Physiological and Morphological Responses of Three Grapevine Rootstocks to Water Stress

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Water deficit stress is one of the most frequent environmental stresses affecting the physiological and growth responses of plants, especially grapevines. However, grapevine varieties and species differ in their tolerance to water stress. To identify the most tolerant grapevine rootstock, a factorial randomised block design with two factors was used. The first factor included the susceptible cv. Sultana (*V. vinifera* L.) scion grafted onto three rootstocks (Yaghouti, Kolahdari and 140 Ru), and the second factor was water stress potential at three levels (control, -1 MPa and -2 MPa). The physiological parameters, such as malondialdehyde (MDA), electrical leakage (EL), proline, soluble sugar, protein, photosynthetic pigments, and antioxidant enzymes were investigated. Our results revealed that increasing water stress enhanced H_2O_2, MDA, EL, proline, soluble sugar and soluble protein, while decreasing chlorophyll (Chl) and carotenoid contents, growth parameters, and plant dry weight. The glutathione peroxidase (GPX) activity was enhanced in response to water deficiency, whereas catalase (CAT) and ascorbate peroxidase (APX) enzymes exhibited higher activity at -1 MPa, which was then reduced under the lowest water potential (-2 MPa). In addition, 140 Ru rootstocks exposed to water stress had lower levels of MDA, H_2O_2 and EL, and higher Chl (a, b), carotenoid, APX and GPX activity, as well as higher shoot dry weight. Overall, the physiological and morphological responses of the three rootstocks propose that grafting the commercial Sultana cultivar onto drought-tolerant rootstocks such as 140 Ru is an effective strategy for improving drought stress tolerance.

INTRODUCTION

Grapevine (*Vitis vinifera*) is one of the most valuable and healthy fruits in the world, enriched with vitamins, sugars and minerals. More than 90 countries grow this fruit for fresh and processed products, as well as for pharmaceutical purposes (Foshati *et al*., 2022; Zhou *et al*., 2022). Iran, a semi-arid region with 316 000 ha of grapevine and an annual production of 1 945 930 tons, ranks 11th in the world (FAO, 2019).

In arid areas, water stress is one of the greatest abiotic stresses that limit grapevine production and quality (Guo *et al*., 2022; Ryckewaert *et al*., 2022). In addition to affecting grape composition and phenology, this environmental issue increases water consumption, resulting in lower transpiration and photosynthesis rates (Conesa *et al*., 2016; Van Leeuwen and Destrac-Irvine, 2017)2017. Water stress often induces oxidative damage, leading to the generation of reactive oxygen species (ROS), such as $O_2^{\cdot}$ and hydrogen peroxide (H_2O_2). Subsequently, several antioxidant enzymes, such as CAT, peroxidases (POD) and superoxide dismutase (SOD), are produced in grapevines to scavenge ROS (Laxa *et al*., 2019; Rajput *et al*., 2021). These products have previously been reported to accumulate in some grapevine cultivars to scavenge ROS (Fahim *et al*., 2022). The primary and secondary metabolism in grapevines are also changed under water deficit (Rienth *et al*., 2021). While moderate water stress enhances the accumulation of some phenolics and sugar compounds, severe water shortage leads to remarkably decreased grape yield and berry quality (in terms of sugar and aroma) (Van Leeuwen *et al*., 2018; Irani *et al*., 2021). Shirazi *et al.* (2020) found that polyethylene glycol (PEG)-induced water stress decreased the chlorophyll a content in grapevine leaves. However, proline contents were enhanced with increased PEG concentration.

Grapevine resistance to water deficit is influenced by soil, rootstock, cultivar, and applied agricultural practices (Gambetta *et al*., 2020; Villalobos-Soublett *et al*., 2022). Rootstocks enable growers to plant cultivars that are more adapted to specific soil and climate conditions (such as water stress, salinity and flooding). Furthermore, they enhance the performance of the scion/rootstock combination, as well as its adaptation; consequently, rootstocks affect the quality and yield of the berries as well (Harris, 2013). Koundouras *et al.* (2009) noted a significant effect of rootstock and irrigation treatments on the flavan-3-ol content of seeds in Cabernet

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Sauvignon berries grafted on 1103P and SO4. Similarly, evidence has shown a significant interaction between rootstocks and scion cultivars, which was attributed to yield, the chemical composition of the berries, the accumulation of sugars in berries, and the aromas (Zombardo *et al*., 2020; Prinsi *et al*., 2021).

In Iran, the main origin of grapevines, this fruit can be found in more than 1 000 varieties (Khadivi-Khub *et al.,* 2014; Panahi *et al.,* 2019), mainly in the northeastern (Khorasan), northwestern (Zagros mountains) and southern (Shiraz) parts of the country (Hadadinejad *et al*., 2012).

Based on the response of their leaf potential to drought stress, grape cultivars can be divided into two groups: isohydric (which are tolerant to abiotic stress via leaf water potential) and anisohydric (which are relatively tolerant to stress by providing osmotic balance mechanisms and flexibility in the cell membrane) (Gerzon *et al*., 2015; Shelden *et al*., 2017; Dayer *et al*., 2020). Notably, the white quince variety (Sultana) is also among anisohydrics (Lovisolo *et al*., 2002).

