustainable grape production.

In the Caspian Basin, where water resources are becoming increasingly scarce, the cultivation of drought-tolerant grape cultivars is essential for ensuring the continued success of the region's viticulture industry (Ristic *et al.,* 2007), which necessitates multifaceted approaches for the implementation of a strategic selection and cultivation of drought-tolerant grapevine cultivars. This includes identifying and evaluating the performance of promising cultivars under local climatic conditions, as well as developing breeding programmes to further enhance drought tolerance (Ristic *et al.,* 2007). Collaboration among researchers, viticulturists, and policymakers is essential for integrating drought-tolerant grape cultivars into the Caspian Basin's agriculture. Prioritising these resilient varieties can strengthen viticulture, preserving the region's cultural heritage, economic stability, and environmental sustainability amid climate change and water scarcity.

This study aimed to provide a comprehensive assessment of the morphophysiological and antioxidant responses of seven Caspian Basin grapevine cultivars to varying levels of water deficit, in order to identify drought-tolerant genotypes and elucidate the underlying physiological mechanisms. The evaluated cultivars included Qazagiski Ramphi (QR), and Kishmish Hisrao (KH), two seeded black grapes from eastern Turkmenistan and the Republic of Azerbaijan, respectively; Supran Bulgar (SB), Muscat Yamtazini (MY), and Besmiamphi Ramphi (BR), all seeded white grapes from southern Russia; and Chefte (CH), a seeded white grape, along with Bidane Sefid (BS), a seedless white grape, both from Iran (Rasoli & Dolati, 2017).

MATERIALS AND METHODS

1. Plant materials and drought treatments

This research was carried out at the grapevine Research Centre of Qazvin, with an elevation of 1250 m above sea level, latitude 36.03 N, and longitude 49.40 E. Qazvin province in northern Iran experiences a hot-summer Mediterranean climate, with hot, dry summers with average highs of 33 °C in July and cold and snowy winters with average lows of around –2 °C in January. The region receives an average annual rainfall of 220 mm (IMO, 2020). The plant materials included seven grapevine cultivars from the Caspian Basin region, representing diverse genetic backgrounds and drought adaptation profiles. The cultivars evaluated were Qazagiski Ramphi (QR), Supran Bulgar (SB), Muscat Yamtazini (MY), Besmiamphi Ramphi (BR), Kishmish Hisrao (KH), Chefte (CH) as a drought-tolerant cultivar, and Bidane Sefid (BS) as the dominant cultivar of the Qazvin region, serving as a control. The adaptability of these cultivars to the climatic conditions of the region had already been evaluated in the West Azerbaijan and Qazvin provinces, which are the two main grape-producing regions of Iran (Rasoli & Golmohammadi, 2009; Rasoli & Dolati, 2017).

In the fall season, three-bud cuttings of the target cultivars with similar diameters were prepared from the national collection of grapevine cultivars. To facilitate callus formation and rooting, the cuttings were kept upside down for one month in sawdust. After successful rooting, the cuttings were transplanted into 10 L pots filled with a 1:1:1 mixture of agricultural soil, sand, and peat moss. The rooted plants were grown in a controlled greenhouse environment with a relative humidity of 60 ± 5 % and a daytime temperature of 25 ± 5 °C. The experiment was designed as a completely randomised factorial arrangement with three replications each containing three pots. The first factor included the seven grapevine cultivars previously mentioned. The second factor consisted of three levels of water deficit: 100 % field capacity (FC) (control), 70 % FC, and 40 % FC (Pranichiankis & Angelakis, 2008). To determine the amount of water used, the control pots of each cultivar were watered to the point of waterlogging, and two days later, the amount of water drained and the water remaining in the pot was weighed and recorded. The 100 % (control), 70 % and 40 % FC treatments were then calculated and applied based on this control value. Each day at sunset, irrigation water was added to maintain the soil at the required field capacity. A gravimetric method was employed, where each pot was weighed individually, and water was added as needed. The duration of each treatment was 30 days, from 15 July to 15 August, and was determined based on the appearance of drought stress symptoms, such as leaf shrinkage and yellowing of the leaf margins (Neri *et al.,* 2003). Following this period, morphophysiological and antioxidant traits were assessed.

2. Measurement of morphophysiological traits

To measure the fresh and dry weight, three mature leaves were weighed using a digital scale (AND GF-300, Japan). The samples were then dried in an oven at 70 °C for 48-hours and weighed again. The length of the main shoot and the average length of all lateral shoots were measured using a ruler, while the internode length and stem diameter were measured at the third internode from the top of the current season's growth using a digital caliper. Canopy temperature was recorded using an infrared thermometer (TES-1327K, Taiwan).

To determine the chlorophyll content, fresh samples of two mature leaves (0.2 g) per plant were cut into 2 mm pieces and extracted with 10 mL of 80 % (*v/v*) acetone in the dark until the leaves were completely decolorised. The absorbance of the extract was measured using a spectrophotometer at wavelengths of 646 nm and 663 nm. The total chlorophyll content was then calculated according to Gao (2006).

3. Measurement of relative water content, membrane stability index and proline content

The relative water content (RWC) of the leaf and root samples was estimated following the method described by Castillo (1996). The samples weighing 0.5 g were prepared from two mature leaves and several root portions with medium diameter, saturated in 100 mL of distilled water for 24 h at 4 °C in the dark, and their turgid weights were recorded. The samples were then oven-dried at 65 °C for 48 h and their dry weights were measured. The RWC of the plant tissue is expressed as a percentage and calculated using the following equation:

$$RWC\ (\%) = [(FM - DM) / (TM - DM)] \times 100 \qquad \text{(eq. 1)}$$

Where, FM, DM, and TM were the fresh, dry, and turgid masses, respectively.

The method of Sairam (1994) was employed to determine the membrane stability index (MSI) of leaf and root samples. The samples (0.2 g) were washed three times with distilled water and incubated with 15 mL of distilled water at 23 °C for 24-hours in the dark. Following vigorous shaking, the electrical conductivity (EC) of the electrolytes was measured using a conductivity meter (WTW Cond 3110, USA). The samples were then autoclaved for 15-minutes at 60 °C, returned to 25 °C, and the EC was measured again. The MSI was calculated using the equation:

$$MSI = (1\text{-}C1/C2) \times 100 \qquad \text{(eq. 2)}$$

where C1 and C2 represent the initial and final EC, respectively.

