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Screening of some grape (*Vitis vinifera* L.) genotypes responses to drought stress using physiological and biochemical traits in greenhouse condition

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ABSTRACT

Purpose: Grapes (*Vitis vinifera* L.) are among the most significant agricultural products cultivated in various regions of Iran, boasting high nutritional value. This study focuses on assessing the genetic diversity of grape genotypes from vineyards in the West Azarbaijan province. Drought is an important environmental factor that limits plant growth and production. Given the abundant grape germplasm in Iran, there is potential to select cultivars and high-yielding genotypes possessing valuable genetic traits to use as resilient bases in commercial grape cultivars. **Research Method:** This research involved the evaluation of 16 grape genotypes in a single phase. For this purpose, 16 grape genotypes were grouped and compared in various dry conditions including (PEG0%, PEG2% and PEG4%). **Findings:** The results showed that vegetative traits, relative water content, and membrane stability decreased in all cultivars, but this decline was less pronounced in the «Garashire, Gezel, and Fakhri genotypes». Protein content and the activity of protective enzymes in the roots and leaves increased significantly across all 16 genotypes, with particularly notable levels observed in the «Garashire genotype». Drought stress had a marked effect on the accumulation of malondialdehyde and hydrogen peroxide in the Asgari and Reddish Tabriz genotypes. The levels of these compounds were higher in these genotypes compared to others, indicating increased lipid peroxidation and reduced stability against drought. **Research limitations:** There was no limitation. **Originality/Value:** The adverse effects of drought were more pronounced at the end of the stress period, especially under a high dose of PEG (4%). Overall, the «Garashire genotype» exhibited the highest tolerance, while the Asgari genotype was the most sensitive to drought.

INTRODUCTION

Grapes, scientifically classified as *Vitis vinifera* L., are part of the Vitaceae family, which is alternatively known as the Sarmentaceae or Ampelidaceae family (Keller & Tarara, 2010; Rasouli et al., 2014; Rasouli et al., 2015; Doulati Baneh, 2015; Jahnke et al., 2021; Kupe et al., 2021, Mirfatah et al., 2024a). Experts suggest that grape cultivation has been prevalent in Iran for at least 2000 years before the Common Era (Akram et al., 2021; Doulati Baneh, 2015; Jahnke et al., 2021; Kupe et al., 2021). Grapes are among the most essential fruits consumed by humans since ancient times. Alongside apples, citrus fruits, and bananas, grapes rank among the most crucial horticultural plants cultivated worldwide (Kupe et al., 2021). Climate plays a significant role in shaping grape diversity and production within specific regions. The ability to precisely select among plant varieties is crucial for breeding and developing new strains, a process reliant on recognizing existing varieties and their diversity. Investigating genetic diversity within plant populations and pinpointing suitable traits for the production and introduction of superior genotypes is essential (Mirfatah et al., 2024b, Zahedi et al., 2023). In the realm of screening, numerous studies and experiments have been carried out in Iran and other nations to identify drought-tolerant or resistant genotypes. The quest for resilient cultivars and genotypes against abiotic and biotic stresses stands as a critical strategy for managing such adversities (Razi et al., 2021). By defining appropriate morphological, physiological, and molecular characteristics for screening, it becomes viable to select cultivars and genotypes suited to the climatic conditions of each particular region (Amiri & Eslamian, 2010). In a study conducted by Haddadinejad et al. (2013), a screening process for drought-tolerant genotypes involved 698 genotypes across three stages (Haddadinejad et al., 2013). These studies play a critical role in identifying genotypes with enhanced tolerance, paving the way for their use as the foundation for commercial cultivars to enhance water efficiency in crop production (Zahedi et al., 2023). In a study by Rasouli et al. (2014), the phenotypic diversity of 32 grape varieties and genotypes was examined over three years. The research encompassed morphological and pomological traits, including phenolic content, surface anti-cancer composition of resveratrol, revealing significant diversity among the studied cultivars and genotypes in terms of various measured traits (Rasouli et al., 2014). In another experiment focusing on morphological variation, 36 grape cultivars and genotypes were assessed using the International Grape Descriptor to select superior genotypes based on traits like spike weight, dry spike weight, berry weight, rachis weight, seed weight, and skin color, revealing substantial variation and high diversity coefficients among cultivars and genotypes (Rasouli et al., 2015; Razi et al., 2021). Additionally, Kazemi et al. (2022) evaluated the phenotypic diversity of 60 grape cultivars and genotypes in the tropical and subtropical region of Khuzestan province in Iran. Their findings showcased significant diversity among grape cultivars and genotypes in Khuzestan province, with local cultivars like «Soltani (Sultan), Bangi (Red), and Yershi Dar» displaying superiority in certain traits compared to foreign cultivars (Kazemi et al., 2022). Asadi et al (2018) found that «Chifte» and «Khalili»cultivars along with Askari and «Pearlet» are relatively better in drought stress tolerance and can be cultivated in areas with limited access to water. These cultivars may also serve as a basis for breeding programs aimed at improving drought resistance.

Polyethylene glycol (PEG), mannitol, and sorbitol have been used to stimulate osmotic stress for in vitro selection (Darko et al. 2019), but PEG has been most frequently used to impose water-deficit stress in plants (Siaga et al., 2016). One of the advantages of using PEG is that it does not penetrate the apoplast, causing water withdrawal not only from the cell but also from the cell wall, replicating the effects of water deficit stress (Shirani Bidabadi et al., 2023). Studies have shown that increased drought stress induced by PEG can lead to a significant reduction in tissue moisture content. Despite its widespread use, there has been a limited focus on investigating the effects of PEG-6000-induced drought stress specifically in viticulture. It is worth noting that PEG-induced osmotic stress can result in a decrease in cell water potential, a key indicator of the stress imposed on plant cells (Elmaghrabi et al., 2017). This property of PEG makes it a valuable tool for researchers and breeders seeking to understand plant responses to drought stress and to select for drought-tolerant plant varieties (Darko et al., 2019).

The current research conducted aimed to explore the morphological diversity among grape cultivars and genotypes sourced from vineyards in West Azarbaijan province, particularly Urmia city in northwest Iran. By delving into the physiological and biochemical responses of these grape cultivars and genotypes to simulated drought stress using PEG 6000, the study aimed to shed light on the mechanisms underlying drought tolerance in these varieties.