The objective of this study was to evaluate the biochemical and physiological characteristics of two traditional grapevines ('Yaghouti' and 'Kolahdari') used as rootstocks for Sultana in response to water stress in comparison with the hybrid rootstock of 140 Ru.

MATERIALS AND METHODS

The experiment was carried out in greenhouse conditions from 2020 to 2021 at the Higher Engineering Education Complex of Esfarayen, North Khorasan province, Iran (36°540′ N, 56°57′ E, altitude 1 260 m). The experiment was organised as a factorial randomised block design with two factors and three replications. The first factor included three grapevine rootstocks (Yaghouti, Kolahdari and 140 Ru), and the second factor was water potential at three levels (-0.1 MPa (control), -1 MPa and -2 MPa). The grapevine (*V. vinifera* L., var. Sultana) was grafted onto three rootstocks (Yaghouti, Kolahdari and 140 Ru). One-year-old grapevine rootstocks of Yaghouti and Kolahdari grafted with 'Sultana' were provided by Kesht and Sanat Jovin Company. The 140 Ru rootstock was supplied by the Horticultural Research Station of Tehran University in June 2020.

A grafting machine equipped with omega cuts was used to mechanically graft rootstocks and scion cuttings, after which they were transferred to a rooting medium consisting of perlite and peat (40:60). Grafted plants were maintained at 25°C for about 20 days to promote callus formation. The rooting hormone was then applied in the form of naphthaleneacetic acid (NAA) hormone (100 mg L^{-1}). The rooted cuttings were cultivated in pots (volume: 2 L) and later repotted into larger pots (volume: 20 L) filled with sandy loam potting soil medium. Grapevine seedlings were grown under greenhouse conditions from 2020-08 to 2021-01, with the temperature maintained at 25°C to 32°C and relative humidity at 45% to 70%. The seedlings were irrigated manually. The drought treatments were applied in 2021-08 to evaluate the FC (field capacity) and soil water potential. Soil samples were collected from several pots and taken to the soil science laboratory at Ferdowsi University of Mashhad, Iran. These soil samples were placed under a pressure plate. A soil water-retention curve was measured and used as input data into the RETC software, and to verify estimated volumetric moisture values. Soil water suction was expressed in MPa.

The moisture content of the soil samples was evaluated daily after the last irrigation. Three similar and homogeneous pots were subjected to water stress, and the estimated time of the occurrence of water stress was calculated. Water stress treatments were applied by interrupting irrigation (Lovisolo *et al*., 2010) at three levels – control (-0.1 MPa), -1 MPa, and -2 MPa. Immediately after sampling, the leaves were placed in liquid nitrogen and kept at -80°C until analysis. The number of leaves and stem diameter of each plant was measured at the end of the experiment.

Chlorophyll and carotenoid compounds

Extraction and estimation of chlorophyll and carotenoids was done according to reference values (Lichtenthaler & Buschmann, 2001). In summary, leaf samples (0.2 g) were first ground and mixed with 10 ml of 99% methanol in a porcelain mortar, after which the resulting solution was centrifuged at 3 000 rpm for 3 min, and finally the absorbance of the supernatant was read by spectrophotometer at wavelengths of 653, 666 and 470 nm to determine the amount of chlorophyll and carotenoids. The amounts of chlorophyll a and b, total chlorophyll (a + b) and carotenoid were calculated by the following equations:

$$\text{Chl a} = 15.65\ A666 - 7.340\ A653$$
$$\text{Chl b} = 27.05\ A653 - 11.21\ A666$$
$$\text{Carotenoid} = 1\,000\ A470 - 1.8\ \text{Chl a} - 85.02\ \text{Chl b}$$

Proline

The proline content of the samples was evaluated using the method of Bates *et al*. (1973). A total of 0.1 gram dry leaves was mixed with 3% sulfosalicylic acid. The supernatant was treated with ninhydrin and acetic acid, heated for one hour, and then absorbance at 520 nm was measured by UV-visible spectrophotometer (Biochrom S 2100). The proline contents were estimated as mg g^{-1} DW.

Soluble sugar

The extraction and evaluation of soluble sugars was done as per phenol-sulphuric acid. A 2 mL aliquot of a carbohydrate solution was mixed with 1 mL of 5% aqueous phenol solution in a test tube. Subsequently, 5 mL of concentrated sulphuric acid was added rapidly to the mixture. After allowing the test tubes to stand for 10 min, they were vortexed for 30 s and placed in a water bath at room temperature for 20 min for colour development. Light absorption at 490 nm was then recorded on a spectrophotometer (Dubois *et al*., 1956), and the contents of these compounds were estimated as mg g^{-1} DW.

Total soluble protein

Analyses of total soluble proteins were carried out with 0.12 g of fresh leaf samples ground with potassium phosphate buffer (50 mM, pH 7.0) (Braford, 1970). The supernatant was used to evaluate protein and enzymes.