Proline content was measured using the method described by Fahim *et al.* (2022). Leaf and root tissues were finely ground in liquid nitrogen, and 10% sulfosalicylic acid was added to the ground sample. The mixture was then filtered, and the filtered solution was combined with a ninhydrin reagent and acetic acid. This mixture was incubated for 1 hour at 100 °C. Subsequently, toluene was added to the mixture, which was then vortexed. The absorbance of the resulting red supernatant solution was measured at 520 nm using a spectrophotometer.

4. Measurement of total protein, hydrogen peroxide and antioxidant enzymes

The total proteins were extracted from the root samples, and their content was determined using the colorimetric Bradford assay (Bradford, 1976). The absorbance of the extracted proteins was measured at 595 nm, with bovine serum albumin used as the standard. The total protein values were expressed as mg/g FW.

The hydrogen peroxide (H_2O_2) concentration in the root samples was determined following the method described by Loreto and Velikova (2001). Root samples weighing 0.3 g were homogenised in 3 mL of 1 % (*w/v*) trichloroacetic acid solution. The homogenate was then centrifuged at 10,000 × *g* at 4 °C for 10 min. Next, 0.75 mL of the supernatant was added to 0.75 mL of 10 mM potassium phosphate buffer (pH 7.0) and 1.5 mL of 1 M potassium iodide. The H_2O_2 concentration in the supernatant was determined by comparing its absorbance at 390 nm to a standard calibration curve and expressed as µmol/g FW.

For measuring the enzyme activities, the roots of the grapevine plants were collected, and approximately 0.5 g of root tissue from each sample was weighed and finely ground in a mortar using liquid nitrogen. From the frozen powdered sample,

100 mg was homogenised in 1.0 mL of a sodium phosphate buffer (0.5 M, pH 7.8) containing 1 mM EDTA and 2% (*w/v*) PVP-40. The homogenised samples were then centrifuged at 10,000 × *g* for 20 min at 4 °C, and the enzyme activities were measured using the supernatant. The specific enzyme activities were expressed as units/mg protein. The activity of POD and ascorbate peroxidase (APX) was determined following the method described by Karami *et al.* (2017). The activities of guaiacol peroxidase (GPX) and CAT were measured using the procedure outlined by Irani *et al.* (2021).

5. Statistical analysis

The statistical analysis was carried out using SPSS (version 21), and mean comparisons were made using the Least Significant Difference (LSD) test. Before analysis, appropriate transformations were applied to the data to satisfy the assumptions of normality and homogeneity of variance.

To select grapevine cultivars with high drought tolerance, a ranking system was developed based on the mean comparison of the interaction effects of cultivar and water deficit for the studied traits (Doulati *et al.,* 2010). For this purpose, statistical groupings were translated into numerical scores, with alphabetical letters denoting statistical significance converted as follows: 'a' = 1, 'b' = 2, 'c' = 3, and so on. For groups containing multiple letters (*e.g.*, 'abc'), the score was computed as the arithmetic mean of the corresponding letter scores. An exception applied to H_2O_2, which typically increases under drought stress; here, scoring was reversed: the lowest letter was assigned 1, while 'a' received the highest score. To standardise trait scales, scores were normalised by dividing by the group's maximum, yielding values from 0 to 1. The normalised scores for all traits were then averaged for each cultivar, and cultivars were ranked accordingly. The lowest mean score indicated the most drought-tolerant cultivar, while the highest indicated the most drought-sensitive.

RESULTS AND DISCUSSION

1. Morphophysiological traits of shoots and leaves

According to the results of the analysis of variance, the interaction effect of cultivar and water deficit was significant for certain traits, such as main shoot length, leaf fresh weight, and leaf dry weight. Therefore, the comparison of the mean of the interaction effects is presented for these variables. However, for other traits, such as internode length and canopy temperature, the interaction effects of cultivar and water deficit were not significant. In these cases, the significant independent effects of the treatments have been expressed.

The results demonstrated a statistically significant difference in main shoot length between cultivars under the varying water deficit treatments ($p < 0.001$). The highest shoot lengths were observed in the control treatment (100 % FC) for cultivars BS and QR (Table 1). However, as the severity of the water deficit increased, the shoot length was observed to decrease accordingly. This finding aligns with the well-established understanding that water availability is a critical factor influencing plant growth and development (Farooq *et al.,* 2009). Specifically, the exposure to drought stress for the QR and SB cultivars led to a greater than 50 % reduction in the main shoot length under the 40 % FC treatment, as compared to the control group. This substantial decrease in shoot length under severe water deficit conditions suggests that the growth of these cultivars may be more sensitive to drought stress than others. Furthermore, the lowest shoot lengths were recorded in both the 40 % and 70 % FC water deficit treatment levels for cultivars QR, KH, BR, SB, and BS.

The statistical analyses revealed a significant difference in both leaf fresh weight and leaf dry weight among cultivars under the various water deficit conditions ($p < 0.001$ for both). As the intensity of the water deficit increased, the leaf fresh and dry weights were observed to decrease accordingly, with the lowest values for both parameters recorded under the 40 % FC conditions (Table 1). Interestingly, in the 70 % FC treatment, MY exhibited the highest leaf dry weight, suggesting the presence of differential drought tolerance mechanisms among the evaluated cultivars, potentially involving adaptations that maintain leaf biomass under moderate water deficit conditions (Lata & Prasad, 2011). Furthermore, under the most severe 40 % FC water deficit treatment, the BS cultivar displayed the lowest leaf fresh and dry weights, while the CH cultivar maintained higher leaf fresh and dry weights compared to the other cultivars. This observation indicates the presence of cultivar-specific adaptations to drought stress, with the CH cultivar potentially possessing more effective strategies for maintaining leaf biomass production under severe water deficit conditions (Fang & Xiong, 2015).

Drought conditions, lead to a decrease in leaf turgidity and in overall water content (Chaves *et al.,* 2003). This reduction in leaf water status directly impacts the leaf fresh weight. In addition, drought stress can also lead to a decrease in the accumulation of dry matter in the leaves (Sofo *et al.,* 2004a), as drought-induced reductions in photosynthesis and the subsequent impairment of carbon assimilation can limit the plant's ability to produce and translocate carbohydrates to the leaves, resulting in a decline in leaf dry weight (Flexas *et al.,* 2006). The extent of the reduction in leaf fresh and dry weight under drought stress can vary depending on the plant species, cultivar, and the severity and duration of the drought event (Anjum *et al.,* 2011). More drought-tolerant plants may exhibit smaller decreases in these parameters, as they have developed adaptive mechanisms to maintain leaf water status and carbon assimilation under water-limited conditions (Blum, 2005).

