

Biochemical, physiological, and molecular responses of diverse olive cultivars to different irrigation regimes

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Abstract

Olive is an essential industrial crop in the Mediterranean region with valuable economic and agricultural concerns. Despite its drought resistance, its productivity is restricted by extreme drought stress. Olive cultivars display considerable variation in response mechanisms to drought stress. Accordingly, the impact of mild and extreme water deprivation over two seasons compared to full irrigation requirements on growth and physiological characteristics of three diverse olive cultivars. Three olive cultivars, ‘Manzanillo’, ‘Eggizi-Shami’, and ‘Tofaahi’, were evaluated under three irrigation regimes 100% ET_c, 75% ET_c, and 50% ET_c. Characteristics of shoot and root, as well as physio-chemical constituents, were determined. Besides, the gene expression of dehydration-responsive element binding (DREB), dehydrin (DHN), and catalase (CAT) genes in olive cultivars were explored under different irrigation regimes. The results indicated a substantial impact of irrigation level on all studied parameters. The mild and extreme drought stress treatments caused a gradual reduction in nitrogen, phosphorus, and potassium content, relative water content, root and shoot length, root and leaf numbers, branch count, and leaf area across both seasons. Conversely, proline content was considerably increased under drought treatments compared to well-watered conditions. Similarly, the assessed cultivars exhibited significant variation in all studied parameters, with ‘Eggizi-Shami’ demonstrating superiority. Under mild and extreme drought stress conditions, the cultivar ‘Eggizi-Shami’ displayed the highest proline content and most growth characteristics. Besides, the real-time quantitative PCR (RT-qPCR) analysis displayed significant alterations in gene expression of the tested three genes related to drought response (DHN, DREB, and CAT). The RT-qPCR analysis revealed that under drought stress conditions (75% and 50% ET_c), ‘Eggizi-Shami’ exhibited higher expression compared to the other two cultivars (‘Tofaahi’ and ‘Manzanillo’). Combining the results of morphological and physiological parameters with gene expression analysis of drought-related genes can offer highly validated information about drought-tolerant olive cultivars. This integrated approach serves as an innovative methodology to identify and confirm genes involved in abiotic stress.

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Keywords: catalase; chemical constituents; *DHN*; *DREB*; drought tolerance; olive (*Olea europaea* L.); qPCR; vegetative characteristics

Introduction

Olive (*Olea europaea* L.) is an evergreen crop cultivated in the Mediterranean basin. It is a vital industrial crop with substantial economic and agricultural concerns in the Mediterranean region. Its total cultivated area in 2021 was about 10.34 million hectares produced 23.05 million tonnes (FAOSTAT, 2023). The cultivated area in Egypt in 2021 was 99102 hectares producing 976063 tonnes (FAOSTAT, 2023). Drought stress has become a recent problem in arid and semi-arid environments, particularly under current climate change. The threat of water shortage to world food security is one of the most severe, especially in arid regions worldwide (Al Rashed *et al.*, 2023). This is translated to be a restricting factor for the fruit industry, principally with the growing global population and intensified use of freshwater which makes competition with agricultural production (Agüero Alcaras *et al.*, 2021). Agricultural sectors, especially horticulture, are experiencing increased water demand (Selote and Khanna-Chopra, 2004). Under these conditions, the economic productivity of applied water in agricultural production should be increased (Mahmud *et al.* 2023). Accordingly, olive production in Mediterranean regions that suffer from water scarcity is an ideal choice for cultivation in arid areas, especially newly reclaimed land, due to their high stress tolerance.

Olive grows in a dry-subtropical climate, although, its productivity is affected by several abiotic factors (Cherbiy-Hoffmann *et al.*, 2012). In Mediterranean climates, high temperatures and water scarcity are common meteorological features (Boussadia *et al.*, 2008). Accordingly, olive cultivation is limited mainly by water deficit and salinity stresses in arid and semi-arid regions which cause significant yield losses. Olive has a greater tolerance to drought than many other fruit tree species, but its drought tolerance varies considerably among genotypes (Bosabalidis and Kofidis, 2002). However, its tolerance to different environmental stresses is still limited compared to other cultivated species. Water deficit by applying less than full requirements of crop evapotranspiration (ETc) of highly drought-tolerant genotypes during certain stages or throughout plant growth could allow lowering applied water without considerable reductions in yields (Agüero Alcaras *et al.*, 2021). Accordingly, it is valuable to explore the genotypic variations to demonstrate their tolerance mechanisms to different stresses (Gracia *et al.*, 2012; Ponce-Molina *et al.*, 2012; Mansour *et al.*, 2018; Abaza *et al.*, 2020; Rehman *et al.*, 2023; Ghazy *et al.* 2023).

