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Research Article

Assessment of biochemical and physiological responses of several grape varieties under water deficit stress

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Abstract

Grapes (*Vitis vinifera* L.) are a crucial crop globally, particularly in arid regions where water scarcity is a concern. This study investigated the physiological and biochemical responses of several grape varieties to water deficit stress. The research was conducted within the controlled environment in a factorial experiment with a completely randomized setup comprising three replicates. The first variable, irrigation, consisted of two levels: a 15-day water stress and a control group receiving irrigation maintained at field capacity. The second variable was the grape variety, encompassing a total of 15 commercial varieties. Water deficit stress reduced chlorophyll content in all varieties. 'Bidaneh Sefid' variety had the highest chlorophyll content (8.76 $\mu g.g^{-1}$ FW). Furthermore, this variety demonstrated superior relative water content under stress (79.22%), whereas 'Keshmesh' variety exhibited such performance under normal conditions. The results illustrated a proline content range spanning from 48.48 to 61.01 $\mu mol.g^{-1}$ FW. Notably, water deficit stress resulted in elevated proline content, with the highest mean observed in the 'Khalili' variety under such stress conditions. Water scarcity impacted grape traits significantly: reduced chlorophyll, relative water content, increased leakage, higher proline, carbohydrate, anthocyanin, and phenols, with varying antioxidants. 'Bidaneh Sefid' variety had most chlorophyll, 'Khalili' highest proline under stress, 'Maskeh' variety excelled in carbohydrate. 'Fakhri', 'Filam Seedless', 'Shiregi' varieties topped anthocyanins; 'Khalili' variety showed best antioxidant activity. The findings highlight specific grape varieties that exhibit desirable traits under water deficit stress, such as higher chlorophyll content, proline accumulation, and carbohydrate accumulation. These varieties could serve as valuable genetic resources for breeding programs aimed at developing drought-tolerant grape cultivars. Additionally, the identification of varieties with enhanced antioxidant capacity and anthocyanin levels holds potential for the development of grape-based products with possible health benefits.

Keywords: chlorophyll content; drought tolerance; flavonoid content; proline content; water scarcity stress

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Introduction

Grapes (*Vitis vinifera* L.) are a perennial plant from the Vitaceae family that require warm and dry summers for growth. Cultivating grapes in semi-arid and arid conditions is common, and unfavourable conditions can adversely affect grape growth and development (Zandkarimi *et al*., 2015). The cultivation of grapes is globally significant because of its versatility and wide range of uses. Grapes are not a fruit that can be eaten directly but they are also the main ingredient, in wine production, which is a thriving industry worldwide (Alston and Sambucci, 2019). In addition, grapes are used in making food products like raisins and jams. Some types of grapes can withstand water scarcity and drought conditions making them valuable crops in areas with irrigation resources (Gambetta *et al*., 2020). This agricultural practice provides farmers with income. Furthermore, ongoing genetic research aims to improve grapevine characteristics such as disease resistance and product quality highlighting the importance of grape cultivation, in the landscape (Carrasco *et al*., 2022). Cultivating grapes in arid regions is of paramount importance due to several key factors. Drought-resistant grape varieties enable water-efficient production, addressing water scarcity challenges (Wang *et al*., 2021).

Drought stress represents the primary constraint on global agricultural yields, as highlighted by Cotrina Cabello *et al*. (2023). Given Iran's predominant arid climate, it is virtually certain that at least one critical phase of the plant's life cycle will inevitably confront drought stress, as observed by Zeydalinejad *et al*. (2023). Extensive efforts are currently underway to elucidate drought-tolerant mechanisms through molecular techniques, yielding reports on genes responsive to drought (Puppala *et al*., 2023). Several genes play a crucial role in plant defense mechanisms by participating in stress perception, signal transduction, transcriptional regulation networks, and dehydration resistance (Hrmova and Hussain, 2021).

Water-different stress resulting from deficit irrigation is one of the most significant environmental stresses that affect plant morphology, physiology, and biochemistry, ultimately exerting major effects on agricultural productivity (Seymen *et al*., 2023). Drought stress influences mesophyll metabolism and reduces photosynthetic capacity through the decreased synthesis of the ribulose-1,5-bisphosphate enzyme and reduced rubisco activity or both. In response to drought, the closure of stomata, accompanied by growth inhibition, is among the first plant responses (Momayyezi *et al*., 2020). The mechanisms of photosynthesis in chloroplasts are largely complex, and during the early stages of drought, a significant limitation in photosynthesis arises from stomatal closure (Sharma *et al*., 2020).

Under stress conditions, one of the most prevalent reactions observed in plants is the excessive production of various types of organic compounds known as compatible solutes. These low-molecular-weight solutes, which are highly soluble and generally non-toxic, are often present in relatively high concentrations. These organic osmolytes typically encompass carbohydrates like sugars, amino acids, proteins, and amino acids such as proline (Mehta and Vyas, 2023). Proline, in particular, is a well-recognized amino acid that accumulates extensively in plants, especially in response to environmental stressors (Aghighi Shahverdi *et al*., 2019). Besides its function as an osmolyte for regulating cellular water potential, proline plays a crucial role in stabilizing subcellular structures like membranes and organelles, mitigating the effects of reactive oxygen species (Afshari *et al*., 2022). In a study conducted by Cui et al. (2020), the 'Yanshan-1' cultivar of common grapes exhibited elevated levels of proline, soluble sugars, and chlorophyll content, accompanied by reduced accumulation of malondialdehyde (MDA) compared to the 'He'an' cultivar. Additionally, the accumulation of superoxide anion free radicals (O_2^-) decreased in 'Yanshan-1' but increased in 'He'an'. Rahmani *et al*. (2023) reported that drought stress significantly reduced the membrane stability index and the relative water content, and increased electrolyte leakage, catalase, hydrogen peroxide, proline, ascorbic acid, guaiacol peroxidase, protein, sodium, and potassium levels of commercial grapevine cultivars. In light of the significance of grapes and the selection of drought-resistant varieties, the present research was conducted to evaluate the physiological and biochemical responses of 15 commercial grape varieties to water deficit stress.