The statistical analysis also revealed a significant difference in total leaf chlorophyll content between cultivars across the different water deficit treatments ($p = 0.005$). Compared to the well-irrigated control, a sharp decrease in total chlorophyll content was evident in both water deficit treatment levels (40 % and 70 % FC) (Table 1). The results are

TABLE 1. The interaction effects of cultivar and water deficit on main shoot length, leaf fresh and dry weight, and total chlorophyll in grapevine plantlets.

Cultivar	Water deficit (% FC)	Main shoot length (cm)	Leaf fresh weight (g)	Leaf dry weight (g)	Leaf total chlorophyll (mg/g FW)
		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p = 0.001$
CH	100 (Control)	29 ab	2.68 cd	0.63 def	2.76 bcd
MY		27 b	3.17 bc	0.72 cde	3.95 a
QR		33 a	3.29 abc	1.54 ab	1.18 f
KH		27 b	2.66 cd	1.39 b	1.97 def
BR		26 b	2.74 cd	0.95 c	2.96 bc
SB		31 ab	3.51 ab	0.26 g	2.64 bcd
BS		34 a	3.86 a	1.28 b	3.50 ab
CH	70	21 c	2.00 ef	0.96 c	1.49 ef
MY		18 cd	2.87 cd	1.71 a	2.17 cde
QR		20 c	2.01 ef	0.93 cd	1.48 ef
KH		19 cd	1.81 ef	0.7 cde	1.29 ef
BR		16 c-f	2.42 de	0.71 cde	1.47 ef
SB		13 ef	1.79 efg	0.35 fg	1.58 ef
BS		11 f	1.71 fgh	0.58 ef	1.60 ef
CH	40	15 def	1.88 ef	0.62 def	1.17 f
MY		17 de	1.58 fgh	0.5 efg	1.23 ef
QR		15 def	1.11 hi	0.43 efg	1.16 f
KH		16 c-f	1.16 ghi	0.52 efg	1.14 f
BR		16 c-f	1.93 ef	0.58 ef	1.02 f
SB		14 def	1.67 fgh	0.51 efg	1.22 f
BS		18 cd	1.07 i	0.22 g	1.41 ef

A *p*-value of less than 0.05 indicates a significant difference in the analysis of variance. Different letters in each column represent significant differences at $p = 0.01$, LSD test. The abbreviations are as following: BR (Besmiamphi Ramphi), BS (Bidane Sefid), CH (Chefte), FC (Field capacity), KH (Kishmish Hisrao), MY (Muscat Yamtazini), QR (Qazagiski Ramphi), and SB (Supran Bulgar).

consistent with previous studies that have demonstrated the functionality of the photosystem is affected by drought stress (Liang *et al.,* 2018). Under drought stress, the photosynthesis rate is reduced due to a decrease in the chlorophyll content of leaves and stomatal closure (Liang *et al.,* 2018). Farooq *et al.* (2009) showed that water stress can lead to a reduction in chlorophyll content, which is a crucial component of the photosynthetic apparatus. Notably, under the 70 % FC treatment, the MY cultivar exhibited the highest amount of total chlorophyll content compared to the other cultivars evaluated. This finding is particularly interesting, as this same cultivar also displayed the highest leaf dry weight when subjected to 70 % FC treatment and appeared to be less affected by the water deficit in terms of reduced shoot length development. These results suggest that the MY cultivar may possess enhanced drought tolerance mechanisms, potentially involving the maintenance of chlorophyll content and leaf biomass under mild water deficit conditions (Basu *et al.,* 2016). This cultivar-specific response could have important implications for viticultural management and crop selection in water-scarce regions, as the identification of drought-tolerant cultivars is crucial for ensuring sustainable crop production (Lobell & Gourdji, 2012). Water stress significantly reduces chlorophyll content due to oxidative damage, decreased synthesis, and enhanced degradation of chlorophyll molecules. This reduction triggers a chain reaction: limited chlorophyll content inhibits photosynthesis, with penalties in carbohydrate production and resulting in lower fresh and dry leaf weights. These adaptations, though crucial for stress survival, ultimately restrict plant growth and productivity (Huang *et al.,* 2019; Alhoshan *et al.,* 2020). Chlorophyll content plays a pivotal role in photosynthetic efficiency, water use efficiency, and drought resilience. Strategies to stabilise chlorophyll through nutrient management can enhance plant productivity under stress. Severe drought-induced chlorophyll loss often signals oxidative damage, impairing plant health. However, some

genotypes counteract this by activating antioxidants and preserving photosynthetic functions (Baccari *et al.,* 2020).

The cultivar BS exhibited the highest internode length (Figure 1A) and the lowest lateral shoot length (Figure 1C). This cultivar-specific response indicates the presence of differential growth strategies among the evaluated plant genotypes, potentially reflecting adaptations to cope with varying water availability (Tardieu, 2013). Additionally, a significant difference was observed among the water deficit treatments ($p = 0.014$): the 70 % FC and 40 % FC treatments had shorter internodes than the control (Figure 1B). This finding suggests that the severity of the water deficit can directly impact internode elongation, with more severe drought conditions leading to a reduction in this growth parameter (Márquez-García *et al.,* 2012). Under drought stress, plants often exhibit a reduction in internode length, as they prioritise the allocation of resources to maintain other essential functions, such as root growth and water uptake (Chaves *et al.,* 2003). This decrease in internode elongation can lead to a more compact and stunted plant architecture, which is a common drought avoidance strategy (Flexas *et al.,* 2006). Furthermore, a significant difference was detected between the water deficit treatments in lateral shoot length ($p < 0.001$), and the 40 % FC treatment had the lowest lateral shoot length (Figure 1D). These results suggest that lateral shoot length is greatly affected by water deficit, with the lateral shoot length in the control being almost 4 times that of the 40 % FC treatment.