Drought stress adjusts physio-chemical constituents at any period of plant growth (Khan *et al.*, 2022). It induces chlorophyll degradation, unbalances mineral nutrients, reduces cell turgidity, declines photosynthesis activity, and causes substantial oxidative damage (Desoky *et al.*, 2023). The adverse impacts of drought stress on physiological processes inactivate damage to membrane lipids and impair nucleic acids as well as metabolic enzymes, which ultimately leads to a considerable reduction in plant development (Farooq *et al.*, 2009; Mansoor *et al.* 2023). Hence, water scarcity limits plant growth and productivity at varying levels, depending on the severity of stress (Mansour *et al.*, 2023). Under drought stress, olives absorb water from the soil to maintain protoplasm hydration through osmoregulation. In addition to preserving turgidity, this mechanism contributes to the root's ability to contact the soil and maintain its biological function (Boudiar *et al.*, 2022). Through osmoregulation, olive leaves and roots establish gradient water potentials and the plants can maintain a regular net photosynthesis rate (Vitagliano and Sebastiani, 2002). The response of olive plants to environmental stresses such as drought through osmotic adjustment needs to be explored (Brito *et al.*, 2019). Variations in biochemical and physiological responses to water deficit are related to restrictive leaf gas exchange and stomatal regulation under water stress, antioxidant defense efficiency, and association with low water potential (Guerfel *et al.*, 2009; ElSayed *et al.*, 2022; Mardinata *et al.*, 2021). Due to their mode of expression in

response to water scarcity, DHNs were identified as dehydration-induced proteins (Riyazuddin *et al.*, 2022). In regulating DHN expression under drought stress, DREB transcription factors also regulate DHNs (Agarwal *et al.*, 2006). However, drought stress exacerbates membrane integrity by increasing catalase and lignin biosynthesis enzyme expressions (Tiwari *et al.*, 2019). Most subcellular compartments contain these proteins, including the nucleus, cytoplasm, plastid, and mitochondrion (Hanin *et al.*, 2011). Under environmental stresses, including water scarcity the adaptation mechanisms are stimulated. Studying candidate genes and exploring their alteration in response to environmental stress facilitate recognition of plant adaptation ability (Karan *et al.*, 2012; Abd El-Moneim, 2020; Abd El-Moneim *et al.*, 2020; Abd El-Moneim *et al.*, 2021). Subsequently, this could assist in unravelling genetic variability for boosting plant growth and adaptation to environmental stresses (Mirouze and Paszkowski, 2011). Hence in the present study, the morphological and physiological response of diverse olive cultivars were assessed under different water regimes, besides their gene expression of *DHN*, *DREB*, and *CATALASE* associated with drought stress conditions were explored.

Materials and Methods

Experimental site and irrigation treatments

The experiment was performed from May to July of 2020 and 2021 at Arish University, Egypt (30° 43'N, 32° 15'E). Seedling one-year-old of three olive cultivars ('Manzanillo', 'Eggizi-Shami', and 'Tofaahi') were used in this study. Plastic containers with a 20 cm diameter and a depth of 30 cm were employed. The containers were filled with a constant weight (6 kg), and the plants were pruned to single shoots. Each replication consisted of three transplants in a factorial experiment with a completely randomized design (Klute, 1986). The cultivars were assessed under three water irrigation levels based on crop evapotranspiration (ET_c) replacement following the crop coefficient approach (Allan *et al.*, 1998). Daily reference evapotranspiration was determined from climate data using the FAO-56 Penman-Monteith equation (Allan *et al.*, 1998). Daily meteorological data were collected from weather stations (Table 1). The amount of full ET_c (100%ET_c) diminished by 25% and 50% to applying the mild (75%ET_c) and extreme (50%ET_c) water deficit conditions. The water used was fresh with an electric conductivity (EC_w) of 1.84 dS m⁻¹.