Materials and Methods

The experiment was conducted at the research greenhouse of Bojnourd Islamic Azad University, Iran, located at coordinates N: 37° 27', E: 57° 19', for 2018-2020. In the late winter of 2018, two-year-old seedlings of 15 grape varieties ('Asghari', 'Bidaneh Sefid', 'Divane', 'Fakhri', 'Flim Seedless', 'Garmeh', 'Keshmeshi', 'Khalili', 'Kolahdari', 'Lal', 'Maskeh', 'Sahebi', 'Sanjari', 'Sefid-Berian', and 'Shiregi') were transplanted into new plastic pots with dimensions of 35 cm in diameter and 40 cm in depth. These pots were filled with a mixture of sand, peat, and garden soil in equal proportions. Before subjecting the plants to drought treatment, all pots were irrigated to reach field capacity (FC).

The experimental design employed a factorial approach within a completely randomized design (CRD), with two factors: water deficit treatments (watering to field capacity as a control and a 15-day watering interval as stress levels) and the 15 grape varieties. Water deficit stress was induced by withholding water until wilting signs became apparent on the leaves, which typically occurred after about two weeks, in June 2018 (Siemens and Zwiazek, 2003). Natural sunlight served as the primary light source, and the greenhouse maintained a maximum temperature of 34 °C and a minimum temperature of 18.5 °C. Throughout the experiment, there was no application of fertilizers. The details of the soil composition utilized can be found in Table 1. Following the application of stress (stress treatments were applied up to one week before sampling), leaf samples were collected, rapidly frozen in liquid nitrogen, and subsequently stored at -80 °C for later use.

Table 1. The physical and chemical characteristics of the soil at the experimental site

Soil texture	pH	EC ($dS.m^{-1}$)	N (%)	P ($mg.kg^{-1}$)	K ($mg.kg^{-1}$)	Fe ($mg.kg^{-1}$)
Sandy loam	7.8	3.1	0.05	8.4	380	4

Measurement of total chlorophyll

To assess the levels of total chlorophyll, the technique outlined by Lichtenthaler and Wellburn (1983) was employed. The optical density (O.D.) of the resulting extract was then determined at wavelengths of 646.8 and 663.2 nm, allowing us to estimate the concentrations of total chlorophyll using a spectrophotometer (Perkin Elmer Lambda 25, USA). The quantity of pigment present in each sample was calculated using the following formula:

Eq. 1. Total chlorophyll ($\mu g.g^{-1}$ FW) = 20.2 (O.D. of 645) + 8.02 (O.D. of 663) × V × 1000

In these equations, W represents the fresh weight of the extracted tissue in g, V signifies the final volume of the 80% acetone extract, and O.D. corresponds to the optical density at a specific wavelength.

Measurement of relative water content (RWC)

To assess the RWC, three young and fully developed leaves were harvested from the upper part of the plant canopy. The samples were immediately transported to the laboratory, and their initial moisture content was determined (Wf). Next, these samples were immersed in distilled water at room temperature and kept in the dark for 16 to 18 h. Following this soaking period, the samples were promptly and precisely reweighed (Wt). Subsequently, leaf segments were subjected to a temperature of 70 °C in an oven (MEMMERT's UNpa110 model from Germany) for 48 h to measure their dry weight (Wd). Ultimately, the RWC was calculated using the following formula (Rahmani *et al*., 2023):

Eq. 2. RWC (%) = [(Wf-Wd)/(Wt-Wd)] × 100

Measurement of electrolyte leakage

Electrolyte leakage (EL) was determined following the method outlined by Sairam *et al.* (2002) and calculated using the equation:

Eq. 3. EL (%) = $[EC_1/EC_2] \times 100$

Where EC_1 and EC_2 represent the electrolytic conductance before and after immersion in a boiling water bath, respectively.

Measurement of proline content

Proline content was quantified spectrophotometrically by measuring absorbance at 515 nm, following the procedure described by Irigoyen *et al*. (1992). The concentration of proline was determined by referencing a standard proline curve.

Measurement of carbohydrate

The alcoholic extract for carbohydrate measurement was obtained using the method by Irigoyen *et al*. (1992). The absorbance of the samples at 625 nm was recorded using a spectrophotometer. Subsequently, the type and quantity of carbohydrate in the unknown samples were determined by comparing them to standard samples.

Measurement of anthocyanin

The Wagner (1979) method was employed to measure the anthocyanin content. Approximately 1.0 g of plant sample was thoroughly ground in a mortar with 10 mL of acidified methanol and the extract obtained was kept for 24 h at a temperature of 25 °C. After centrifugation, the absorbance of the samples was measured at a wavelength of 550 nm, and the anthocyanin content was calculated using an extinction coefficient of 33,000.

Measurement of total phenolic compound content

The measurement of total phenolic compound content was conducted using the method of Velioglu *et al*. (1998). Approximately 1.0 g of the samples were completely extracted in 5 mL of 80% methanol containing 1% hydrochloric acid for 2 h on a shaker at room temperature. To this, 100 µL of the sample extract, and 750 µL of Folin's reagent were added, and the resulting mixture was kept at room temperature for 5 min. Then, by adding sodium carbonate, the absorption of the sample was read at a wavelength of 725 nm, and the concentration of phenolic compounds was calculated using gallic acid.