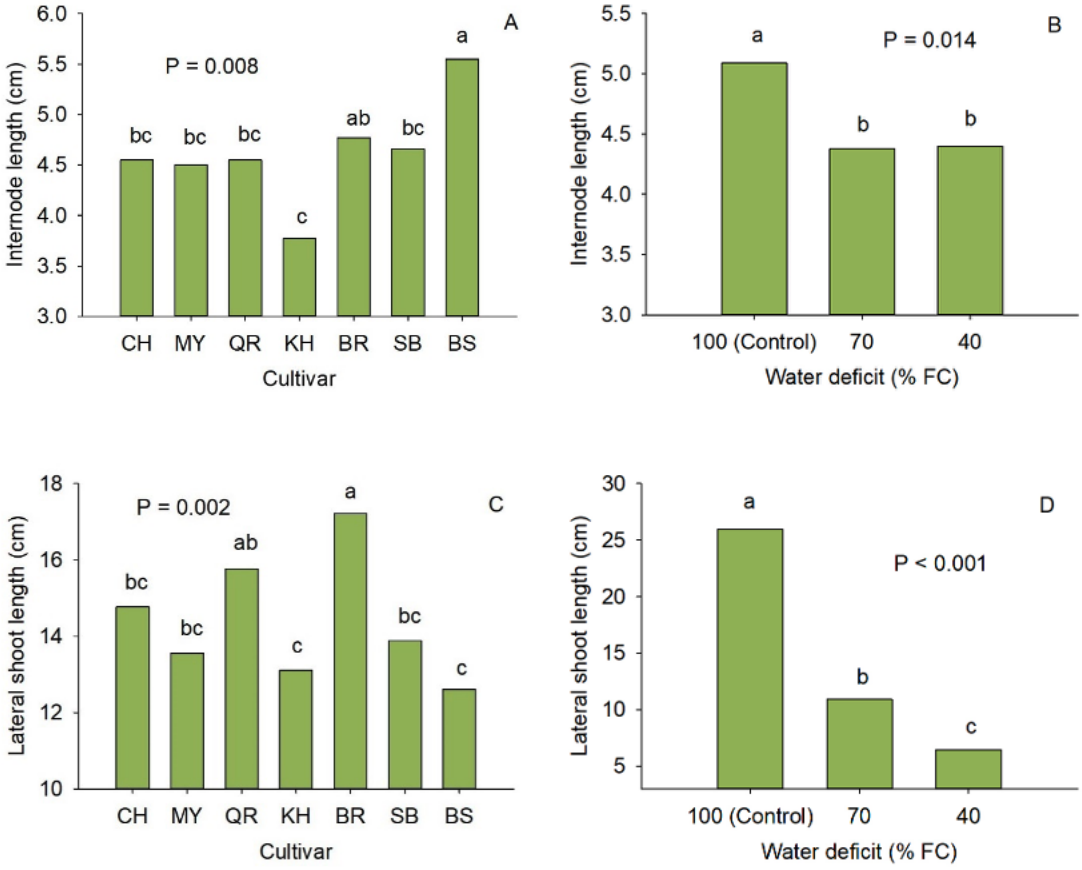


FIGURE 1. The main effects of cultivar and water deficit on internode (A and B) and lateral shoot length (C and D) in grapevine plantlets. A and C illustrate the response of the cultivars to the average FC, while B and D depict the response of all cultivars under water deficit conditions. A *p*-value of less than 0.05 indicates a significant difference in the analysis of variance. Different letters represent significant differences at $p = 0.01$, LSD test. The abbreviations are as following: BR (Besmiamphi Ramphi), BS (Bidane Sefid), CH (Chefte), FC (Field capacity), KH (Kishmish Hisrao), MY (Muscat Yamtazini), QR (Qazagiski Ramphi), and SB (Supran Bulgar).

The observed differences in internode length and lateral shoot length between cultivars and water deficit treatments highlight the importance of understanding the adaptive responses of various plant genotypes to drought stress (Gill & Tuteja, 2010). The cultivar-specific variations in growth characteristics could be attributed to the presence of different drought tolerance mechanisms, such as altered resource allocation, morphological adaptations, or the activation of stress-responsive signalling pathways (Shinozaki & Yamaguchi-Shinozaki, 2007). These results have important implications for the selection and cultivation of drought-tolerant crop varieties in water-limited environments. By identifying cultivars that can maintain desirable growth characteristics, such as the BS cultivar with its relatively

high internode length, breeders and agronomists can develop more resilient plant materials capable of thriving under water-scarce conditions (Blum, 2010).

The analysis revealed a statistically significant effect of the water deficit treatments on stem diameter, lateral shoot number, and canopy temperature ($p < 0.001$ for all parameters) (Figures 2A–C). Notably, the plants subjected to the 40 % FC treatment exhibited the lowest stem diameter and lateral shoot number, while simultaneously displaying the highest canopy temperature. As water becomes scarce, the reduction in cell turgor and the subsequent decrease in cell expansion can result in a smaller stem diameter, which may limit the plant's ability to transport water and nutrients effectively (Anjum *et al.,* 2011). This response is typical of plants under water stress, as they attempt to conserve water by reducing transpiration and growth (Farooq *et al.,* 2009). Based on these findings, the results suggest that under conditions of water deficit the canopy temperature increases, which can potentially introduce an additional stress on the plant. The increase in canopy temperature is a result of reduced evaporative cooling due to stomatal closure, a common adaptation strategy of plants to cope with water scarcity (Meron *et al.,* 2010).