Furthermore, the research aimed to highlight and promote genotypes that exhibit promising traits associated with drought resistance, potentially offering valuable insights for grape cultivation in regions prone to water scarcity or drought conditions like Urmia city in Iran.

MATERIALS AND METHODS

Plant materials and growth conditions

Cuttings from 16 grape genotypes «including Lal, Ghezel, Asgari, Grashireh, Siah Sardasht, Tabarzeh Sefid, Tabarzeh Ghermez, Bidaneh Sefid, Bidaneh Ghermez, Fakhri, Hosseini, Rish Baba, Pastili, Hybrid H6, Chugao and Taefi» were initially prepared. After disinfection with 1.5% benomyl, the cuttings were treated with indole butyric acid (IBA) at a concentration of 100 ppm for 5–10 seconds. The treated cuttings were then placed in a perlite medium within a thermal bedding system, maintained at 22–28°C with 80–100% humidity to promote rooting, which typically occurred within 2–4 weeks.

After rooting, two healthy cuttings were selected and transferred to containers filled with perlite and Hoagland solution. The cuttings were maintained under the same conditions for two weeks, until 3 to 4 fully developed leaves had emerged. The modified Hoagland solution for grapes contained the following nutrient concentrations:0.125 mM KNO_3, 0.125 mM $Ca(NO_3)_2$, 0.05 mM $MgSO_4.7H_2O$, 0.0125 mM KH_2PO_4, 5.75 µM H_3BO_3, 1.34mM $MnCl_2.4H_2O$, 0.1 µM $ZnSO_4.7H_2O$, 0.038 µM $CuSO_4.5H_2O$, 0.025 µM $Na_2MoO_4.2H_2O$, and 8.88 µM Fe-EDTA (Abbaspour et al., 2013). After the two-week period and the development of four leaves, drought stress treatment was initiated using polyethylene glycol (PEG 6000) at concentrations of 0%, 2%, and 4%, in combination with the Hoagland nutrient solution. These treatments were applied to the four-leaf plants grown in 2-liter pots filled with perlite for duration of two weeks. To prevent osmotic shock, PEG 6000 was gradually added to the Hoagland nutrient solution until the target concentration was reached (Mohsen et al., 2020). The experiment followed a randomized complete block design with three replications, where each treatment included three replicates, and each replicate contained two plants. The length, wet and dry weight of shoots and roots were determined. Dry and fresh tissues of roots and leaves were kept at -80°C until biochemical assessments.

Leaf relative water content (LRWC)

The LRWC was assessed following the methodology detailed by Shams et al. (2019). Leaf segments, each measuring 10 mm, were initially weighed to establish the fresh mass (FM). These segments were then immersed in distilled water at room temperature and left to float for a period of 24 hours to determine the turgor mass (TM). Subsequently, the leaf segments were subjected to oven-drying at 70°C for 48 hours and the resulting weight was recorded as the dry mass (DM). The LRWC percentage was calculated using the following equation 1:

$$\mathrm{LRWC}(\%) = \frac{\mathrm{FM - DM}}{\mathrm{TM - DM}} \times 100 \qquad (1)$$

Electrical conductivity (EC)

The quantitative assessment of these indices was carried out using the method outlined by Sairam (1994). In this procedure, 2 grams of leaf tissue were first washed in bi-distilled water to eliminate any electrolytes attached to the surface. The samples were then immersed in a water bath at 40°C for 80 minutes, during which the electrical conductivity (EC) was gauged using an EC meter. Subsequently, the samples were transferred to a water bath at 70°C for 75 minutes, and the electrical conductivity was once again measured. The desired indices were calculated based on the following equation 2:

$$\mathrm{EC}(\%) = \frac{\mathrm{EC1}}{\mathrm{EC2}} \times 100 \qquad (2)$$

Oxidative stress markers (MDA, H_2O_2)

Malondialdehyde (MDA) was determined following the protocol established by Rao and Sresty (2000). Frozen grape leaves (300 mg) were homogenized in 0.1% trichloroacetic acid (TCA) on ice. The homogenized solution was then centrifuged at 4°C for 10 minutes at 10,000g, and the resulting precipitate was extracted twice using the same solvent. Subsequently, 0.5 ml of the supernatant was combined with 1.5 ml of 20% TCA, followed by the addition of 0.5% thiobarbituric acid. The mixture was heated at 95°C for 25 minutes, cooled to room temperature (RT), and then centrifuged at room temperature for 10 minutes. The sample was measured at 532 nm and adjusted for non-specific absorption at 600 nm.

Hydrogen peroxide (H_2O_2) levels were assessed according to the method of Alexieva et al. (2001). A homogenization process involving 300 mg of leaf sample with 3 ml of 0.1% (w/v) TCA was carried out in a cool environment. Following this, centrifugation was performed for 15 minutes at 21,000×g. A mixture of 1 mL of 1 M potassium iodate and 500 µl of 10 mM K_2PO_4 buffer (pH 7) was combined with 500 µL of the supernatant. The absorbance was then measured at 390 nm to determine the concentration of hydrogen peroxide.

Total Protein

The total protein content was determined using the method outlined by Bradford in 1976. To conduct this analysis, 0.25 g of leaf tissue was homogenized with 2.5 mL of 50 mM KH_2PO_4 buffer (pH=7), and the resulting homogenate was centrifuged at 15,000 g for 20 minutes at 4°C. Subsequently, 2.5 mL of Coomassie Brilliant Blue G-250 was added to 20 µL of the supernatant and vortexed. After an incubation period of 10 minutes, the samples were measured at 595 nm. Bovine serum albumin (BSA; Sigma A7906) served as the standard protein for calculating the total protein content in the leaf samples.

Antioxidant enzymatic activities

For the antioxidant enzyme assays, Grapevine leaves (0.2 g each) were ground to a fine powder using liquid nitrogen and then extracted in 3 mL of a buffer comprising 50 mM KH_2PO_4 (pH=7.0), 0.1 mM EDTA, and 1% polyvinylpyrrolidone (PVP) (w/v). The homogenate was subsequently filtered and centrifuged at 4°C for 15 minutes at 15,000×g. The supernatant was used for Ascorbate peroxidase (APX) and Catalase enzyme activity (CAT) and Guaiacol peroxidase (GPX) activity assay (Ulusu et al., 2017).