Measurement of total flavonoid content

The measurement of total flavonoid was carried out using the aluminum chloride colorimetric method by Zhishen *et al*. (1999). A volume of 500 µL of the alcohol extract was diluted to 5 mL with distilled water. By adding sodium nitrite, aluminum chloride, and finally, sodium hydroxide, the absorbance of the sample was measured at a wavelength of 510 nm. The flavonoid content in the samples was calculated using a standard quercetin-3-rutinoside curve.

Measurement of DPPH

The free radical scavenging activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical method, following the procedure described by Wagner *et al*. (1979). A total of 1.0 g of plant samples, obtained from various concentrations (200, 130, 80, and 40 $mg.mL^{-1}$) in ethanol, were mixed with 1,000 µL of a 80% methanol solution containing 1% hydrochloric acid. The mixture was shaken for complete extraction at room temperature for 2 h. Subsequently, 100 µL of the resulting solution was mixed with 900 µL of a 1,000 µM DPPH methanolic solution. The mixture was then kept at room temperature for 5 min. The absorbance was measured at 517 nm using a spectrophotometer after dilution with methanol as needed. The scavenging activity of the plant extracts on the DPPH radical was calculated as a percentage of inhibition using the formula:

Eq. 4. $[\text{Inhibition } (\%) = [(A_0 - A_1) / A_0] \times 100\backslash]$

Where, A_0 is the absorbance of the control (DPPH solution without the sample); A_1 is the absorbance in the presence of the sample.

Statistical analysis

After evaluating the normality of data distribution through the Kolmogorov-Smirnov and Shapiro-Wilk tests, a statistical analysis of the desired characteristics was carried out using the Statistical Analysis System software (SAS Institute, Cary, NC, USA, version 9.2). Mean values were compared using the least significant difference (LSD) test at a significance level of $p < 0.05$. Furthermore, Pearson correlation analysis among the attributes was performed using SAS software. Cluster analysis and principal component analysis (PCA) were conducted using Minitab version 18 software.

Results

Total chlorophyll content

The results of the analysis of variance indicated that the water deficit stress and varieties had significant effects on chlorophyll content at the 1% and 5% probability levels, respectively (Table 2). Based on the findings, water scarcity stress resulted in a substantial 34.4% decline in the total chlorophyll content. Within the grape varieties, 'Bidaneh Sefid' stood out with the highest chlorophyll content, averaging 8.76 $\mu g.g^{-1}$ FW. Conversely, the 'Asgari' exhibited the lowest chlorophyll content, averaging only 3.89 $\mu g.g^{-1}$ FW. Except for the aforementioned varieties and 'Senjeri', the other tested varieties displayed the highest chlorophyll content and were grouped statistically similarly to the top-performing treatment (Figure 1).

Table 2. ANOVA the effect of water deficit stress on some physiological and biochemical traits of commercial grape varieties (*Vitis vinifera* L.)

SOV	df	Mean square (MS)								
		Total chlorophyll	RWC	Electrolyte leakage	Proline	Total carbohydrate	Anthocyanin	Phenol	Flavonoids	DPPH
Water deficit (W)	1	408.8**	2.34ns	4127.2**	4.16ns	4349.6**	21.9**	4197.9**	28.7**	20312.1**
Varieties (V)	14	9.12*	109.1ns	1265.7**	15.0ns	1441.3**	0.11**	119.2**	15.8**	405.9**
W × V	14	5.79ns	364.5**	163.8**	31.5**	1223.5**	0.14**	13.8**	9.35**	18.48**
Error	60	4.86	110.8	33.5	11.17	34.18	0.04	4.34	0.02	0.29
CV (%)	-	23.26	17.39	9.45	6.50	4.11	5.50	6.60	7.99	8.02

ns: non-significant; * and **: significant at α=0.05 and 0.01, respectively.

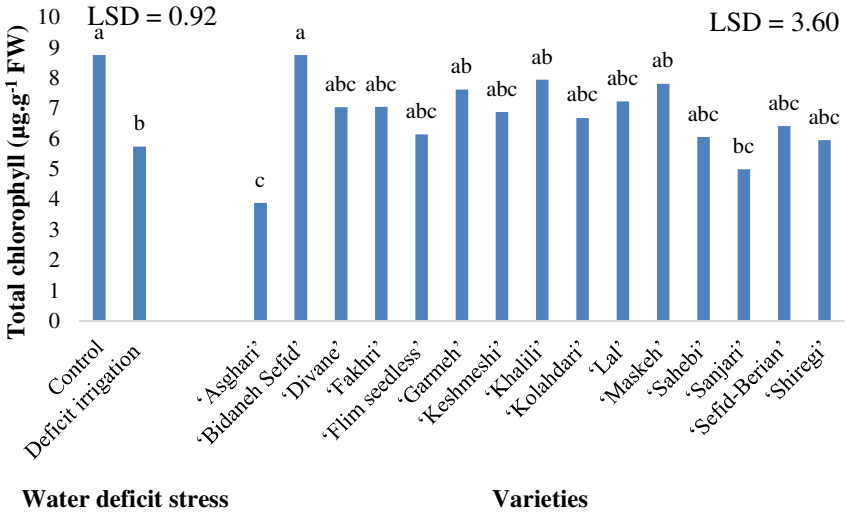


Figure 1. Effect of water deficit stress and varieties on total chlorophyll content of commercial grape (*Vitis vinifera* L.)

The water deficit stress levels and varieties were compared separately. Means with similar letters do not show a significant difference at the 5% probability level based on the Least Significant Difference (LSD) test.