Table 1. Meteorological data of the experimental site

Month	Min Temp (°C)	Max Temp (°C)	Temp Mean (°C)	Humidity (%)	Wind speed (km/day)	Sunshine (H)	Radiation (MJ/m ² /day)
May	13.7	30.2	22.0	68.0	204.0	9.8	24.4
June	15.2	30.6	22.9	72.0	200.0	11.9	27.8
July	16.0	31.0	23.5	74.0	191.0	11.4	26.8

Soil and water analysis

Soil samples were taken from the containers at 5 cm in diameter and 5 cm in height. Soil water content was recorded at the end of every month subtract after irrigation from before irrigation. Soil bulk density was determined according to (Blake and Hartge, 1986). Soluble Na⁺ and K⁺ were determined in the soil extract for 1: 5 (soil: water) soil suspension photometrical, while the soluble Ca⁺⁺ and Mg⁺⁺ were determined using EDTA according to Richards (Richards, 1954) and adjusted to the soil saturation extract (Sonmez *et al.*, 2008). The sodium adsorption ratio of the water was determined according to Richards (Richards, 1954). The volumetric soil water content was recorded by the gravimetric method after and before the applied irrigation water (Allan *et al.*, 1998).

Phytochemical constituents of leaves

A leaf sample was taken from the sixth and seventh leaves of the shoot, then washed several times with tap water, and dried to constant weight at 70 °C. According to (Piper, 1950), the sample was digested in a mixture of sulphuric and perchloric acids. As a percentage of dry weight, the following measurements were determined. The total nitrogen percentage according to (Novamsky *et al.*, 1974). The phosphorus content was recorded using a Flame photometer according to (Temminghoff and Houba, 2004) and (Brown and Lilleland, 1946). Calorimetrically, free proline concentrations were determined using ninhydrin reagent, according to (Bates *et al.*, 1973).

Measurements of vegetative characteristics

According to (Karimi *et al.*, 2018) shoot length (cm) was estimated from the soil surface to the seedling upper. Besides, the root length was determined twice throughout the trial, at the start and the end of the water deficit period. Number of branches and leaves were recorded during the experiment. The leaf area (cm²) of the basal 6th and 7th leaves (15 leaves) was estimated at the termination of the water deficit period. According to (Sala *et al.*, 2015) relative water content was recorded at the termination of the water deficit period.

RNA extraction and gene expression analysis

RNA was extracted from the leaves of the three cultivars under control and experimental conditions following the method of Kim *et al.* (2006). Three biological replications from each sample were used. The extraction was performed from fresh leaves samples using Triazole[®] reagent and purified utilizing phenol-chloroform extraction. The quality of the RNA was checked through agarose gel electrophoresis using a 2 µg RNA loading buffer. The concentrations of total RNA were measured using a Quantus™ Fluorometer (Promega, USA). The purified total RNA (1.5 and 2 µg) was employed for first-strand cDNA synthesis performed using M-MLV[®] reverse transcriptase following the kit manual; after completion, cDNA was reserved at -20 °C until quantification.

The primers performed for the RT-qPCR analysis are listed in Table 2. Reactions were applied on three cDNA samples and three replications. The qPCR cycle program was conducted using an initial denaturation temperature of 92 °C for 2 min and repeated 40 cycles at 92 °C for 5 sec. The annealing phase was applied at 56 °C for 15 sec and the extension phase at 72 °C for 26 sec. *GAPDH* was utilized as reference genes for sample normalization (Nonis *et al.*, 2012; Ray and Johnson, 2014).

Table 2. Oligonucleotide primers used in RT-PCR quantification

Gene type	Gene ID (accession)	Direction	Sequence (5'→3')
House Keeping	Polyubiquitin UBQ (AF429430)	F	GGTGGCCTCTAAATGTTCTTCTACTG
		R	ACAGACTTCATTAGAAAAGACAATCA
Water Drought	Dehydration responsive TF DREB (EF635424)	F	ACATGTTCTCCGCTCAGCTT
		R	GTGCCTCGTCTCCTTGAAAA
	Dehydrin DHN (KR349290)	F	GGTTTGAAGGGGAAGGTTTC
		R	CTTCCTCAGCCTTCTTGTGG
ROS scavenging activity	Catalase CAT (JQ429793)	F	GGATCCAGCCAGACAAGAGA
		R	TTGGCCTTACATTGAGACGA

Statistical analysis

The equation on the graph per microgram from mRNA-converted cDNA was employed to estimate the number of cDNA molecules (Livak and Schmittgen, 2001). As a measure of relative quantification, Ct values were used to estimate gene expression. Using the fold change method, target genes were compared to the control. The data of two years were analysed using R statistical software version 4.1.2. The differences among genotypes and applied treatments were distinguished by Tukey's HSD test ($P < 0.05$).