Enzyme activity

Catalase activity was evaluated by preparing enzymes in 1.0 ml of the reactant (65 mM/ml of hydrogen peroxide at 60 mM/1 sodium phosphate-potassium, pH = 7.4) at 37°C for three minutes. Work was stopped with ammonium molybdate, after which absorption was measured in the yellow compound in molybdates and hydrogen peroxide at 374 nm for the blank (Hadwan & Ali, 2018).

APX activity was evaluated following the decline in absorbance at 290 nm, and the reaction contained 0.1 M HEPES–KOH buffer (pH 7.8), 1 mM EDTA, 3.4 M H_2O_2 and 0.5 mM ASA (Ranieri *et al*., 1998). GPX activity was evaluated by the method of Paglia and Valentine (1967). The reaction mixture contained 10 mM glutathione, 1 mM NaN_3, 1 mM EDTA, 1.5 mM NADPH, 0.1 M phosphate buffer (pH 7.0), 0.1 ml of cell lysate and one unit of glutathione reductase. GPX activity was estimated as the rate of NADPH oxidation at 340 nm.

Relative water content (RWC)

Leaf samples were collected and weighed (fresh weight (FW)) and then immersed overnight in distilled water at 4°C. After cold incubation, the leaves were dried with paper and weighed [turgid weight (TW)] and finally dried in an oven at 80°C for 48 h. The dry weight (DW) of the leaves was then recorded. The relative water content of the leaves was calculated using the following equation (Yamasaki & Dillenburg, 1999):

$$RWC = (FW - DW)/(TW - DW)) \times 100$$

Malondialdehyde (MDA)

Malondialdehyde (MDA) was estimated on the basis of the method of Esterbauer and Cheeseman (1999). A total of 0.2 g dry weight of leaves and 1.5 mL of 5% TCA was centrifuged at 13 000 g for 20 min. Supernatant was mixed with 2 mL 0.5% thiobarbituric acid solution and heated in a water bath at 100°C for 25 min. Sample absorbance was read at 450, 532 and 600 nm using a blank containing all reagents.

H_2O_2 content evaluation

A modified method of Velikova *et al*. (2000) was used to measure H_2O_2 content. To do that, 0.3 g leaf powder and 2 mL of ice-cold 0.1% trichloroacetic acid (TCA) (w/v) were homogenised, and centrifuged (12,000× *g*) for 15 min at 4 °C. 1 mL of 1 M potassium iodide and 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0) were added to the supernatant (0.5 mL). The absorbance of solution was expressed at 390 nm.

Electrolyte leakage (EL)

An amount of 0.1 g of leaf tissue was soaked in 15 ml of distilled water for 24 h. The electrical conductivity of the samples was recorded with a conductivity meter, as EC1. Samples were heated at 100°C for 30 min and, after they had cooled down, the electrical conductivity of the samples was evaluated and recorded as EC2. Electrolyte leakage (EL) was estimated using the following (Dionisio-Sese & Tobita, 1998):

$$EL = (EC1 / EC2) \times 100$$

Statistical analysis

Data were statistically analysed using the PROC GLM in SAS Software (Version 9.1, SAS Institute Inc., Cary, NC). The assumption of homogeneity of variance was tested before analysing the data. The data were subjected to a mean comparison using Duncan's multiple range test at the 5% probability level.

RESULTS

According to our results, rootstock and water stress affected the total fresh weight of the plants (Table 1). The highest fresh weight was observed in three rootstocks under non-stress conditions (control) (Table 1). The fresh weight of the plants decreased with increasing drought stress. The highest fresh weight was recorded in Sultana grafted on 140 Ru and Yaghouti rootstocks under the control conditions, while the lowest value was recorded in Yaghouti and Kolahdari rootstocks at severe drought stress (-2 MPa) (Fig. 1).

There were significant effects of rootstock and water stress on the dry weight of grapevine (Table 1). Accordingly, water deficit had a negative effect on dry weight and -2 MPa resulted in the lowest dry weight of the plant (Table 1). The higher dry weight was observed in rootstock 140 Ru at all water potentials (Table 2).

In addition, the number of leaves and stem diameter were significantly affected by water stress and rootstock (Table 1). Sultana scions grafted on Yaghouti and 140 Ru rootstocks had the most leaves per plant, while 140 Ru rootstocks had the largest stem diameters (Table 2). Nevertheless, the diameter of the stem was not significantly different between Sultana scions grafted on Yaghouti and Kolahdari rootstocks. Moreover, water stress negatively affected these parameters, so the highest and lowest numbers of leaves and stem diameter were achieved in the control and -2 MPa, respectively (Table 1).

According to the results, the main effects of water stress and rootstocks were significant on RWC (Table 1). Water stress considerably decreased RWC content; accordingly, the lowest value was observed at -2 MPa (Table 1). Among the rootstock vines, the highest and lowest RWC was found in 140RU and Kolahdari, respectively (Table 2).