Leaf relative water content (RWC)

The outcomes of the variance analysis indicated that the primary impacts of water deficit and grape variety on RWC did not show statistical significance. Nonetheless, a notable interaction effect of water deficit and the variety was observed, reaching statistical significance at the 1% confidence level (Table 2). In the context of water deficit stress, the 'Bidaneh Sefid' grape variety displayed the highest leaf RWC, reaching 79.22%. In contrast, under without-stress conditions, the 'Keshmesh' variety ranked second with a RWC of 77.79%. Conversely, the 'Kolahdari', when subjected to water deficit stress, and the 'Maskeh' variety under non-stress conditions, exhibited the lowest RWC, measuring 47.51% and 47.85%, respectively (Table 3).

Electrolyte leakage

Electrolyte leakage was significantly influenced by water deficit, grape cultivars, and the significant interaction effect between these two factors, demonstrating meaningful differences at the 1% probability level (Table 2). Analysis of mean comparisons revealed that exposure to water deficit stress led to a rise in the average electrolyte leakage levels. Specifically, the highest mean value was noted in the 'Garmeh' variety under water deficit stress conditions, reaching 85.83%, while the lowest mean was documented in the 'Divane' variety under normal conditions, registering at 19.67% (Table 3).

Proline content

The experimental findings indicated that the interaction effect of variety and water deficit on proline content was statistically significant ($p<0.01$). However, the individual effects of these two factors were not statistically significant, as detailed in Table 2. Moreover, the results illustrated a proline content range spanning from 48.48 to 61.01 µmol.g^{-1} FW. Notably, water deficit stress resulted in elevated proline content, with the highest mean observed in the 'Khalili' variety under such stress conditions. Conversely, the lowest mean proline content was recorded in the 'Flim Seedless', 'Garmeh', 'Khalili', 'Lal', and 'Shiregi' varieties under non-stress conditions. It's noteworthy that under water deficit stress conditions, the 'Bidaneh Sefid', 'Fakhri', 'Garmeh', and 'Maskeh' varieties exhibited the lowest proline content (Table 3).

Total carbohydrate content

Statistical analysis of variance revealed that the influence of water deficit stress, grape variety, and their interaction significantly affected the total carbohydrate content at the 1% probability level (Table 2). Notably, the 'Maskeh' variety exhibited the highest total carbohydrate content, reaching an average of 209.63 $\mu g.g^{-1}$ FW under water deficit stress conditions. Interestingly, this variety did not display a similarly elevated average under non-stress conditions. In contrast, 'Bidaneh Sefid' exhibited the lowest content for this trait under non-stress conditions, measuring 115.2 $\mu g.g^{-1}$ FW (Table 3).

Table 3a. Effect of water deficit stress on some physiological and biochemical traits of commercial grape varieties (*Vitis vinifera* L.)

Water deficit × varieties		RWC (%)	Electrolyte leakage (%)	Proline ($\mu mol.g^{-1}$ FW)	Total carbohydrate ($\mu g.g^{-1}$ FW)
Control	'Asghari'	69.3±8.93a-d	26.63±6.16lm	50.28±0.9bcd	131.42±4.9hij
	'Bidaneh Sefid'	50.32±9.68fg	52.6±3.8ijk	50.97±1.73bcd	115.26±5.62m
	'Divane'	54.88±7.18c-g	19.67±5.51m	50.46±2.27bcd	135.52±2.82h
	'Fakhri'	57.52±10.27c-g	65.19±2.98e-h	54.93±5.24bc	176.02±9.08c
	'Flim Seedless'	68.43±13.27a-e	52.68±3.23ijk	49.09±0.34d	123.87±5.68j-m
	'Garmeh'	53.08±2.68d-g	60.93±3.23ghi	49.18±0.42d	127.12±4.87h-l
	'Keshmeshi'	77.79±15.15ab	75.7±2.01bcd	51.26±1.98bcd	121.08±10.96klm
	'Khalili'	66.1±2.89a-f	61.67±6.79ghi	49.45±0.4d	118.74±6.17lm
	'Kolahdari'	64.41±4.63a-g	61.23±2.87ghi	52.37±4.13bcd	125.84±12.17i-l
	'Lal'	63.86±8.26a-g	69.35±5.21c-g	48.48±0.15d	145.14±15.05g
	'Maskeh'	47.85±27.92g	61.26±5.6ghi	50.59±1.62bcd	133.7±4.91hi
	'Sahebi'	55.7±10.68c-g	27.07±6.9lm	53.31±3.5bcd	148.49±1.5g
	'Sanjari'	62.07±10.15a-g	58.53±4.25hij	55.31±4.29b	162.63±8.4d
	'Sefid-Berian'	50.62±16.42fg	58.23±7.59hij	53.77±4.26bcd	128.85±3.2h-k
	'Shiregi'	68.54±7.08a-e	66.89±2.01d-h	48.65±0.36d	133.17±9.19hij
Water deficit stress	'Asghari'	51.49±10.15efg	35.67±2.02l	54.96±2.43bc	128.39±0.28h-k
	'Bidaneh Sefid'	79.22±6.5a	75.33±6.26bcd	48.81±0.55d	133.55±1.72hi
	'Divane'	61.14±11.13b-g	50.13±15.79jk	51.96±1.53bcd	126.48±2.09h-l
	'Fakhri'	56.98±3.94c-g	66.35±3.74d-h	48.83±0.85d	150.87±1fg
	'Flim Seedless'	50.8±5.63fg	79.57±5.07ab	52.79±5.4bcd	130.31±0.81h-k
	'Garmeh'	67.27±6.11a-f	85.83±5.59a	48.69±0.07d	151.7±3.63efg
	'Keshmeshi'	63.85±4.77a-g	77.07±1.81abc	52.81±1.16bcd	127.63±6.72h-l
	'Khalili'	60.74±10.77b-g	76.5±3.5abc	61.01±13.01a	129.64±0.85h-k
	'Kolahdari'	47.51±6.9g	72.64±3.04b-f	52.07±2.94bcd	159.99±1.81def
	'Lal'	53.56±11.99d-g	80.95±3.64ab	53.46±2.26bcd	149.63±1.91g
	'Maskeh'	63.71±16.84a-g	61.65±5.87ghi	48.95±0.8d	209.63±3.05a
	'Sahebi'	56.91±8.62c-g	47.53±12.87k	49.81±0.43cd	160.91±1.77de
	'Sanjari'	71.36±8.94abc	73.23±3.43b-e	50.27±1.28bcd	132.85±2.32hij
	'Sefid-Berian'	70.88±4.41abc	74.83±4.48bcd	50.01±0.3bcd	193.35±2.41b
	'Shiregi'	50.21±5.34fg	63.47±4.11fgh	50.12±0.78bcd	150.5±0.66fg
	LSD = 0.05	17.19	9.46	5.46	9.54