Ascorbate peroxidase (ASPX, EC 1.11.1.11)

Analysis of ASPX activity was determined using the method of Karabal in (2003). The reaction was monitored by observing a decrease in absorbance at 290 nm over a 3-minute period. The reaction mixture, with a total volume of 3 mL, comprised 1450 µL of 50 mM phosphate buffer (pH=7), 750 µL of ascorbic acid, 750 µL of 30% H_2O_2, and 50 µL of plant extract. The enzyme activity was computed using the extinction coefficient of ascorbate (2.8 mM^{-1} cm^{-1} at 290 nm).

Catalase (CAT, EC 1.11.1.6)

activity was tested in a reaction mixture (final volume: 3 mL) containing 1450 µl of 50 mM KH_2PO_4 buffer (pH=7), 1500 µl of 30% H_2O_2 and 50 µl of plant extract. The disappearance of H_2O_2 was followed at 240 nm (3 min) (Schimadzu UV-1800, Japan) (H_2O_2: 0.036 $\mu mol^{-1}cm^{-1}$) (Ulusu et al., 2017).

Gayacol peroxidase (GPX, EC 1.11.1.7)

The activity of GPX was measured using Chance and Maehly method (1955). The reaction mixture includes 100 mM potassium phosphate buffer solutions (pH=7), guaiacol 10 mM dissolved in double distilled water, 70 mM hydrogen peroxide dissolved in 100 mM potassium phosphate (pH=7), sterile double distilled water and enzyme extract It was measured for 180 seconds. The mixture without enzyme extract was used as a blank. The specific activity of the peroxidase enzyme was reported as the number of micromoles of hydrogen peroxide decomposed per minute per milligram of protein.

Statistical analysis

Statistical analyses were done using the Statistical Package for Social Sciences (SPSS). The data were subjected to analysis of variance (ANOVA) and the means were compared with Duncan’s multiple range test ($P \leq 0.05$). Significant differences between treatment means are indicated by different letters.

RESULTS

Based on the obtained results, characteristics such as fresh weight, dry weight, length, relative water content, ion leakage, malondialdehyde, hydrogen peroxide, protein and antioxidant enzymes in aerial and terrestrial organs showed significant variation among genotypes. Considering the diversity observed in these traits, performing more detailed statistical analyzes can be useful for evaluating traits with significant diversity in different cultivars and genotypes.

Physiological parameters

The impact of drought stress on the growth characteristics of grape genotypes is outlined in Table 1. As the percentage of PEG increased, the fresh weight, dry weight of shoots and roots, and stem height of all 16 grape genotypes decreased. It is noteworthy that in most genotypes, root height notably increased with higher PEG percentages. The Garashireh, Fakhri, and Gezel genotypes exhibited the highest root heights (38.54 cm, 37.63 cm, and 36.53 cm respectively), while the Asgari, Tabarzeh Ghermez, Taefi, and Tabarzeh Sefid genotypes showed lower root heights compared the others (10.32 cm, 16.21 cm, and 16.16 cm respectively). In the Siah Sardasht genotype, the height decreased while the root height remained unchanged. Overall, the analysis of variance indicated that PEG-induced stress led to a reduction in the fresh weight of both aerial and terrestrial organs. Bidaneh Ghermez and Pastili genotype exhibited the highest percentage of shoot fresh weight reduction (PEG4%: 68%) compared to the control (PEG0%). The Asgari genotype showed the lowest dry weights for both shoot (PEG4%: 0.38g) and root (PEG4%: 0.2g). Conversely, the Garashireh genotype displayed the highest dry weights for shoot (PEG4%: 1.10g) and root (PEG4%: 1.03g) (Table 1).

Table 1. Comparison of the average effects of drought stress induced by PEG6000 on the fresh weight, dry weight, and height of 16 grape genotypes.