Furthermore, water stress and rootstocks interacted significantly with MDA and EL (Table 1). Our results indicate that the lowest MDA content and EL percentage were found in the non-stress conditions (control) in the three rootstocks. Water deficiency increased MDA and EL up to 3.36 (µmol g^{-1} FW) and 70.11%, respectively compared with the control (Table 1). The highest concentrations of MDA and EL were observed in Yaghouti and Kolahdari rootstocks at the lowest water potential (-2 MPa) (Fig. 2).

According to the results, the main effects of water stress and rootstocks were significant on H_2O_2 content (Table 1). On the other hand, water deficit significantly enhanced H_2O_2 content, and the highest values were found at -2 MPa (Table 1). Among the rootstock vines, the highest and lowest H_2O_2 content was found in Kolahdari and 140RU, respectively (Table 2).

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TABLE 1
The effect of water stress on some morphophysiological traits of three grapevine rootstocks.

	Plant fresh weight (g plant $^{-1}$)	**Plant dry weight** (g plant $^{-1}$)	**Stem diameter** (mm)	**Number of leaves**	**RWC** (%)	**MDA** (µmol g^{-1} FW)	**EL** (%)	**H_2O_2** (µmol g^{-1} FW)	**Proline** (µmol g^{-1} FW)
Water stress (W)	**	**	**	**	**	**	**	**	**
Rootstock (R)	**	**	**	**	**	**	**	**	**
W × R	*	ns	ns	ns	ns	**	*	ns	**
					Water stress				
Control	78.44a	27.22a	7.70a	60.47a	83.79a	1.52c	15.62c	44.27c	3.12c
Moderate (-1 MPa)	45.43b	20.88b	6.62b	28.51b	74.83b	2.21b	19.24b	53.08b	6.04b
Severe (-2 MPa)	30.88c	15.33c	5.74c	16.78c	65.99c	3.09a	27.29a	70.69a	12.41a

	Soluble sugar (mg g^{-1} FW)	**Soluble protein** (mg g^{-1} FW)	**Chl-a** (mg g^{-1} FW)	**Chl-b** (mg g^{-1} FW)	**Carotenoids** (mg g^{-1} FW)	**CAT** (Unit mg^{-1} protein)	**APX** (Unit mg^{-1} protein)	**GPX** (Unit mg^{-1} protein)
Water stress (W)	**	**	**	**	**	**	**	**
Rootstock (R)	ns	ns	**	*	*	ns	ns	ns
W × R	**	ns	**	ns	**	*	**	**
				Water stress				
Control	14.53c	0.779c	0.527a	0.395a	1.375a	0.85c	1.44c	0.89c
Moderate (-1 MPa)	19.02b	0.997b	0.530a	0.408a	1.367a	1.28a	2.61b	1.16a
Severe (-2 MPa)	29.61a	1.211a	0.423b	0.301b	1.219b	1.04b	3.58a	1.17a

**, * and ns indicate significance at $P \leq 0.01$ and $P \leq 0.05$, and not significant.
Means within each column with different letters denote significant differences.
Na: sodium, Cl: chlorine, RWC: relative water content, MDA: malondialdehyde, EL: electrolyte leakage, Chl: chlorophyll, CAT: catalase, APX: ascorbate peroxidase, GPX: glutathione peroxidase.

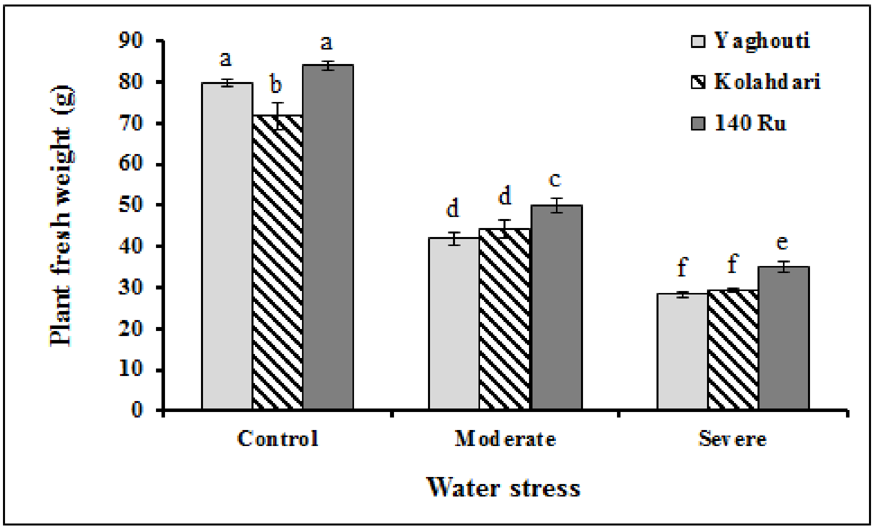

FIGURE 1
Change in fresh weight of three grapevine rootstocks under normal (control), moderate (-1 MPa) and severe (-2 MPa) water stress conditions. The values are the means (n = 3) ± standard error. Different letters indicate significant differences at P < 0.05.