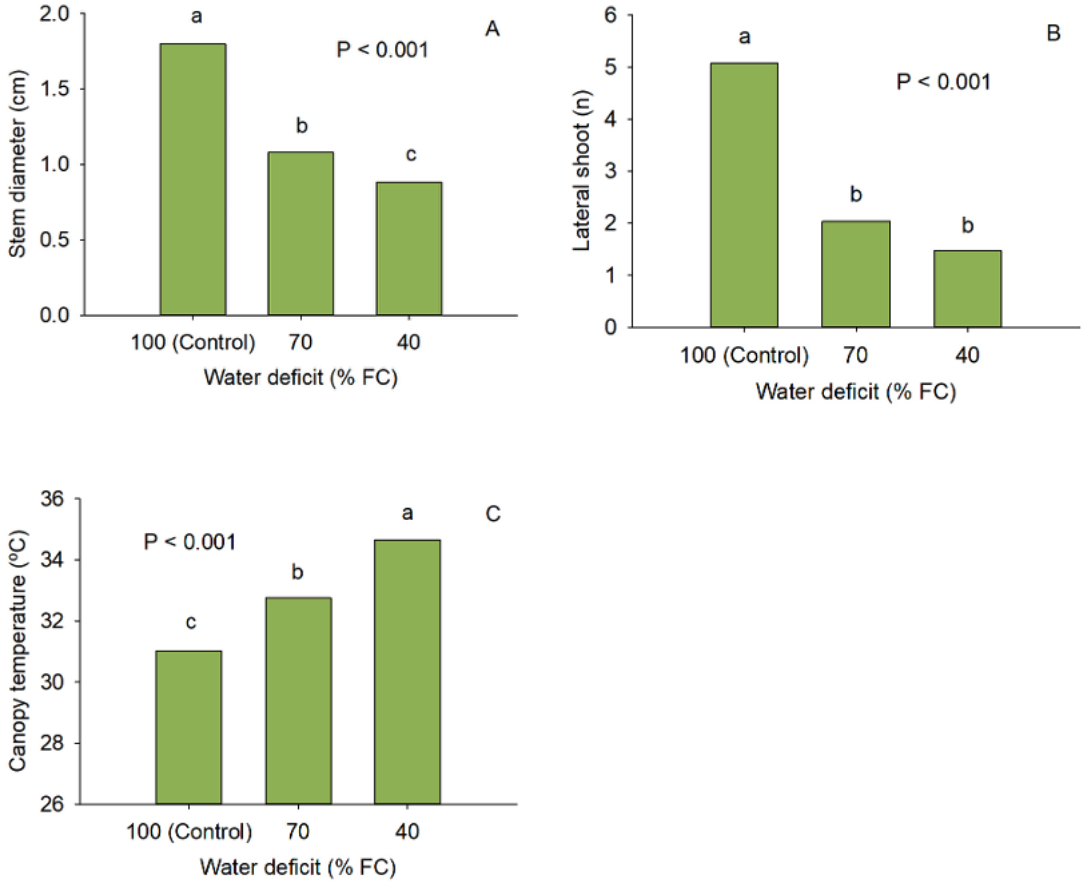


FIGURE 2. The effects of water deficit on stem diameter (A), lateral shoot number (B), and canopy temperature (C) in grapevine plantlets. A *p*-value of less than 0.05 indicates a significant difference in the analysis of variance. Different letters represent significant differences at $p = 0.01$, LSD test. FC represents field capacity.

2. Relative water content, membrane stability index and proline content of leaves and roots

The results of this study demonstrated a significant difference among the studied cultivars in RWC of both leaves ($p < 0.001$) and roots ($p = 0.006$) (Table 2). The RWC of leaves can reflect the level of drought tolerance and water absorption efficiency (Anjum *et al.,* 2011). Our results showed that RWC decreased with increasing drought stress in both plant tissues. In the control treatment, the MY cultivar exhibited the highest leaf RWC (84.0 %), and the KH cultivar the lowest. Under the 70 % FC and 40 % FC treatments, the CH and QR cultivars displayed the highest leaf RWC. The effect of water deficit was more pronounced on root RWC, with the QR and KH cultivars showing the highest root RWC in both water deficit treatments. The ability of some cultivars (such as CH and QR) to maintain higher RWC in leaves under drought stress indicates their potential for better adaptation to water-limited environments (Blum, 2005). Similarly, the higher root RWC observed in QR and KH cultivars under drought conditions likely reflects their enhanced ability to sustain water uptake and transport, a vital trait for plant survival and productivity (Feng *et al.,* 2022).

TABLE 2. The interaction effects of cultivar and water deficit on relative water content of leaves and roots in grapevine plantlets.

Cultivar	Water deficit (% FC)	Relative water content (%)		Membrane stability index (%)	
		Leaf	Root	Leaf	Root
		$p < 0.001$	$p = 0.006$	$p = 0.021$	$p = 0.003$
CH	100 (Control)	75.7 bc	83.3 abc	69.9 ab	68.7 abc
MY		84.0 a	88.0 ab	67.1 abc	64.9 a-d
QR		74.3 bcd	88.2 ab	73.7 a	77.5 a
KH		68.1 efg	89.5 a	75.2 a	74.0 ab
BR		78.6 b	81.6 a-d	66.6 abc	65.5 a-d
SB		74.2 bcd	88. 7 ab	57.3 bcd	56.3 cde
BS		72.7 cde	88.9 ab	67.2 abc	66.1 a-d
CH	70	74.2 bcd	74.1 def	69.0 abc	67.8 a-d
MY		71.9 cde	70.4 fg	67.5 abc	66.4 a-d
QR		70.1 def	82.7 a-d	58.1 bcd	57.1 cde
KH		69.8 d-g	80.2 b-e	68.5 abc	67.4 a-d
BR		66.3 fg	59.7 h	75.1 a	77.0 a
SB		67.4 efg	80.4 b-e	57.5 bcd	56.6 cde
BS		70.6 c-f	77.8 c-f	66.7 abc	65.6 a-d
CH	40	70.6 c-f	60.3 h	47.9 d	47.1 e
MY		69.2 d-g	61.7 h	65.8 abc	64.8 a-d
QR		72.7 cde	63.2 gh	55.3 cd	54.4 de
KH		59.3 h	72.6 ef	58.6 abc	57.7 cde
BR		66.2 fg	57.4 h	64.3 abc	63.3 bcd
SB		68.4 efg	59.4 h	62.1 abc	61.1 bcd
BS		64.3 g	55.2 h	64.6 abc	63.5 bcd

A *p*-value of less than 0.05 indicates a significant difference in the analysis of variance. Different letters in each column represent significant differences at $p = 0.01$, LSD test. The abbreviations are as following: BR (Besmiamphi Ramphi), BS (Bidane Sefid), CH (Chefte), FC (Field capacity), KH (Kishmish Hisrao), MY (Muscat Yamtazini), QR (Qazagiski Ramphi), and SB (Supran Bulgar).

The results showed that under both well-watered (100 % FC) and water-deficit (40 % FC) conditions, the RWC was higher in the roots compared to the leaves. The differential response of RWC between leaves and roots observed in this research is consistent with findings of Blum (2005) and Chaves *et al.* (2003). Under drought stress, leaves typically show a more pronounced decrease in RWC compared to roots (Blum, 2005). The reduction in leaf RWC is a key response to conserve water and prevent excessive water loss through transpiration (Farooq *et al.,* 2009). Lower leaf RWC triggers physiological adjustments, such as stomatal closure, to limit water loss; however, severe drought can lead to further reductions in leaf RWC, causing wilting, leaf senescence, and eventual leaf abscission (Chaves *et al.,* 2003). As the results of this study show, roots generally maintain a higher RWC compared to leaves during drought stress (Tardieu, 2013). The maintenance of root RWC is crucial for sustaining water uptake and transport to the above-ground parts of the plant. By maintaining a higher RWC in roots, plants can continue to absorb water and transport it to the leaves, allowing them to better tolerate periods of water deficit (Gleason *et al.,* 2017). Consistent with the findings of this study, severe drought may also cause a significant decline in root RWC, impairing the plant's water absorption efficiency (Koç *et al.,* 2019). This contrast in RWC dynamics between leaves and roots is a key physiological mechanism that enables plants to survive and adapt to drought conditions (Sade *et al.,* 2012).