Genotype & Dry (PEG)	Root length (cm)	Shoot length (cm)	Root dry weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Shoot fresh weight (g)
Hosseini						
0%	23±2h-m	35±2.6g-k	0.8±0.05efg	1.1±0.09def	7.8±0.3e-i	12.7±0.4def
2%	28±2.6d-i	34±2.9g-l	0.8±0.09e-h	1±0.03f-j	6.9±0.2g-l	7.6±0.9i-m
4%	31±2a-f	34±2h-l	0.4±0.03n-r	0.8±0.07i-m	5.9±0.4j-o	5.6±0.8k-p
Ghezel						
0%	22±2i-n	47±8.1bc	1.1±0.05c	1.6±0.07bc	11.7±1.9b	18.34±0.1b
2%	32±2.7a-f	41±2.6c-g	0.8±0.06e-j	1.1±0.06e-h	10.3±0.6bc	10.3±0.8fg
4%	36±2.1abc	31±3.1e-i	0.4±0.03n-r	0.9±0.08g-k	7.7±0.1e-j	9.3±0.3gh
Tabrzeh Sefid						
0%	29±1.6d-i	20±2.1q-u	0.4±0.01n-s	0.8±0.09i-m	5±0.2l-s	4.6±0.08n- t
2%	20±1.8j-n	17±1.2s-v	0.2±0.01stu	0.8±0.07i-m	4.4±0.5m-t	3.4±0.2p-w
4%	25±1.2f-l	16±1.2tuv	0.2±0.01u	0.6±0.02mno	4.4±0.7n-t	1.7±0.4vwx
Tabarzeh Ghermez						
0%	22±1.4i-n	33±2h-l	0.36±0.04p-t	0.7±0.01lmn	4.9±0.1l-s	2.9±0.3r-x
2%	18±1.1mno	28±0.8k-p	0.33±0.01q-u	0.6±0.03mno	3.6±0.3q-u	1.9±0.1vwx
4%	16±0.8m-p	13±0.8uv	0.2±0.01tu	0.5±0.06opq	3.2±0.1stu	1.7±0.2wx
Bidaneh Sefid						
0%	22±1.2i-n	48±3.1bc	0.9±0.07de	1.3±0.04cd	8.6±0.7c-g	12±0.9de f
2%	32±2.3a-f	42±4.2b-f	0.49±0.02m-p	1.2±0.03de	6.4±0.1i-m	8.3±1g-j
4%	34±3.4a-f	35±2.8g-k	0.41±0.04o-s	0.8±0.08i-m	6.1±0.6i-n	5.2±0.6i-q
Bidaneh Ghermez						
0%	30±1c-h	65±2.8a	0.8±0.03def	1.1±0.08e-h	8.6±0.7c-g	15.7±0.6c
2%	30±0.8b-g	44±4.3b-e	0.6±0.01klm	0.7±0.07lmn	6.4±0.1i-m	7±0.5i-m
4%	31±1.2a-f	28±3.3k-p	0.3±0.03q-u	0.6±0.06mno	6.1±0.6i-n	5±0.3j-s
Fakhri						
0%	31±1.8a-f	65±2.6a	0.9±0.06de	1.4±0.08bc	10±0.7bc	18.3±1.3b
2%	35±0.8a-d	43±1.8b-e	0.7±0.05h-l	1.2±0.005de	9.6±0.7cde	9±0.3ghi
4%	37±1.2ab	27±1.8k-p	0.5±0.02lmn	0.9±0.08f-j	9.5±0.7cde	6.6±0.5j-n
Siah Sardasht						
0%	31±2.9a-f	42±2.3b-f	0.9±0.05def	1.3±0.06de	8.3±0.5d-h	8.9±0.6ghi
2%	30±2.6b-g	35±2.3b-f	0.5±0.04mno	0.9±0.06h-l	6.1±0.8i-n	7.3±0.6h-l
4%	31±2.6b-f	33±2.3h-l	0.4±0.02m-p	0.6±0.04mno	4.9±0.5l-s	4.8±0.9m-s
Pastili						
0%	20±1.8j-n	27±1.4l-q	0.8±0.06f-j	1±0.08h-l	6.6±0.6k-l	11.9±0.6ef
2%	27±2.3f-j	24±0.8m-s	0.7±0.04i-l	0.8±0.07mno	5.6±0.2k-p	4.1±0.6o-v
4%	26±1.6f-j	22±2.6o-t	0.4±0.03o-s	0.7±0.04mno	4.3±0.7n-t	3.7±0.7o-w
Lal						
0%	15±0.8nop	31±0.8i-m	0.7±0.02f-j	0.9±0.03 h-l	5.9±0.5i-o	6.6±0.4j-n
2%	19±3.4lmn	24±2.6m-r	0.5±0.05mno	0.6±0.05mno	4.2±0.5n-t	3.1±0.6q-x
4%	23±5.4j-m	19±0.8r-v	0.4±0.01n-q	0.6±0.05mno	4.1±0.3o-t	2.6±0.2s-x
Chugao						
0%	20±2j-n	36±2f-j	0.8±0.1e-j	1±0.06f-i	7.5±0.2f-k	5.9±0.9k-o
2%	26±2.4f-k	31±2.3i-m	0.6±0.02jkl	0.8±0.03j-m	5.9±0.7i-o	4.9±0.3i-s

4%	30±1.1c-h	29±2.3j-n	0.3±0.02o-t	0.9±0.04k-n	5.5±0.4l-q	4.3±0.2n-u
Rish Baba						
0%	18±2.3lmn	31±2i-m	0.7±0.01h-k	0.9±0.05h-l	5.3±0.5l-r	5±0.7i-r
2%	26±2.4f-k	21±1.7p-t	0.7±0.04h-k	0.9±0.07h-l	4.5±0.1m-t	2.7±0.2s-x
4%	23±2h-m	16±0.8tuv	0.4±0.02n-r	0.7±0.08lmn	4.4±0.5n-t	2.2±0.1u-x
Hybrid H6						
0%	20±1/5j-n	49±2.8b	0.8±0.03e-j	1±0.07f-j	6.9±0.7g-l	7.6±0.8h-k
2%	22±2.4i-n	31±1.3i-m	0.7±0.02g-k	0.9±0.06f-j	5±0.6l-s	5.1±0.8i-r
4%	27±2.4e-i	24±2.6m-r	0.6±0.05klm	0.9±0.05h-l	4.1±0.3o-t	3.9±0.5o-w
Taefi						
0%	22±1.2i-n	28±1j-o	0.3±0.03o-t	0.8±0.05i-m	5.3±0.9l-r	5.3±0.7i-q
2%	19±0.8k-n	22±1.4n-t	0.29±0.01stu	0.7±0.02lmn	4.3±0.6n-t	2.4±0.5t-x
4%	20±1.4j-n	15±1tuv	0.20±0.02u	0.5±0.03nop	3.7±0.5p-u	1.7±0.3vwx
Asgari						
0%	18±1.5mno	20±1q-v	0.3±0.01r-u	0.5±0.05nop	3.4±0.3r-u	2±0.2u-x
2%	12±1.5op	16±0.9tuv	0.25±0.01tu	0.4±0.03pq	2.8±0.5tu	1.5±0.1w-x
4%	10±1.2p	13±1v	0.2±0.01u	0.3±0.02q	1.9±0.2u	1±0.1x
Garashireh						
0%	32±1.4a-f	63±2.9a	2.5±0.04a	1.9±0.06a	18.9±0.5a	23.5±1.5a
2%	35±1.7a-d	46±2.1bcd	1.6±0.06b	1.6±0.04bc	10±0.9bcd	17.7±1.2b
4%	38±2a	39±1.7d-h	1±0.03cd	1.1±0.05e-h	9±0.4c-f	14.2±0.7cd

Means followed by different letters in each column indicate significant difference at p ≤ 0.05 (Dunkan test).