Means (±SD) followed by different letters, in the same columns, are significantly different at P ≤ 0.05 by LSD test.

Table 3b. Effect of water deficit stress on some physiological and biochemical traits of commercial grape varieties (*Vitis vinifera* L.)

Water deficit × varieties		Anthocyanin (mg SG.g^{-1} FW)	Phenol (µg.mg^{-1} GA)	Flavonoids (µg.mg^{-1} Q)	DPPH (%)
Control	'Asghari'	3.19±0.18ef	128.24±0.94f-i	14.25±0.1fg	48.53±0.27k
	'Bidaneh Sefid'	3.26±0.16cde	116.6±0.21l	13.27±0.02k	27.47±0.38q
	'Divane'	3.3±0.15cde	125.5±0.89ijk	14.02±0.13ghi	37.42±0.49mn
	'Fakhri'	3.19±0.16ef	126.23±0.17ij	14.16±0.02fg	39.23±0.14l
	'Flim seedless'	2.87±0.06f	116.64±0.19l	13.27±0.02k	27.55±0.32q
	'Garmeh'	3.08±0.14ef	116.92±0.19l	13.29±0.01k	30.54±0.61p
	'Keshmeshi'	3.18±0.13ef	118.68±0.59l	13.66±0.1j	34.68±0.59o
	'Khalili'	3.21±0.19de	126.79±0.55g-j	14.18±0.02fg	49.78±0.19j
	'Kolahdari'	2.87±0.12f	116.76±0.06l	13.28±0.01k	30.21±0.21p
	'Lal'	3.34±0.12cde	127.07±0.68f-j	14.18±0.08fg	37.77±0.17m
	'Maskeh'	3.2±0.16de	125.64±1.02ijk	14.08±0.08fgh	37.57±1.03mn
	'Sahebi'	3.09±0.12ef	122.8±0.29k	13.9±0.02hi	36.75±0.24n
	'Sanjari'	3.07±0.05ef	128.48±0.87f-i	14.3±0.07f	48.25±0.12k
	'Sefid-Berian'	3.15±0.15ef	124.3±0.91jk	13.84±0.04ij	36.75±0.96n
	'Shiregi'	3.07±0.13ef	126.52±0.21hij	14.16±0.01fg	39.47±0.16l
Water deficit stress	'Asghari'	4.16±0.11ab	138.38±2.68cd	19.41±0.11b	80.28±0.07b
	'Bidaneh Sefid'	4.39±0.19a	129.89±0.18e-h	10.99±0.16m	57.77±0.43h
	'Divane'	4.15±0.21ab	139±0.81bcd	14.97±0.19d	67.41±0.57f
	'Fakhri'	4.32±0.16a	141.13±0.92abc	15.69±0.06c	72.75±0.11c
	'Flim seedless'	4.3±0.09a	130.25±0.14ef	11.02±0.13m	57.83±0.3h
	'Garmeh'	4.1±0.38ab	130.03±0.07efg	12.22±0.24l	53.46±0.92i
	'Keshmeshi'	4.23±0.24ab	130.36±1.04ef	13.87±0.24hij	63.22±0.96g
	'Khalili'	3.57±0.39c	132.73±8.18e	19.91±0.07a	82.93±0.75a
	'Kolahdari'	4.28±0.35ab	136.85±5.93d	12.09±0.08l	53.01±0.48i
	'Lal'	4.19±0.13ab	142.38±1.61ab	15.11±0.07d	71.7±0.24d
	'Maskeh'	4.18±0.33ab	140.07±1.24a-d	15.02±0.41d	68.53±0.97e
	'Sahebi'	3.95±0.14b	138.4±1.99cd	14.7±0.1e	63.64±0.34g
	'Sanjari'	4.21±0.12ab	142.57±0.9a	19.3±0.05b	80.53±0.51b
	'Sefid-Berian'	3.52±0.38cd	139.52±2.02a-d	14.7±0.38e	66.73±0.84f
	'Shiregi'	4.34±0.06a	140.49±0.06abc	15.79±0.06c	72.88±0.06c
	LSD = 0.05	0.32	3.40	0.23	0.88

Means (±SD) followed by different letters, in the same columns, are significantly different at P ≤ 0.05 by LSD test.

Anthocyanin content

The experimental results demonstrated that the anthocyanin levels were significantly impacted by factors such as water deficit stress, grape variety, and the interplay between these variables, as outlined in Table 2. When comparing the mean values, it became evident that water deficit stress led to a noteworthy increase in anthocyanin content. More precisely, the 'Fakhri', 'Filam Seedless', and 'Shiregi' varieties exhibited the highest anthocyanin levels under water deficit stress conditions, with values of 4.32, 4.3, and 4.34 mg.g^{-1} FW, respectively. In contrast, the 'Kolahdari' variety showed the lowest under non-stress conditions, measuring 2.87 mg.g^{-1} FW (Table 3).