The analysis revealed a significant difference in MSI of leaves and roots among the cultivars and water deficit treatments ($p = 0.021$ and $p = 0.003$, respectively). MSI decreased with the increase in water deficit, and the CH cultivar exhibited the lowest MSI in both plant tissues (Table 2). The reduction in MSI with increasing water deficit suggests that the plants experienced cellular membrane damage, which is a common response to

drought stress (Anjum *et al.,* 2011). The lower MSI observed in the CH cultivar indicates that it was more susceptible to membrane damage compared to the other cultivars investigated. The differences in MSI among the cultivars could be attributed to their varying mechanisms of drought tolerance, such as osmotic adjustment, antioxidant defense systems, and the ability to maintain membrane integrity (Taiz & Zeiger, 2010).

According to the results, the main effects of cultivar and water deficit on leaf proline content were significant ($p = 0.017$). The QR cultivar produced the highest leaf proline content (Figure 3A), and leaf proline content increased with increasing water deficit (Figure 3B). These findings suggest that the accumulation of proline in leaves is a common adaptive response of plants to drought stress, as proline plays a crucial role in osmoregulation, membrane stabilisation, and antioxidant defense (Szabados & Savouré, 2010). Moreover, the interaction effect of cultivar and water deficit on root proline content was significant ($p < 0.001$). Root proline content increased with the rise in water deficit (Figure 3C). In the 40 % FC treatment, the KH and SB cultivars exhibited the highest root proline content, while the MY and BS cultivars displayed the lowest. Proline accumulation is a vital component of the stress response under water deficit, ensuring cellular function and water balance. This study confirmed that roots accumulate more proline than leaves (Figure 3C), consistent with previous findings that attribute this pattern to the direct interaction of roots with soil water availability (Luo *et al.,* 2019). The capacity of roots to consistently accumulate proline underscores their importance in osmotic adjustment, facilitating water uptake during drought stress (Sofo *et al.,* 2004b).

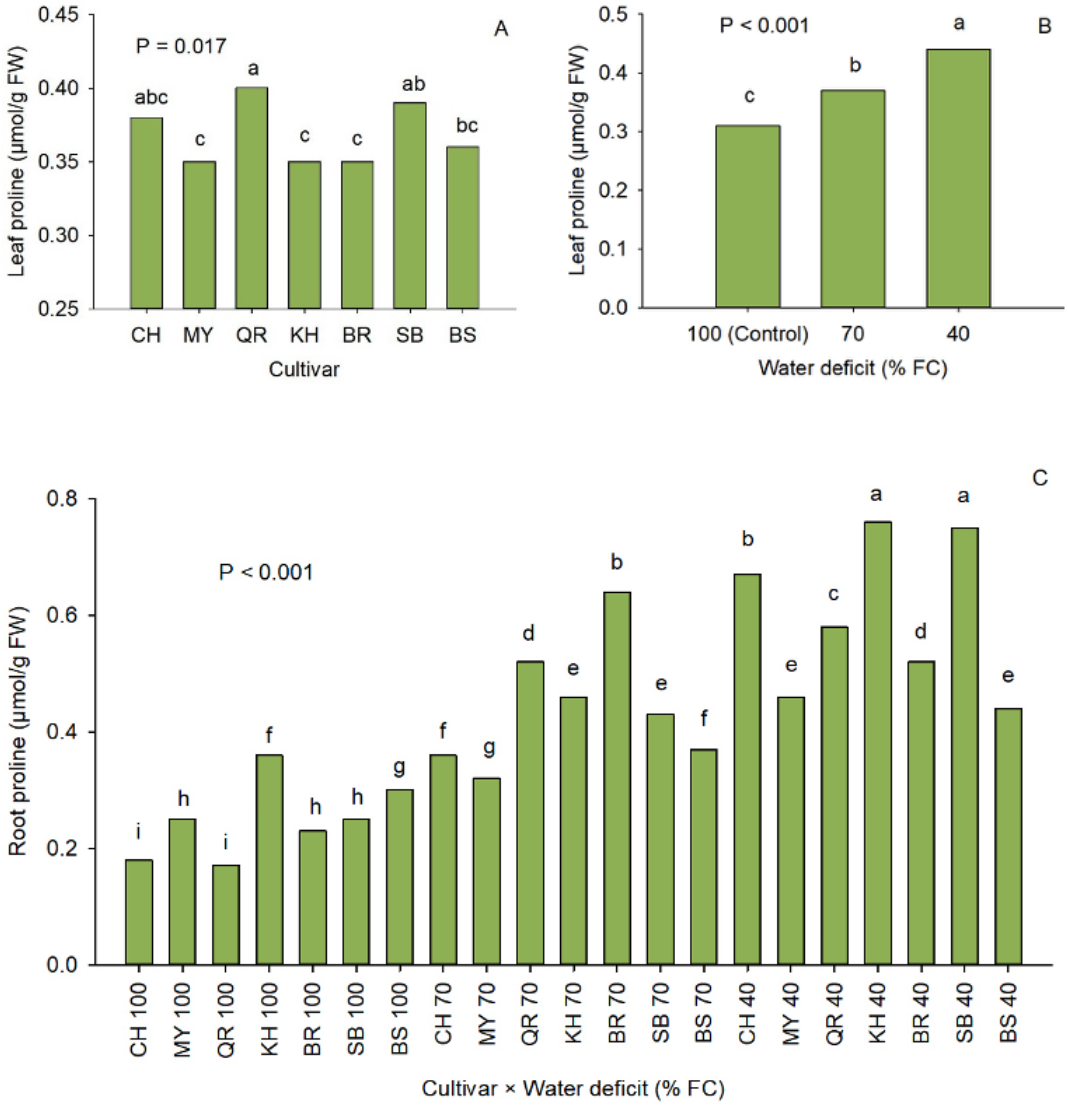


FIGURE 3. The main and interaction effects of cultivar and water deficit on proline content of leaves (A and B) and roots (C) in grapevine plantlets. Figure A illustrates the response of the cultivars to the average FC, while Figure B depicts the response of all cultivars under water deficit conditions. A *p*-value of less than 0.05 indicates a significant difference in the analysis of variance. Different letters represent significant differences at $p = 0.01$, LSD test. The abbreviations are as following: BR (Besmiamphi Ramphi), BS (Bidane Sefid), CH (Chefte), FC (Field capacity), KH (Kishmish Hisrao), MY (Muscat Yamtazini), QR (Qazagiski Ramphi), and SB (Supran Bulgar).