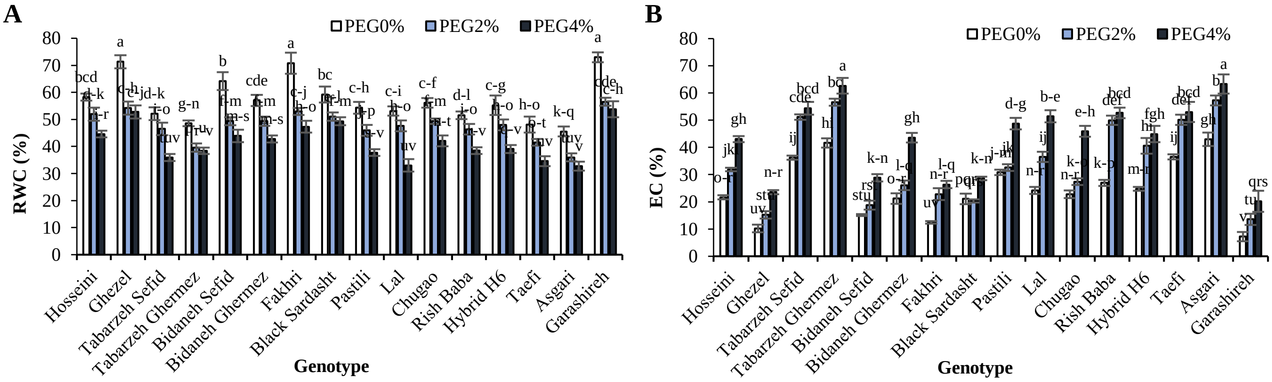

Fig. 1. LRWC (A) and EC (B) in leaves of 16 grape genotypes under different drought treatments (PEG 0%, 2% and 4%). Bars are mean ± standard error.

Drought stress significantly reduces the LRWC and EC in the leaves of all genotypes (Fig. 1). Under PEG4% stress, the Garashireh genotype exhibited the highest RWC percentage at 53.76%, while the Asgari genotype had the lowest LRWC percentage at 32.77%. There was a statistically significant difference in EC percentage among genotypes and treatments ($P \leq 0.05$). The lowest EC percentage under PEG4% stress was observed in the Garashireh (20.20%), Fakhri (26.32%), and Ghezel (23.54%) genotypes, while the highest percentage was recorded for the Asgari (63.29%) and Tabarzeh Ghermez (62.64%) genotypes.

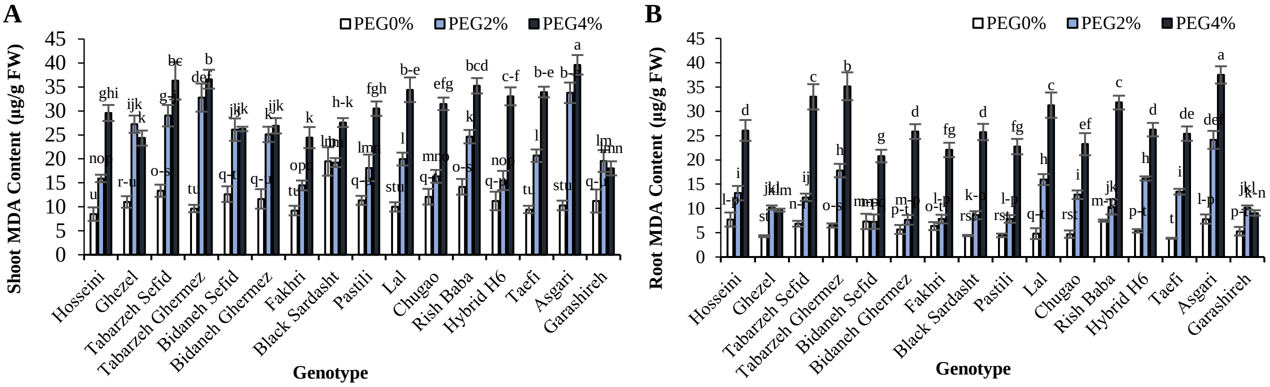

Fig. 2. MDA content (µg.g^{-1} FW) in leaves (A) and roots (B) of 16 grape genotypes under different drought treatments (PEG 0%, 2% and 4%). Bars are mean ± standard error.

MDA, H_2O_2 and protein contents

The effect of drought stress on malondialdehyde (MDA) content of grape genotypes is shown in Figure 2. MDA content increased significantly ($P \leq 0.05$) in the roots and leaves of all genotypes with increasing drought stress. However, this increase in the leaves of Asgari (37.49µg.g^{-1} FW), Tabarze Ghermez (35.16µg.g^{-1} FW), Tabarze Sefid (33µg.g^{-1} FW), and Lal (31.25µg.g^{-1} FW) was relatively more than other genotypes. The roots and leaves of Grashireh, Ghezel, Fakhri and Pastili showed lower MDA content compared to other genotypes (Figure 2). Variance analysis showed that the difference in MDA content in root and leaf between genotypes, treatments and genotype × treatment interaction was statistically significant ($P \leq 0.05$).

The contents of H_2O_2 were investigated in 16 grape genotypes (Fig. 3). Under well-watered conditions, there were no significant changes in the contents of H_2O_2 in the leaves and root. However, as the drought stress prolonged, the contents of H_2O_2 gradually increased in the grapevine leaves. A notable observation in the Asgari genotype is the substantial increase in H_2O_2 levels in the roots (5.08µg.g^{-1} FW) under PEG4% stress conditions.

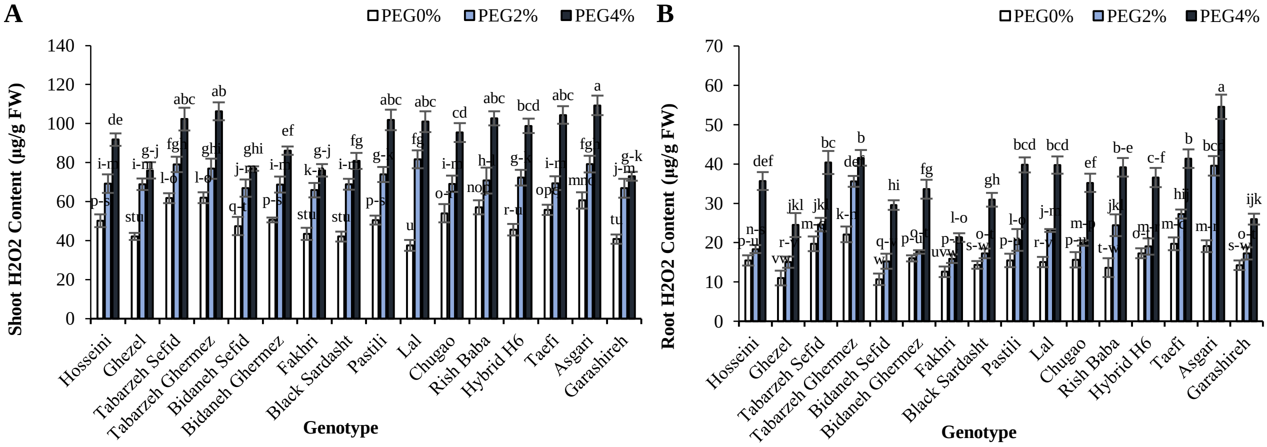

Fig. 3. H_2O_2 content (µg.g^{-1} FW) in leaves (A) and roots (B) of 16 grape genotypes under different drought treatments (PEG 0%, 2% and 4%). Bars are mean ± standard error.