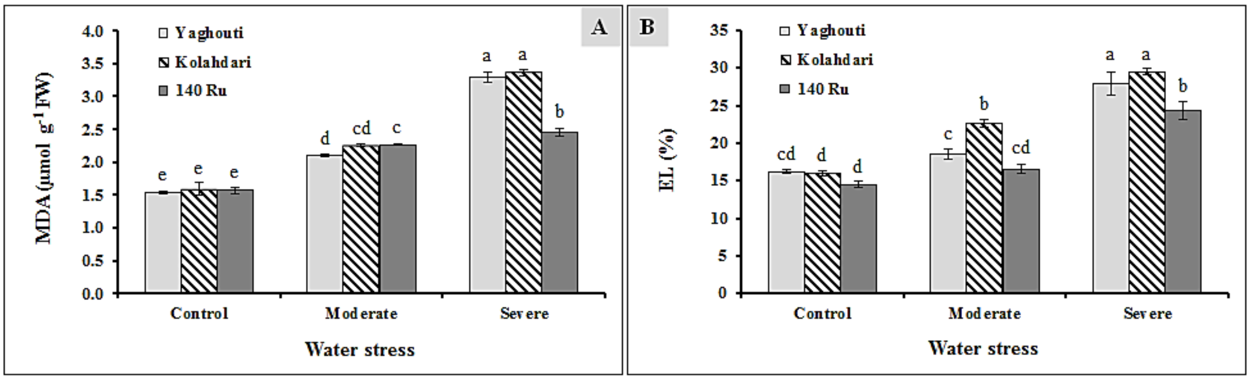

FIGURE 2
Change in malondialdehyde (MDA) (A) and electrolyte leakage (EL) (B) content of three grapevine rootstocks under normal (control), moderate (-1 MPa) and severe (-2 MPa) water stress conditions. The values are the means (n = 3) ± standard error. Different letters indicate significant differences at P < 0.05.

TABLE 2
Change in some morphophysiological traits of three grapevine rootstocks in response to water stress.

	Plant dry weight (g plant $^{-1}$)	**Stem diameter** (mm)	**Number of leaves**	**RWC** (%)	**H_2O_2** (µmol g^{-1} FW)	**Chl-b** (mg g^{-1} FW)
Yaghouti	20.84 ± 0.43b*	6.42 ± 0.18b	34.49 ± 2.24b	75.04 ± 0.72b	57.14 ± 0.90b	0.361 ± 0.021b
Kolahdari	19.27 ± 0.20c	6.58 ± 0.11b	29.87 ± 3.96b	72.53 ± 0.84c	61.24 ± 1.16a	0.341 ± 0.024b
140 Ru	23.22 ± 0.37a	7.16 ± 0.26a	41.79 ± 2.03a	77.09 ± 0.29a	49.54 ± 0.88c	0.403 ± 0.022a

* Means within each column with different letters denote significant differences.
RWC: relative water content, Chl-b: chlorophyll b.

In terms of proline and soluble sugars, there was a significant interaction between water potential treatment and rootstocks (Table 1). The highest and lowest proline activity was obtained in -2 MPa and the control, respectively (Table 1). Yaghouti rootstock had a higher proline concentration (14.99 µmol g^{-1} FW), followed by 140 Ru and Kolahdari at -2 MPa (Figure 3A). Thus, the Yaghouti rootstock had 29% more proline than the Kolahdari rootstock, while no significant differences in proline content were found between the grapevine rootstocks at -1 MPa and the control (Fig. 3A).

The effect of water stress and rootstock on soluble sugars

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in the leaves of grapevine is shown in Fig. 3B. Exposure of 140 Ru rootstocks to low water potential (-2 MPa) led to a significant increase in soluble sugars in the grape leaves. In contrast, the mentioned variety had the lowest concentration of this metabolite at other water potentials. Furthermore, soluble sugars did not differ between Yaghouti and Kolahdari rootstocks at -1 MPa or under control conditions (Fig. 3B).

In grapevine leaves, different water potentials significantly affected total soluble protein, and increasing water stress significantly favoured soluble protein (Table 1). The highest total soluble protein content was observed in the -2 MPa treatment, whereas the lowest value was recorded in the control treatment (Table 1).

A significant interaction was observed between water potential and rootstock between Chl-a and carotenoids (Table 1). Our results show that, although drought stress led to a decrease in Chl-a and carotenoid concentrations in the Yaghouti and Kolahdari rootstocks, the highest values of these pigments in 140 Ru were found in the -1 MPa treatment (Fig. 4).

In contrast, both water potential and rootstock were significant for Chl-b, but their interaction was not significant (Table 1). Chl-b concentrations were highest in the leaves of 140 Ru plants, and water stress negatively affected their concentrations (Table 2). Accordingly, the highest concentration of this pigment was observed in grapevines exposed to the lowest water potential (-2 MPa) (Table 1).