TABLE 3. The interaction effects of cultivar and water deficit on total protein, hydrogen peroxide and antioxidant enzymes activity of roots in grapevine plantlets.

Cultivar	Water deficit (% FC)	Total protein (mg/g FW)	Hydrogen peroxide (µmol/g FW)	Peroxidase (Units/mg protein)	Ascorbate peroxidase (Units/mg protein)	Guaiacol peroxidase (Units/mg protein)	Catalase (Units/mg protein)
		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
CH	100 (Control)	1.18 cde	0.11 p	0.17 k	0.42 gh	0.15 k	0.53 i
MY		1.24 bcd	0.17 n	0.18 k	0.45 fg	0.18 j	0.74 fgh
QR		1.49 a	0.25 k	0.21 jk	0.34 i	0.15 k	0.73 gh
KH		1.27 bc	0.22 l	0.37 ghi	0.26 j	0.36 g	0.70 gh
BR		1.57 a	0.22 l	0.34 hi	0.38 hi	0.23 i	0.76 fg
SB		1.30 b	0.21 m	0.32 i	0.47 f	0.26 h	0.39 j
BS		1.13 def	0.13 o	0.25 j	0.28 j	0.17 j	0.18 k
CH	70	1.12 ef	0.30 j	0.36 ghi	0.28 j	0.15 k	0.92 e
MY		1.15 de	0.51 h	0.38 ghi	0.50 f	0.65 c	1.05 c
QR		1.16 cde	0.89 de	0.58 cd	0.89 cd	0.41 f	1.02 cd
KH		1.18 cde	0.76 g	0.40 gh	0.74 e	0.54 e	0.95 de
BR		1.10 efg	0.86 e	0.42 fg	0.86 cd	0.51 e	0.81 f
SB		0.87 i	1.16 c	0.55 de	1.16 b	0.27 h	0.68 h
BS		1.00 gh	0.47 i	0.47 f	0.47 f	0.17 j	0.37 j
CH	40	1.10 efg	0.82 f	0.48 ef	0.82 d	0.74 b	1.32 b
MY		1.03 fgh	0.92 d	0.71 ab	0.90 c	1.23 a	1.23 b
QR		1.15 de	1.16 c	0.60 cd	1.16 b	0.79 b	1.58 a
KH		1.00 gh	1.15 c	0.75 a	1.15 b	1.19 a	1.49 a
BR		1.11 efg	1.20 bc	0.69 ab	1.20 b	0.58 d	1.21 c
SB		1.03 fgh	1.38 a	0.63 bcd	1.38 a	0.60 d	1.23 b
BS		0.98 h	1.23 b	0.67 abc	1.23 b	0.39 f	1.37 b

A *p*-value of less than 0.05 indicates a significant difference in the analysis of variance. Different letters in each column represent significant differences at $p = 0.01$, LSD test. The abbreviations are as following: BR (Besmiamphi Ramphi), BS (Bidane Sefid), CH (Chefte), FC (Field capacity), KH (Kishmish Hisrao), MY (Muscat Yamtazini), QR (Qazagiski Ramphi), and SB (Supran Bulgar).

3. Total protein, hydrogen peroxide and antioxidant enzymes activity of roots

The results of this study showed a significant interaction effect of cultivar and water deficit on total protein ($p < 0.001$) and H_2O_2 content ($p < 0.001$). The BR and QR cultivars exhibited the highest total protein content under the control treatment, while the lowest total protein content was observed in most cultivars under the 40 % FC treatment (Table 3). These findings align with previous research indicating that drought stress disrupts cellular homeostasis and metabolic activity, ultimately impairing protein synthesis and accumulation. Specifically, Krasensky and Jonak (2012) highlighted how water deficit stress triggers oxidative stress and alters energy metabolism, leading to the degradation or downregulation of protein biosynthetic processes. Our results support this, as the reduced protein levels under severe water deficit suggest a stress-induced suppression of metabolic functions. Furthermore, the observed increase in H_2O_2 under these conditions reinforces the role of oxidative stress as a key mediator of the physiological response to drought. Proteins play crucial roles in various physiological processes, such as photosynthesis, water transport, and stress response pathways. The maintenance of protein homeostasis is, therefore, crucial for the plant's ability to withstand and recover from drought stress. The reduced water availability can impact the plant's ability to synthesise proteins, as the availability of water is essential for various metabolic processes, including protein synthesis (Sofo *et al.,* 2004a). Additionally, drought stress can promote the degradation of existing proteins, either through the activation of proteolytic enzymes or the diversion of resources away from protein synthesis (Anjum *et al.,* 2011).

The SB cultivar displayed the highest H_2O_2 content in the 40 % FC treatment, whereas the CH cultivar had the lowest H_2O_2

TABLE 4. Ranking of grapevine cultivars based on drought tolerance traits.