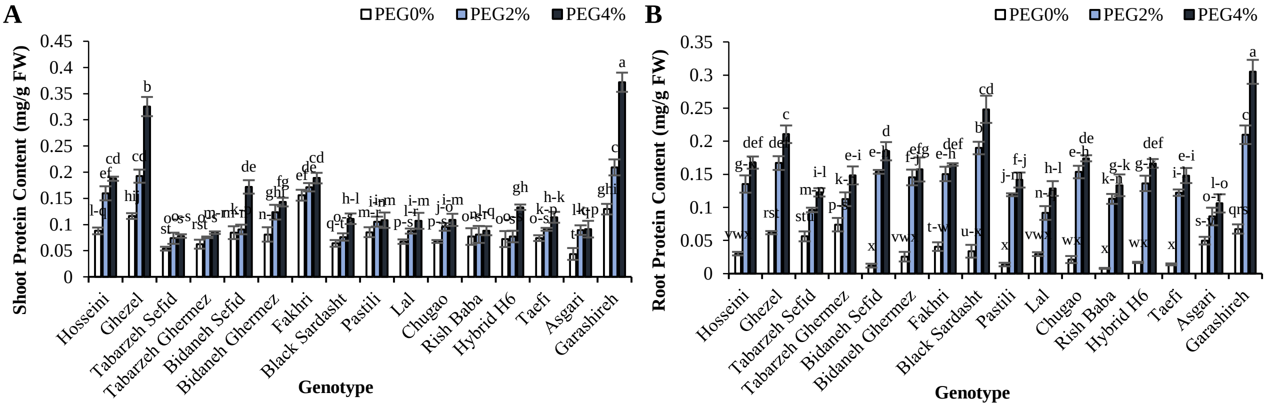

Fig. 4. Protein content (μg.g^{-1} FW) in leaves (A) and roots (B) of 16 grape genotypes under different drought treatments (PEG 0%, 2% and 4%). Bars are mean ± standard error.

Specifically, the amount of H_2O_2 surged by 186% when compared to the control group subjected to PEG (0%) stress. This significant rise in hydrogen peroxide content indicates an elevated level of oxidative stress in the roots of the Asgari genotype under severe osmotic stress induced by PEG (4%) (Fig. 3).

Based on the comparison of average data, a significant difference was found between the 16 genotypes in terms of total protein levels in both the shoot and roots. Additionally, it was observed that as the percentage of PEG increased, the total protein content also increased. The highest amount of protein was observed in the aerial parts of the Garashireh (0.37μg.g^{-1} FW) and Ghezel (0.32μg.g^{-1} FW) genotypes, as well as in the root parts of the Garashireh (0.30μg.g^{-1} FW), SiahSardasht(0.24μg.g^{-1} FW), Ghezel (0.21μg.g^{-1} FW), and Bidaneh Sefid (0.18μg.g^{-1} FW) genotypes under PEG4% stress conditions. Conversely, there was no significant difference in the protein content among the remaining genotypes under the same stress conditions (Fig. 4).

Antioxidant enzyme activities

The experiment results indicate that drought stress leads to an elevation in antioxidant enzyme levels, particularly noticeable at higher PEG concentrations. Across all genotypes, the activity of the APX enzyme was generally higher in the shoots compared to the roots. While PEG2% stress did not show significant differences, a pronounced disparity was observed at PEG4% stress in the leaves of genotypes such as Garashireh (1.93 Unit.mg protein $^{-1}$), Fakhri (0.82 Unit.mg protein $^{-1}$), Bidaneh Sefid (0.54 Unit.mg protein $^{-1}$), Ghezel (1.26 Unit.mg protein $^{-1}$), Hosseini (0.51 Unit.mg protein $^{-1}$), and Bidaneh Ghermez (0.42 Unit.mg protein $^{-1}$), compared to the PEG-free control plants. Notably, Asgari (0.19 Unit.mg protein $^{-1}$), Tabreze Ghermez (0.22 Unit.mg protein $^{-1}$), Rish Baba (0.36 Unit.mg protein $^{-1}$), and Lal (0.23 Unit.mg protein $^{-1}$) genotypes exhibited the lowest APX enzyme activity levels (Fig. 5A and B). A significant divergence in CAT enzyme level increments was noted between the shoots and roots of the genotypes, with the roots of Garashireh (9.19 Unit.mg protein $^{-1}$), Fakhri (10.19 Unit.mg protein $^{-1}$), SiahSardasht (3.44 Unit.mg protein $^{-1}$), Bidaneh Sefid (4.49 Unit.mg protein $^{-1}$), Bidaneh Ghermez (3.57 Unit.mg protein $^{-1}$), Ghezel (5 Unit.mg protein $^{-1}$), and Hosseini (2.40 Unit.mg protein $^{-1}$) genotypes displaying higher CAT activity levels. CAT enzyme levels notably increased with escalating drought stress in the leaves of Garashireh (7.35 Unit.mg protein $^{-1}$), Hosseini (4.38 Unit.mg protein $^{-1}$), Ghezel (5.02 Unit.mg protein $^{-1}$), Fakhri (7.32 Unit.mg protein $^{-1}$), Bidaneh Sefid (7.08), Bidaneh Ghermez (6.28 Unit.mg protein $^{-1}$), Siah Sardasht (4.17 Unit.mg protein $^{-1}$), and Hybrid (H6) (3.34 Unit.mg protein $^{-1}$) raisin plants. However, no significant differences were observed in other genotypes as drought stress levels increased (Fig. 5 C and D). Furthermore, the GPX enzyme activity showed a significantly higher level in shoots compared to roots. The most substantial increase under PEG4% stress was observed in the leaves of the Garashireh genotype (1.06 Unit.mg protein $^{-1}$) and in the roots of the Fakhri (0.43 Unit.mg protein $^{-1}$) genotype (Fig. 5 E and F).