Phenol content

The data analysis showed that water scarcity, variety, and their interaction significantly affected the total phenol content (Table 2). In the mean comparison, considering the interaction of water deficit within grape varieties, the total phenol content exhibited a range from 116.6 to 142.5 µg.mg^{-1} GA, indicating notable variation. These findings revealed that water deficit stress resulted in a substantial increase in the average total phenol content. The 'Sanjari' variety, under water deficit stress conditions, displayed the highest total phenol content. Conversely, the lowest mean for this characteristic was observed in the 'Bidaneh Sefid', 'Flim Seedless', 'Garmeh', 'Keshmeshi', and 'Kolahdari' varieties, with values of 116.6, 116.64, 116.92, 118.6, and 116.7 µg.mg^{-1} GA, respectively.

Flavonoid content

The results of the analysis of variance demonstrated that flavonoid content was significantly influenced by water deficit stress, variety, and their interaction (Table 2). The mean comparisons further reveal a noteworthy rise in flavonoid content under water deficit stress compared to non-stress conditions. The 'Khalili' variety, under water deficit stress, displayed the highest flavonoid content at an average of 19.91 µg.mg^{-1} Q, indicating a substantial 28.7% increase compared to its non-stress conditions. In contrast, the 'Bidaneh Sefid' and 'Flim Seedless' varieties exhibited the lowest flavonoid content under water deficit stress conditions, measuring 10.99 and 11.02 µg.mg^{-1} Q, respectively (Table 3).

Antioxidant activity by DPPH assay

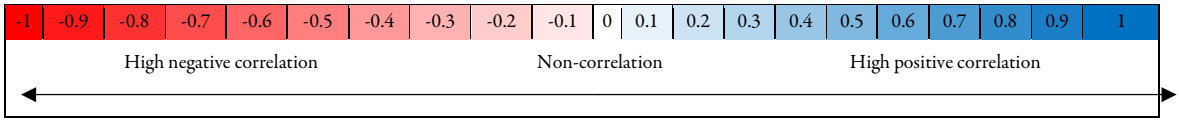
Like many other characteristics, the influence of water deficit stress, variety, and their interplay showed noteworthy variations in antioxidant activity (Table 2). The 'Khalili' variety exhibited the highest antioxidant activity under water scarcity stress conditions, with an average of 82.93%, signifying a substantial 39.9% surge compared to its performance under non-stress conditions. In contrast, the 'Bidaneh Sefid' and 'Flim Seedless' varieties displayed the lowest antioxidant activity, recording 27.47% and 27.55%, respectively, under non-stress conditions (Table 3).

Simple correlation analysis

Simple correlations between traits were presented in Table 4.

Table 4. Simple correlation coefficients (Pearson) between physiological and biochemical of commercial grape varieties (*Vitis vinifera* L.) under water deficit conditions

	Total chlorophyll	RWC	Electrolyte leakage	Proline	Total carbohydrate	Anthocyanin	Phenol	Flavonoids	DPPH
Total chlorophyll	1								
RWC	0.01ns	1							
Electrolyte leakage	0.02ns	0.23ns	1						
Proline	0.01ns	-0.29*	0.01ns	1					
Total carbohydrate	-0.21ns	-0.01ns	0.13ns	-0.08ns	1				
Anthocyanin	-0.34*	-0.10ns	0.36*	-0.06ns	0.24ns	1			
Phenol	-0.50**	-0.08ns	0.21ns	0.04ns	0.49*	0.80**	1		
Flavonoids	-0.26*	-0.05ns	-0.12ns	0.43*	0.02ns	0.15ns	0.47*	1	
DPPH	-0.43*	0.01ns	0.26*	0.25*	0.29*	0.80**	0.91**	0.62**	1



The results indicated the presence of both positive and negative correlations among the measured traits. Total chlorophyll content exhibited significant negative correlations with anthocyanins, total phenols,

flavonoids, and antioxidant activity. Conversely, antioxidant activity showed significant positive correlations with electrolyte leakage, proline, total carbohydrates, anthocyanins, total phenols, and flavonoids.

Principal component analysis (PCA) and cluster analysis

The results of the PCA of physiological and biochemical traits in different grape varieties under various water deficit treatments showed that the first component accounted for 38.5% of the variation, while the second component explained 18.1% of the variation. In other words, the two principal components collectively accounted for 56.6% of the total variation. In the first component, traits such as anthocyanins, total phenols, and antioxidant activity were prominent, while in the second component, amino acid content, proline, and flavonoids had high loadings (Figure 2).