As for antioxidant enzymes, water potential and rootstocks significantly affected CAT, GPX and APX activity (Table 1). CAT activity increased in the leaves of Kolahdari and 140 Ru rootstocks when subjected to -2 MPa pressure. In contrast, enzyme activity in Yaghouti leaves was 7% lower in the -1 MPa than in the 140 Ru under the same conditions (Fig. 5A).

Similarly, APX activity increased significantly under drought stress (Table 1). Accordingly, the highest activity was found in the 140 Ru and Yaghouti rootstocks at -2 MPa, while the lowest value was recorded in all three rootstocks in the controls (Fig. 5B).

On the other hand, grapevine rootstocks differed in their activity of GPX enzyme under water stress. The water potential of -2 MPa increased the GPX activity in 140 Ru rootstock, while it reduced the GPX activity of Yaghouti rootstock (Fig. 5C).

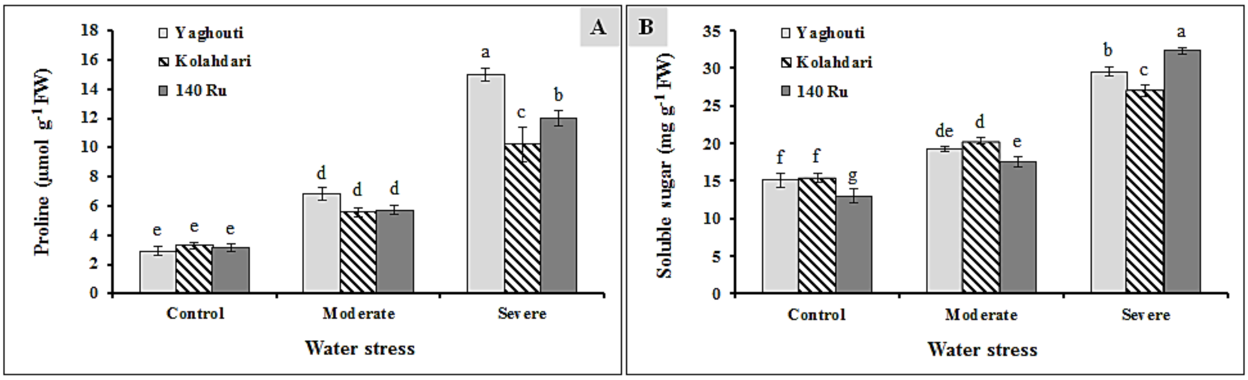

FIGURE 3
Change in proline (A) and soluble sugar (B) content of three grapevine rootstocks under normal (control), moderate (-1 MPa) and severe (-2 MPa) water stress conditions. The values are the means (n = 3) ± standard error. Different letters indicate significant differences at $P < 0.05$.

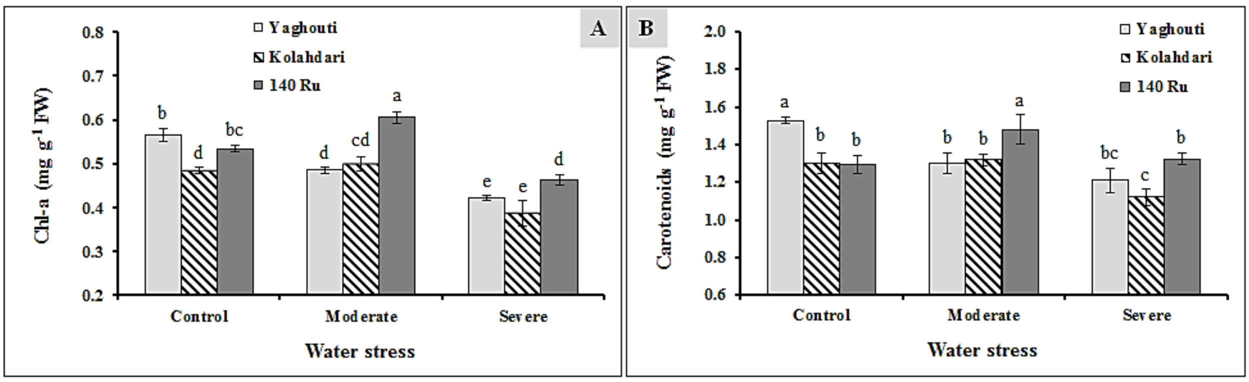

FIGURE 4
Change in chlorophyll a (Chl-a) (A) and carotenoid (B) content of the three grapevine rootstocks under normal (control), moderate (-1 MPa) and severe (-2 MPa) water stress conditions. The values are the means (n = 3) ± standard error. Different letters indicate significant differences at $P < 0.05$.