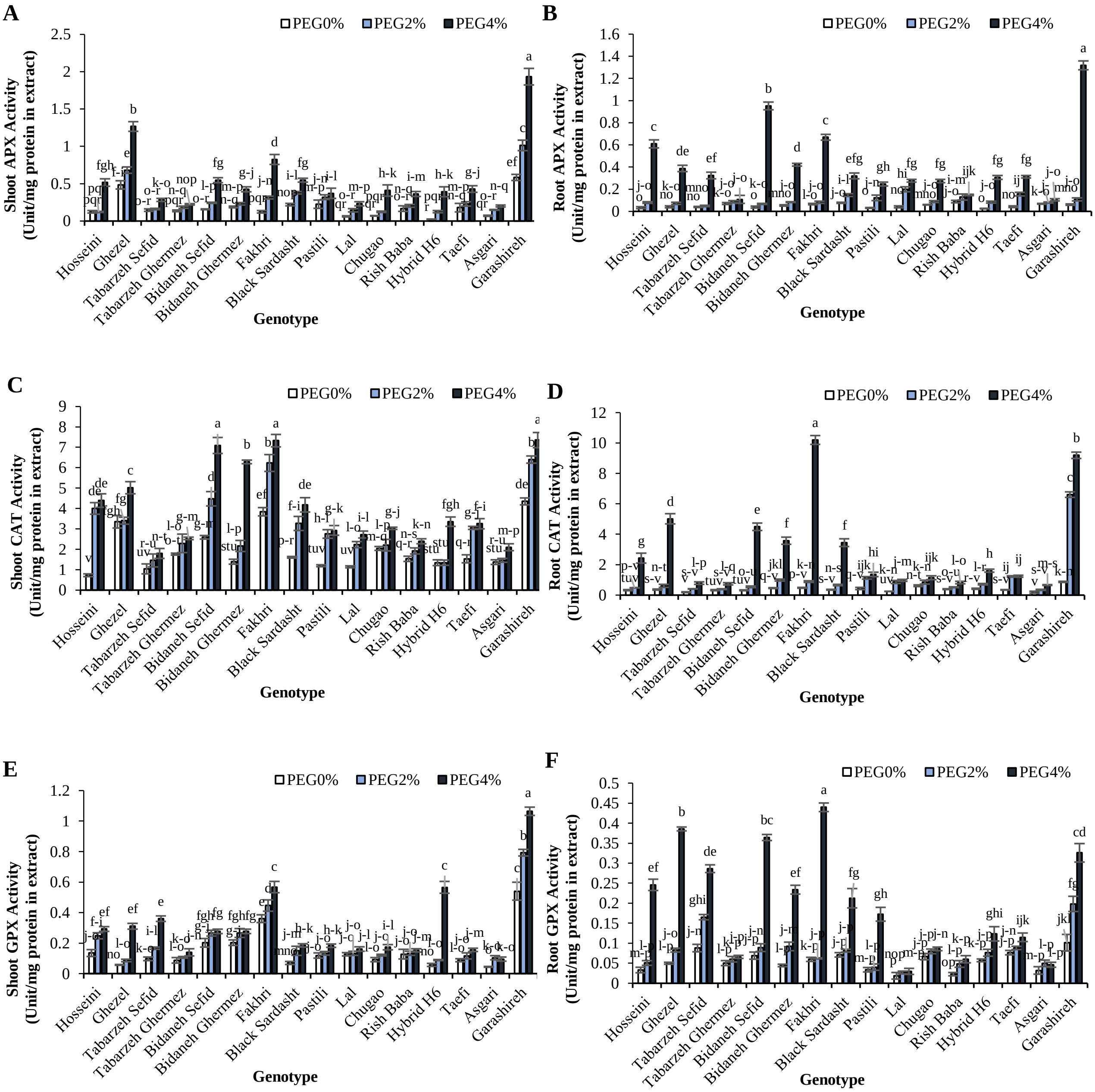

Fig. 5. Ascorbate peroxidase (APX) activity in leaf (A) and root (B), Catalase (CAT) activity in leaf (C) and root (D) and Guaiacol peroxidase (GPX) activity in leaf (E) and root (F) of 16 grape genotypes under different drought treatments (PEG 0%, 2% and 4%). Bars are mean ± standard error.

DISCUSSION

Drought stands out as the predominant abiotic stress factor that significantly constrains the growth and productivity of crop plants. Consequently, focusing on breeding programs aimed at developing genotypes that exhibit tolerance to drought stress plays a crucial role in mitigating the substantial yield losses associated with this stressor (Levitt, 1980; Karimi et al., 2012). Through targeted breeding efforts, the cultivation of drought-tolerant crop varieties can help safeguard agricultural productivity and food security in the face of drought-induced challenges. The study examined 16 grape cultivars under in vitro conditions subjected to PEG-induced drought stress. Efforts have been directed towards identifying stress-tolerant plants under in vitro conditions across a diverse array of plant species, encompassing cereals, vegetables, fruits, and other economically significant plant varieties (Rai et al., 2011; Bigdeloo et al., 2018). By evaluating grape cultivars in this controlled environment, the research aims to elucidate stress responses and potentially identify drought-tolerant genotypes that could be valuable for future cultivation practices. The study observed significant reductions in fresh and dry weight stem and root length as the PEG concentration in the culture medium increased. Oukabli et al. (2008) also noted restricted growth under drought stress conditions. PEG serves to lower water potential, mimicking drought stress without inducing toxic effects or plant uptake (Rumbaugh & Johnson, 1981; Kent & Lauchli, 1985; Bigdeloo et al., 2018). LRWC is regarded as a crucial indicator for evaluating plant tissue water status (Kramer, 1983). Numerous studies have demonstrated a decline in LRWC in response to drought stress (Augé et al., 2003; Liu et al., 2008; Sarvari et al., 2017). In the current investigation, LRWC decreased with escalating PEG concentrations in the medium. Notably, the Garashireh genotype exhibited a smaller decline in LRWC with increasing stress levels compared to other genotypes, with a decrease ranging from 26% to 20% in contrast to the control group. Conversely, the Siah Sardasht variety displayed lower LRWC levels than Garashireh under normal conditions, yet its LRWC decreased by 16% under PEG-induced 4% stress. This suggests that the Siah Sardasht genotype coped better with PEG stress in terms of LRWC compared to the Garashireh genotype. According to the research by Asadi et al. (2019), the Galati and Melai cultivars demonstrate better drought tolerance compared to the Fakhri variety. These cultivars can be cultivated in areas with limited water availability.