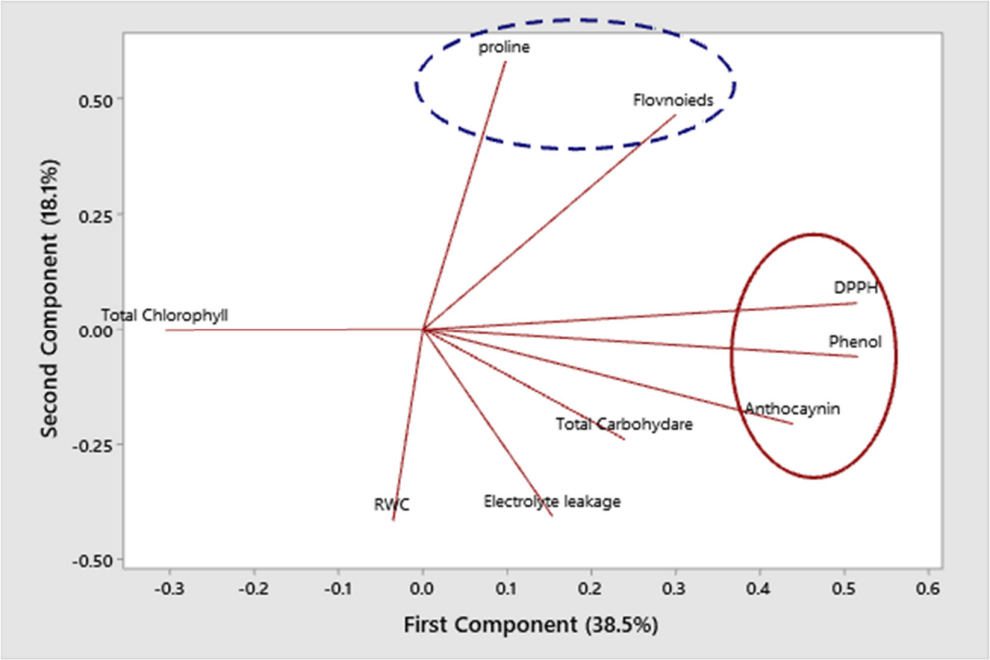


Figure 2. Principal component analysis of physiological and biochemical traits in different grape varieties under various water deficit stress

The cluster analysis results are presented in Table 5 and Figure 3.

Table 5. Cluster analysis of different grape varieties based on physiological and biochemical traits under water deficit treatments

Variable	Cluster1	Cluster2	Cluster3	Grand centroid
Total chlorophyll	5.603	**8.868**	6.794	7.247
RWC	**63.512**	62.268	58.026	60.537
Electrolyte leakage	55.371	**69.358**	58.041	61.279
Proline	**53.548**	50.805	50.953	51.423
Total carbohydrate	133.944	131.635	**153.017**	142.075
Anthocyanin	3.567	**3.654**	3.642	3.631
Phenol	**132.866**	124.299	132.789	129.974
Flavonoids	**16.891**	12.695	14.594	14.421
DPPH	**65.051**	43.575	53.470	52.488
Number of varieties	3	5	7	-

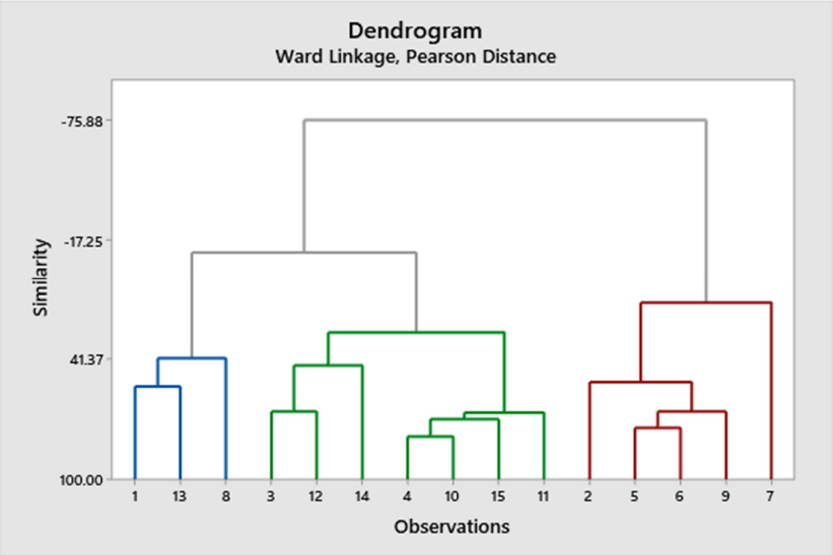


Figure 3. Dendrogram of the cluster analysis results for different grape varieties under water deficit stress (1. 'Asghari'; 2. 'Bidaneh Sefid'; 3. 'Divane'; 4. 'Fakhri'; 5. 'Flim Seedless'; 6. 'Garmeh'; 7. 'Keshmeshi'; 8. 'Khalili'; 9. 'Kolahdari'; 10. 'Lal'; 11. 'Maskeh'; 12. 'Sahebi'; 13. 'Sanjari'; 14. 'Sefid-Berian'; 15. 'Shiregi')

According to these findings, grape varieties were divided into three clusters. Cluster 1 consisted of three varieties ('Asghari', 'Sanjari', and 'Khalili'), Cluster 2 included five varieties ('Bidaneh Sefid', 'Flim Seedless', 'Garmeh', 'Kolahdari', and 'Keshmeshi'), and Cluster 3 comprised seven varieties ('Divane', 'Sahebi', 'Sefid-Berian', 'Fakhri', 'Lal', 'Shiregi', and 'Maskeh'). Cluster 1, which had the fewest varieties, showed superiority in terms of RWC, proline levels, total phenols, flavonoids, and antioxidant activity. On the other hand, Cluster 2 excelled in total chlorophyll content, electrolyte leakage, and anthocyanin levels. Lastly, Cluster 3 stood out in terms of total carbohydrate content.

Discussion

The current research was conducted to evaluate the physiological and biochemical responses of important commercial grape varieties to various water deficit treatments. The results indicated that the measured traits were significantly influenced by both grape variety and water deficit stress. Grape varieties exhibit different responses to water deficit stress, and generally, water deficiency leads to a reduction in the RWC and total chlorophyll content (Ye *et al.*, 2022). The plant's water condition can impact various physiological aspects like leaf turgor potential, plant growth, stomatal conductance, transpiration rate, photosynthesis, and respiration. Earlier studies have demonstrated a notable reduction in RWC during periods of water stress (Zafari *et al.*, 2020).

In the present study, water deficit stress led to a reduction in the total chlorophyll content. It is suggested that the decrease in relative chlorophyll levels under water deficit stress is not only due to a reduction in chlorophyll synthesis but also attributed to an increase in chlorophyllase activity, peroxidase activity, and phenolic compounds (Yadav *et al.*, 2020; Attaran Dowom *et al.*, 2022). According to these researchers' conclusions, the reduction in chlorophyll levels under stress conditions is associated with an increase in the production of oxygen-free radicals in the cell. These free radicals lead to oxidation and, consequently, the degradation of pigments. An additional factor contributing to the decline in chlorophyll levels under stress conditions is the heightened nitrogen utilization for proline synthesis (Ye *et al.*, 2022).