Rank	5	1	3	2	4	6	7
Mean	0.842	0.762	0.784	0.782	0.789	0.851	0.884
Catalase	0.58	0.42	0.38	0.46	0.75	0.83	1
Ascorbate peroxidase	1	0.64	0.39	0.5	0.39	0.21	0.57
Guaiacol peroxidase	0.84	0.25	0.5	0.38	0.56	0.75	1
Peroxidase	1	0.7	0.52	0.63	0.59	0.56	0.59
Hydrogen peroxide	0.6	0.73	0.88	0.73	0.88	1	0.77
Total protein	0.72	0.69	0.53	0.72	0.75	1	0.97
Root proline	0.67	1	0.58	0.5	0.5	0.5	0.92
Leaf proline	1	1	1	1	1	1	1
Root MSI	0.88	0.59	1	0.76	0.47	0.82	0.65
Leaf MSI	0.92	0.62	1	0.77	0.46	0.77	0.62
Root RWC	0.81	0.91	0.63	0.56	1	0.72	0.78
Leaf RWC	0.56	0.7	0.67	1	0.96	0.89	0.85
Canopy temperature	1	1	1	1	1	1	1
Lateral shoots number	1	1	1	1	1	1	1
Stem diameter	1	1	1	1	1	1	1
Lateral shoot length	1	1	1	1	1	1	1
Internode length	1	1	1	1	1	1	1
Leaf total chlorophyll	1	0.83	1	1	1	1	0.96
Leaf dry weight	0.64	0.56	0.76	0.8	0.76	1	1
Leaf fresh weight	0.69	0.66	0.88	0.84	0.63	0.81	1
Main shoot length	0.76	0.71	0.76	0.76	0.86	1	0.9
Cultivars	CH	MY	QR	KH	BR	SB	BS

The abbreviations are as following: BR (Besmiamphi Ramphi), BS (Bidane Sefid), CH (Chefte), KH (Kishmish Hisrao), MSI (Membrane stability index), MY (Muscat Yamtazini), QR (Qazagiski Ramphi), RWC (Relative water content), and SB (Supran Bulgar).

content in the control treatment. H_2O_2 is a common reactive oxygen species that can accumulate in plants under drought stress, leading to oxidative damage to cellular components (Sharma *et al.,* 2012). The differential accumulation of H_2O_2 among the cultivars indicates their varying capacities to mitigate oxidative stress and maintain cellular homeostasis under water deficit conditions (Turkan, 2011). The decline in chlorophyll content under water stress in this study likely correlates with elevated endogenous H_2O_2 levels, which contribute to the oxidative degradation of chlorophyll.

Furthermore, the interaction effect of cultivar and water deficit on the activity of antioxidant enzymes was also significant ($p < 0.001$ for all enzymes). The activity of all four studied enzymes (POD, APX, GPX, and CAT) increased with increasing drought stress (Table 3). This finding suggests that the plants responded to the oxidative stress induced by water deficit through the upregulation of their antioxidant defense systems (Gill & Tuteja, 2010). The CH, MY, and QR cultivars showed the lowest POD activity in the control treatment, whereas the KH cultivar had the highest activity in the 40 % FC treatment. The KH cultivar exhibited the lowest APX activity in the control treatment, and the SB cultivar had the highest activity in the 40 % FC treatment. The QR cultivar in the control treatment and the CH cultivar in the control and 70 % FC treatments displayed the lowest

GPX activity, while the KH and MY cultivars in the 40 % FC treatment showed the highest activity. The BS cultivar in the control treatment showed the lowest CAT activity, and the KH and QR cultivars in the 40 % FC treatment the highest. These results indicate that the different cultivars exhibit varying capacities to activate their antioxidant defense systems in response to water deficit stress (Turkan, 2011). The cultivars that maintain higher antioxidant enzyme activities under drought conditions, such as KH and QR, may be more tolerant to oxidative damage and better able to adapt to water-limited environments (Flexas *et al.,* 2006). For example, increased POD activity can contribute to the maintenance of cell wall integrity and the regulation of auxin levels, which are important for plant growth and adaptation under drought conditions. Similarly, enhanced APX and GPX activities can help mitigate the damaging effects of H_2O_2, while elevated CAT activity can facilitate the decomposition of H_2O_2 to water and oxygen (Mittler, 2006).

4. Ranking of grapevine cultivars for drought tolerance

The ranking of the cultivars based on their responses across multiple drought tolerance-related traits revealed that MY was the most tolerant, followed by KH and QR (Table 4). Interestingly, these cultivars demonstrated even greater drought tolerance than CH, which was included in the study as a known drought-tolerant cultivar for the region. By contrast, the dominant cultivar in the region, BS, was identified as the most sensitive to drought stress. These findings provide valuable information for the regional growers and vineyard managers. The superior drought tolerance of MY, KH, and QR suggests that these cultivars may be more suitable for cultivation in water-limited areas, potentially offering increased resilience and productivity compared to the commonly grown BS cultivar. The results of this analysis can help inform decision-making processes related to cultivar selection, vineyard management, and the development of more sustainable viticulture practices in the face of increasing water scarcity and drought conditions. Moreover, it can also guide the ongoing efforts to improve drought tolerance in grapevine through breeding and genetic enhancement programs. The identification of the most drought-tolerant cultivars and the underlying physiological and biochemical mechanisms can inform the selection of parental lines and the development of targeted strategies for cultivar improvement (Blum, 2010).

CONCLUSION

The controlled environment of pot studies enables precise observation of traits, providing a rapid method for screening and identifying drought-tolerant cultivars. This study provides crucial insights into how grapevine cultivars respond to water deficits through morphophysiological and biochemical adaptations. Increasing the drought severity resulted in reduced shoot length, leaf weights, and chlorophyll content, thus highlighting its detrimental effects on key growth and physiological parameters. However, some cultivars, such as MY, maintained higher chlorophyll levels and leaf dry weights, demonstrating inherent drought-tolerant mechanisms. Severe reductions in lateral shoot length were particularly notable, underscoring their value as markers of water stress tolerance. Physiological traits like RWC and MSI corroborate the cultivar-specific strategies for sustaining water status and membrane integrity. The accumulation of osmoprotectants like proline and enhanced antioxidant enzyme activity reflected the biochemical responses underpinning these adaptations. The findings of this study have significant implications for grapevine breeding and management. Drought-tolerant cultivars (*e.g.*, MY, KH and QR) can serve as parental lines in breeding programmes for improving resilience. Additionally, insights into physiological and biochemical responses can inform management strategies such as deficit irrigation to enhance grapevine performance under water-limited conditions. By advancing understanding of drought responses in grapevine genotypes, this research contributes to sustainable viticulture in the context of increasing water scarcity and climate change. This research was conducted under controlled greenhouse conditions using non-fruiting plantlets, which may not fully replicate field-scale environmental dynamics. Therefore, extrapolating these findings to mature, fruit-bearing vines requires caution, as drought resilience mechanisms may differ between vegetative growth and reproductive development. Despite these limitations, the findings can guide breeders, researchers, and growers in developing drought-tolerant cultivars and implementing effective management practices. Additionally, these results underscore the need for longitudinal studies that compare drought responses across different developmental stages, as well as field trials under varying pedoclimatic conditions.

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