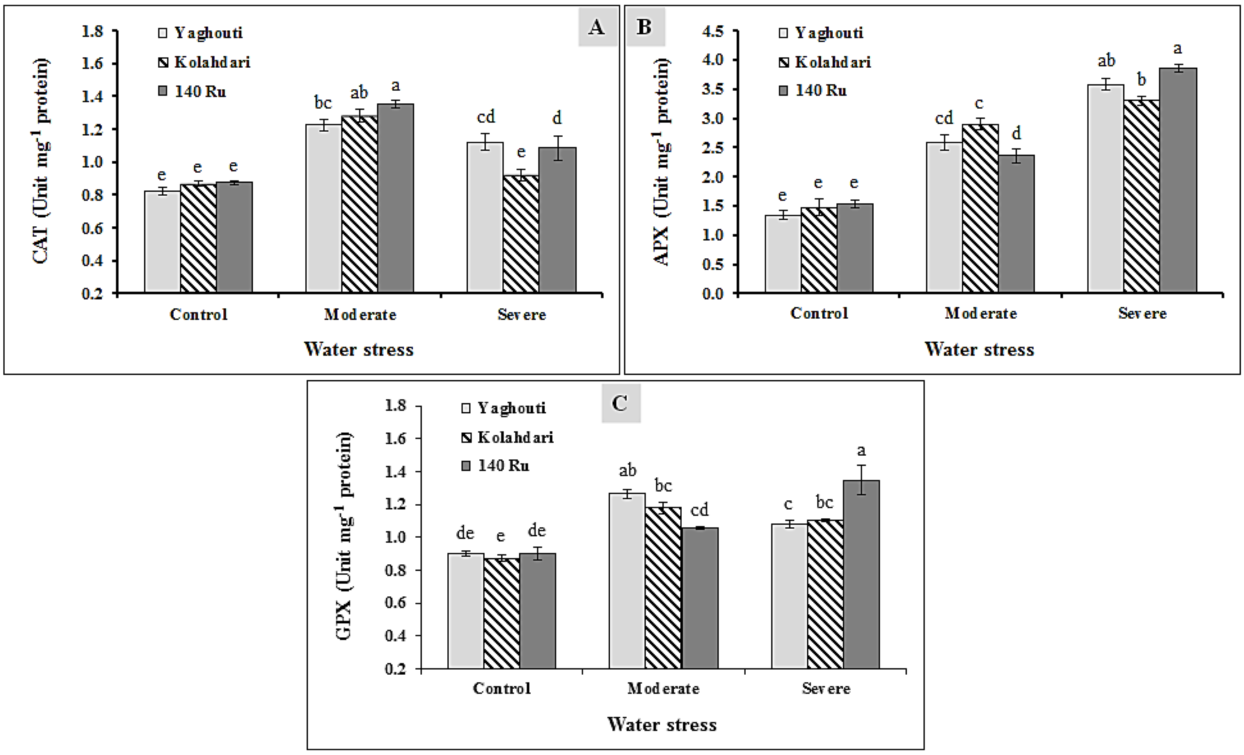

FIGURE 5
Change in catalase (CAT), ascorbate peroxidase (APX) and glutathione peroxidase (GPX) activity in the three grapevine rootstocks under normal (control), moderate (-1 MPa) and severe (-2 MPa) water stress conditions. The values are the means (n = 3) ± standard error. Different letters indicate significant differences at $P < 0.05$.

DISCUSSION

In the present study, increasing water stress reduced growth parameters (Tables 1 and 2). Several studies have noted the negative impact of water stress on cell division in many species, including grapes (Ojeda *et al*., 2001; McCarthy *et al*., 2002) and olives (Ojeda *et al*., 2001). Plant growth indicators such as cell division, cell size, cell wall composition, plant size and dry weight are negatively affected by drought stress (Medyouni *et al*., 2021). In addition, drought stress reduces dry weight by increasing growth inhibitors and decreasing growth hormones, resulting in decreased photosynthesis (Rezayian *et al*., 2020). According to the results of this study, Sultana scions grafted on 140 Ru can be introduced as drought-tolerant combinations, probably because of the increased activity of CAT and APX, the increased chlorophyll content, the increased number of leaves, the larger stem diameter, and the higher dry weight, which may influence hormone transport between roots and scions.

The results show that the lowest amounts of MDA and EL were found in the non-stress conditions (control) in the three rootstocks. Lower levels of these compounds in cells can be associated with the better tolerance of the plants to drought stress (Nazir *et al*., 2022). The H_2O_2, MDA and EL of grapevine cv. Sultana grafted on three rootstocks were significantly affected by increasing water stress. In previous studies, lower levels of MDA and H_2O_2 were observed in olive and poplar plants under drought stress (Yang & Miao, 2010; Petridis *et al*., 2012). Various abiotic stresses, such as drought stress, induce the production of different types of ROS such as H_2O_2, which damage membrane lipids (Garg & Manchanda, 2009). Since this compound (H_2O_2) is a relatively long-lived molecule, it is easier to measure in tissue samples. In addition, MDA is a product of lipid peroxidation, which is often used to assess oxidative stress. It appears that the higher MDA content in water-stressed plants can be associated with higher H_2O_2 concentrations. Consequently, higher production of these compounds causes more serious oxidative damage. In this study, MDA and H_2O_2 levels increased remarkably when water limitation occurred. However, this response varied depending on the rootstock and water stress (Table 1). In addition, the Sultana scion grafted on 140 Ru rootstock was the most drought-tolerant grafting combination because it had the lowest H_2O_2 content. The decrease in this compound under water stress conditions may be due to the activation of antioxidant enzyme activities, particularly CAT, which detoxify H_2O_2 and decrease its accumulation (Umar & Siddiqui, 2018). Consequently, the 140 Ru rootstock protects the scion against oxidative stress under water deficit. Due to the more developed root surface of the 140 Ru rootstock, it is capable of exploring larger and deeper soil volumes, which contributes to its drought tolerance.