The researchers stated that grape varieties with higher RWC seem to be capable of experiencing fewer damages resulting from dehydration and reduced water content under stressful conditions. It appears that these varieties can be classified as drought-resistant. The results demonstrated that different grape varieties have varying responses to the reduction in RWC during water deficit stress. This difference may be attributed to the varieties' ability to absorb water from the soil or their capacity to close stomata less and increase cellular sap concentration under drought stress conditions, as reported by previous studies (Yadav *et al*., 2020; Wahab *et al*., 2022).

In drought-tolerant varieties, cellular membranes may incur damage due to drought stress (Sebastian *et al*., 2022). However, because the extent of this damage is nearly at a manageable threshold, it is possible to restore cells to their initial state and regain membrane fluidity by creating suitable conditions and providing water to the plant. Under water deficit stress, the integrity of the cell membrane is compromised, and when leaves rehydrate in an aqueous environment, solutes leak out of the cells. Therefore, membrane stability is assessed by examining ion permeability (Sebastian *et al*., 2022). The results indicated that water deficit stress disrupts the biological activity of the cell membrane, reducing its fluidity and either deactivating or slowing down the ion pumping rate across the membrane. Consequently, ion leakage also increases (Hniličková *et al*., 2019). Water deficit stress induces alterations in the phospholipids of the membrane, leading to increased unsaturated fatty acids. Under severe water-deficit stress, certain regions of the bilayer phospholipids undergo a hexagonal phase transition, transforming the membrane structure into a porous one, and allowing substance leakage to occur. Generally, water deficit stress results in increased lipid peroxidation and ultimately reduces membrane stability in various plant cells (Hniličková *et al*., 2019; Sebastian *et al*., 2022). In the present study, the 'Asghari' is considered a resilient variety because it exhibited the lowest electrolyte leakage under water deficit conditions.

Prior studies have shown that water deficit stress leads to an elevation in plant proline content. This increase in proline contributes to enhancing plant resilience to water stress by facilitating osmotic adjustment, preventing enzyme damage, and scavenging hydroxyl radicals (Zafari *et al*., 2020). The increase in proline content in plants subjected to stress is a form of adaptation to overcome stressful conditions. Jafarzadeh *et al*. (2013) mentioned that proline content increased with the severity of water deficit stress, but after a certain point, the average of this trait decreased. In the results of the current research, proline content also showed significant differences among different grape varieties under both stressful and non-stressful conditions. Some varieties had high proline levels under stress, while others had higher levels under normal conditions. Therefore, based on these findings, proline content cannot be used as a reliable indicator of water deficit resistance in this study.

The scientists observed that the presence of flavonoids and anthocyanins is influenced by the grape's specific variety, its stage of ripeness, and its genetic background, which is essentially its inherent genetic potential (He *et al*., 2010). It seems that this factor might be one of the contributing factors to the differences in metabolite levels among various grape varieties. Oxidative stress, as a secondary stress, occurs following drought stress, and plants respond by producing and accumulating antioxidant compounds to protect their photosynthesis system and cell structure (Wahab *et al*., 2022). When plants are exposed to water deficit stress, the levels of various reactive oxygen species in them increase. Subsequently, antioxidant genes and antioxidant activity work to eliminate these increased reactive oxygen species. This enhances the antioxidant defense system, ultimately leading to increased drought tolerance in plants (Yadav *et al*., 2020; Zafari *et al*., 2020; Afshari *et al*., 2022; Wahab *et al*., 2022). Phenolic and flavonoid changes under drought stress conditions in different grape varieties have been the subject of scientific discussion (Pérez-Álvarez *et al*., 2021). It is known that drought stress triggers a cascade of biochemical responses in plants, including alterations in secondary metabolites such as phenolics and flavonoids. These compounds play vital roles in the defense mechanisms of plants against environmental stresses (Aguirre-Becerra *et al*., 2021). Studies have shown that different grape varieties exhibit varied responses to drought stress, with some showing higher accumulation of phenolics and

flavonoids as a protective mechanism (Król *et al*., 2014). Understanding the dynamic nature of these secondary metabolites under drought stress is crucial for developing strategies to enhance grapevine adaptation and improve crop performance in water-limited environments (Oguz *et al*., 2022).

Conclusions

In summary, this study revealed that water deficit stress significantly influenced a range of physiological and biochemical traits in grapes. Notable effects included a reduction in chlorophyll content, variable impacts on RWC, increased electrolyte leakage, elevated proline, carbohydrate, anthocyanin, and phenol levels, as well as varying antioxidant activity. Moreover, correlations between these traits were identified, and grape varieties were categorized based on their responses. Among the grape varieties examined, 'Bidaneh Sefid' exhibited the highest chlorophyll content, 'Khalili' displayed the highest proline content under water deficit stress, and 'Maskeh' demonstrated the highest total carbohydrate content under similar stress conditions. Furthermore, 'Fakhri', 'Filam Seedless', and 'Shiregi' varieties exhibited the highest anthocyanin levels under water deficit stress, while 'Khalili' showcased the highest antioxidant activity under the same stress conditions. These findings shed light on grape adaptations to water deficit stress and highlight specific grape varieties with remarkable responses to these conditions.

Authors' Contributions

Conceptualization, SS and AA; Data curation, SS, AA, AM and MS; Formal analysis, AA; Methodology, AA and MS; Writing – original draft, SS and MS; Writing – review & editing, AA and AM. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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