It has been observed that water stress reduces the relative water content in grapes. These findings are consistent with the results reported by other researchers (Khalil & Badr Eldin, 2021; Fahim et al., 2022; Zeng et al., 2022; Fayek et al., 2022; Bidabadi et al., 2023). Among others. These results indicate that reduced water availability can create challenges for plant roots in terms of water and soil uptake, forcing plants to regulate water loss through transpiration. This adjustment may lead to a decrease in LRWC in grape leaves, which may not necessarily indicate optimal conditions for grape plants (Al-Tabbal et al., 2020).

Leaf RWC serves as a crucial indicator of plant water status, closely tied to cell volume and reflecting the balance between water supply to leaves and their transpiration rate (Deka et al., 2018). It is considered a valuable metric for assessing the level of water stress experienced by leaves. RWC is an indicator of the metabolic activity within plant tissues (Yan et al., 2016) and typically exhibits a significant decrease under water stress conditions. When the soil moisture levels are inadequate and roots face water scarcity, plants may struggle to compensate for water loss through transpiration. Consequently, the RWC of leaves tends to decrease as a result of this water stress (Al-Tabbal et al., 2020). Monitoring RWC can provide insights into the water status and physiological condition of plants, especially under challenging environmental conditions like water scarcity.

Drought stress can lead to an increase in leaf electrolyte leakage, which is often indicator of cellular injury. Under drought conditions, reactive oxygen species (ROS) accumulate due to the stress on cell membranes, ultimately causing damage. This stress-induced ROS production can result in membrane lipid peroxidation, leading to increased membrane permeability and ion leakage, disrupting membrane structure and function (Hnilickova et al., 2018). The extent of this damage can be assessed by measuring electrical conductivity resulting from ion leakage. Research of Min et al. (2019) indicated that polyethylene glycol (PEG) can elevate electrolyte leakage in leaves and young grapevine seedlings. Studies of Zeng et al. (2022) demonstrated that drought stress in grape leaves increases the production of hydrogen peroxide (H_2O_2) and free radicals, leading to higher levels of malondialdehyde (a byproduct of membrane lipid peroxidation) and increased ion leakage, aligning with similar findings in other studies. Furthermore, Fahim et al. (2022) observed a significant increase in electrolyte leakage in 14 commercial grape varieties under heightened drought stress, echoing the results of previous studies. Altinci and Cangi (2019) research on six commercial wine grape varieties in vitro revealed membrane destruction, cellular damage, and inactivation of ion pumps on the cell membrane due to drought stress, resulting in elevated electrolyte leakage. These findings collectively underscore the impact of drought stress on membrane integrity and ion balance in grapevines.

In the current study, it was observed that increasing the concentration of PEG induced drought stress in grapevines led to an increase in the activity of antioxidant enzymes such as ascorbate peroxidase (APX), catalase (CAT), and guaiacol peroxidase (GPX). These findings align with previous reports on grapes (Pontin et al., 2021; Fahim et al., 2022; Shirani Bidabadi, 2023). Fahim et al. (2022) noted that under decreasing water levels, antioxidant enzymes like superoxide dismutase (SOD) and CAT, as well as enzymes from the glutathione-ascorbate cycle such as APX and POD, increase to counteract the effects of reactive oxygen species (ROS).The activation of enzymatic defense mechanisms, including the increase in antioxidant enzyme activity, in response to drought stress is crucial for detoxifying ROS and combating the rise in free radicals within plant cells under stressful conditions, often linked to H_2O_2 production (Fahim et al., 2022). Inducing enzyme activity in antioxidants represents a general adaptation strategy employed by plants to mitigate oxidative stress-induced damage (Noctor & Foyer, 2016). Plant cells are equipped with a free radical scavenging system comprising both antioxidant enzymes and non-enzymatic antioxidants for protection against oxidative damage (Keivanfar et al., 2019). In research of Zeng et al. (2022), it was found that drought stress significantly increased SOD activity while decreasing CAT activity compared to the control group of the examined grapes. Another study reported by Shirani Bidabadi et al. (2023) indicated a decrease in CAT enzyme activity in grape leaves under drought stress, which was attributed to a reduction in iron concentration, a cofactor for the CAT enzyme. Conversely, an increase in CAT activity was observed in Cabernet Sauvignon grapes following severe drought stress for 16 days, as reported by Shirani Bidabadi (2023).

CONCLUSION

The main goal of measuring the traits was to evaluate diversity and identify superior grape cultivars for breeding programs. Limited water access compromises plants' water absorption, leading to decreased cell water potential, which can produce damaging free radicals. These radicals can harm cell membranes, degrade photosynthetic pigments, and reduce photosynthesis efficiency, ultimately stunting growth and productivity. Thus, adequate water availability is critical for healthy plant growth. The «Garashireh genotype» has been identified as drought-tolerant among tested grapevines. Its notable drought tolerance mechanisms include:

- Reduced Malondialdehyde (MDA) Content: Lower MDA indicates less oxidative stress and better cellular integrity.
- Enhanced Antioxidant Enzymes: Increased activity of these enzymes helps scavenge reactive oxygen species (ROS), protecting cells from damage.
- Lower Ion Leakage: This suggests better membrane stability and reduced cell damage under drought stress.
- Decreased Hydrogen Peroxide Levels: This reduction indicates effective ROS scavenging, mitigating oxidative stress.
- Tolerance to PEG Stress: The genotype can withstand various PEG doses, showing adaptability to osmotic stress.
- Maintenance of Vegetative Apparatus: The «Garashireh genotype» sustains growth despite drought, demonstrating resilience.

These mechanisms underscore the «Garashireh genotype» adaptability to drought, making it a strong candidate for regions facing water scarcity.

Conflict of interest

The authors have no conflict of interest to report.

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