We observed higher EL in the less tolerant Yaghouti and Kolahdari rootstocks (Fig. 2B). Drought stress decreases the integrity of cell membranes, and therefore the movement of ions inside and outside cells can be used as an indicator of damage to a variety of tissues (Blokhina *et al*., 2003; Masoumi *et al*., 2010).

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In plants, soluble sugars and proline play a vital role in the defence system by increasing tolerance to water stress (Szabados & Savouré, 2010; Shen *et al*., 2014; He *et al*., 2017). Studies have shown that these products act as osmotic molecules that contribute mainly to an improvement in cell turgor, thus protecting plants in the event of water shortage (Szabados & Savouré, 2010). The highest concentration of soluble sugars was detected in the leaves of 140 Ru at low water potential compared to the other treatments (Fig. 3B). The rootstock of 140 Ru responds osmotically to a water deficit (Barrios-Masias *et al*., 2015). As a result, the grafting of commercial cultivars onto drought-tolerant rootstock (s) such as 140 Ru can be considered a promising strategy to improve tolerance to drought stress. In contrast, the Kolahdari rootstock reacted poorly under drought stress due to its low concentration of soluble sugars.

The increased content of soluble sugar under drought stress can be explained by the degradation of polysaccharides, including starch, to glucose, which mainly contributes to the increase in cellular turgor pressure and osmotic potential under drought stress. According to our results, the Sultana leaves grafted onto Yaghouti exposed to -2 MPa exhibited a high proline concentration. Similarly, Moghadam *et al*. (2011) documented that water stress enhanced proline content in canola. This phenomenon can be attributed to the variations in the enzyme activities involved in proline biosynthesis and degradation and the inhibition of oxidation. Furthermore, water stress increased overall protein production in our study (Table 1). It has been found that the plant can resist environmental stress by accumulating proline and protein (Hong *et al*., 2000). The aggregation of different types of protein in grapevine leaves under drought stress was also reported by Król and Weidner (2017), which is consistent with the results of this study.

Furthermore, the enzymes CAT and APX (except in the case of 140 Ru) responded similarly to water stress and showed higher activity at moderate water stress, which then decreased at severe water deficit (Table 1). Our results are in agreement with those of Antoniou *et al*. (2017). During water stress, the synergy of enzymes is crucial to protect the plant against oxidative damage. However, under severe stress, the activities of these enzymes are decreased considerably. This decrease could be due to the fact that the content of reactive oxygen species exceeds the capacity of the antioxidant enzyme system of the plant.

The highest concentration of chlorophylls and carotenoids was observed in 140 Ru under a water potential of -1 MPa, thereby increasing photosynthetic efficiency. These results are consistent with previous findings that grafting onto a tolerant rootstock improved the photosynthetic efficiency of plants under drought stress (Penella *et al*., 2014). Chlorophyll (a and b) and carotenoids decreased significantly in Sultana grafted on Kolahdari and Yaghouti rootstocks under water stress. This decrease in photosynthetic pigments under stress is a common phenomenon also found in some other grapevine rootstocks (Madadi *et al*., 2021), mung bean (Sadiq *et al*., 2017), carrot (Razzaq *et al*., 2017), canola (Akram *et al*., 2018) and apple rootstock (Alizadeh *et al*., 2011). This phenomenon may be due to the instability of protein complexes and the destruction of chlorophyll caused by the enhanced activity of chlorophyllase enzyme (Kabiri *et al*., 2014; Kapoor *et al*., 2020). Carotenoids boost the plant's reaction to oxidative stress. The percentage content of carotenoids increases with a decline in chlorophyll (Bhandari *et al*., 2016). The results show that rootstock 140 Ru was the most drought tolerant due to several factors, including the highest accumulation of carotenoid content.

CONCLUSIONS

Our study reveals that increasing water stress markedly improved H_2O_2, MDA, EL, proline, soluble sugar, total soluble protein and the activity of some enzymes, such as APX and GPX, in plants, but decreased RWC, fresh weight, dry weight, Chl and carotenoid contents. The characteristics of the grapevines studied varied widely, suggesting that different rootstocks can withstand drought stress using different mechanisms. For instance, white quince transplanted onto ruby rootstock had a higher increase in proline than other rootstock types. Overall, 140 Ru was introduced as the most drought-tolerant rootstock based on the lowest MDA and H_2O_2 concentration, and the highest soluble sugar content, carotenoid content, fresh and dry weight, total soluble protein content at higher water stress. Hence, it can be concluded that grafting commercial cultivars onto drought-tolerant rootstock(s) such as 140 Ru is a useful strategy for improving tolerance to drought stress.

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