Results*Soil water content*

The volumetric soil water content (θ %) in both seasons was significantly reduced by increasing drought stress levels (Table 3). It is noticed that the volumetric soil water content gradually increased with time from May to July. Generally, the lowest soil water content was recorded under 50% ETc throughout May while the highest values were assigned for 100% ETc throughout July.

Table 3. Crop evapotranspiration and volumetric soil water content (θ %) in both seasons

Irrigation level	May		June		July	
	2020	2021	2020	2021	2020	2021
Full irrigation (100% ETc)	5.70 a	6.13 a	6.55 a	6.98 a	6.56 a	7.24 a
Moderate drought (75% ETc)	3.84 b	4.16 b	4.90 b	5.13 b	5.18 b	5.56 b
Extreme drought (50% ETc)	2.27 c	2.40 c	3.65 c	3.72 c	3.83 c	4.05 c

Means followed by different letters under the same factor differ significantly based on Tukey's HSD test ($P \leq 0.05$)

Physio-chemical constituents

The analysis of variance for physio-chemical constituents indicated that the effects of irrigation level, assessed cultivars and their interaction were significant (Table 4). There was a substantial difference in the proline content of the three cultivars as well as under irrigation treatments. The highest proline content was assigned under extreme drought stress (50% ETc) compared to moderate drought (75% ETc) and full irrigation (100% ETc) conditions (Table 4). Moreover, the assessed cultivars possessed significant variation in proline content with the superiority of 'Eggizi-Shami'. Moreover, the significant interaction displayed that the cultivars 'Eggizi-Shami' displayed the highest values of proline content under moderate and extreme drought stress conditions compared to the other cultivars (Figure 1A). The highest phosphorus, nitrogen, and potassium contents were recorded under well-watered conditions (Table 4). The cultivars 'Eggizi-Shami' and 'Manzanillo' recorded the uppermost values of nitrogen, phosphorus content, and potassium. The cultivars 'Eggizi-Shami' displayed the highest values of nitrogen, phosphorus, and potassium contents under mild and extreme drought stress conditions (Figures 1B-D).

Table 4. Impact of different irrigation levels on physio-chemical constituents of the assessed olive cultivars

Studied factor	Proline content (mg/g FW)		Nitrogen content (%)		Phosphorus content (%)		Potassium content (%)		
	2020	2021	2020	2021	2020	2021	2020	2021	
Irrigation level									
Full irrigation (100% ETc)	19.89 c	20.11 b	1.980 a	2.043 a	0.203 a	0.197 a	1.057 a	1.113 a	
Moderate drought (75% ETc)	20.56 b	20.89 b	1.867 b	1.943 b	0.190 b	0.180 b	0.977 b	1.067 a	
Extreme drought (50% ETc)	21.96 a	22.22 a	1.800 b	1.867 b	0.183 b	0.182 b	0.830 b	0.901 b	
Cultivar									
Manzanillo	22.44 a	22.55 a	1.833 b	1.900 b	0.190 b	0.183 c	0.947 b	1.043 b	
Eggizi-Shami	23.00 a	23.22 a	2.033 a	2.110 a	0.200 a	0.190 a	1.057 a	1.167 a	
Tofaahi	16.79 b	17.44 b	1.780 b	1.843 b	0.187 b	0.187 b	0.860 b	0.870 c	
ANOVA	df	P value							
Applied Irrigation (AI)	2	<0.001	0.040	0.013	<0.001	<0.001	0.007	0.006	<0.001
Cultivar (C)	2	<0.001	<0.001	0.038	<0.001	<0.001	0.041	0.017	<0.001
AI×C	4	<0.001	0.003	0.032	<0.001	<0.001	0.0027	0.0213	0.028

Different letters denote substantial differences according to Tukey's HSD test ($P < 0.05$)

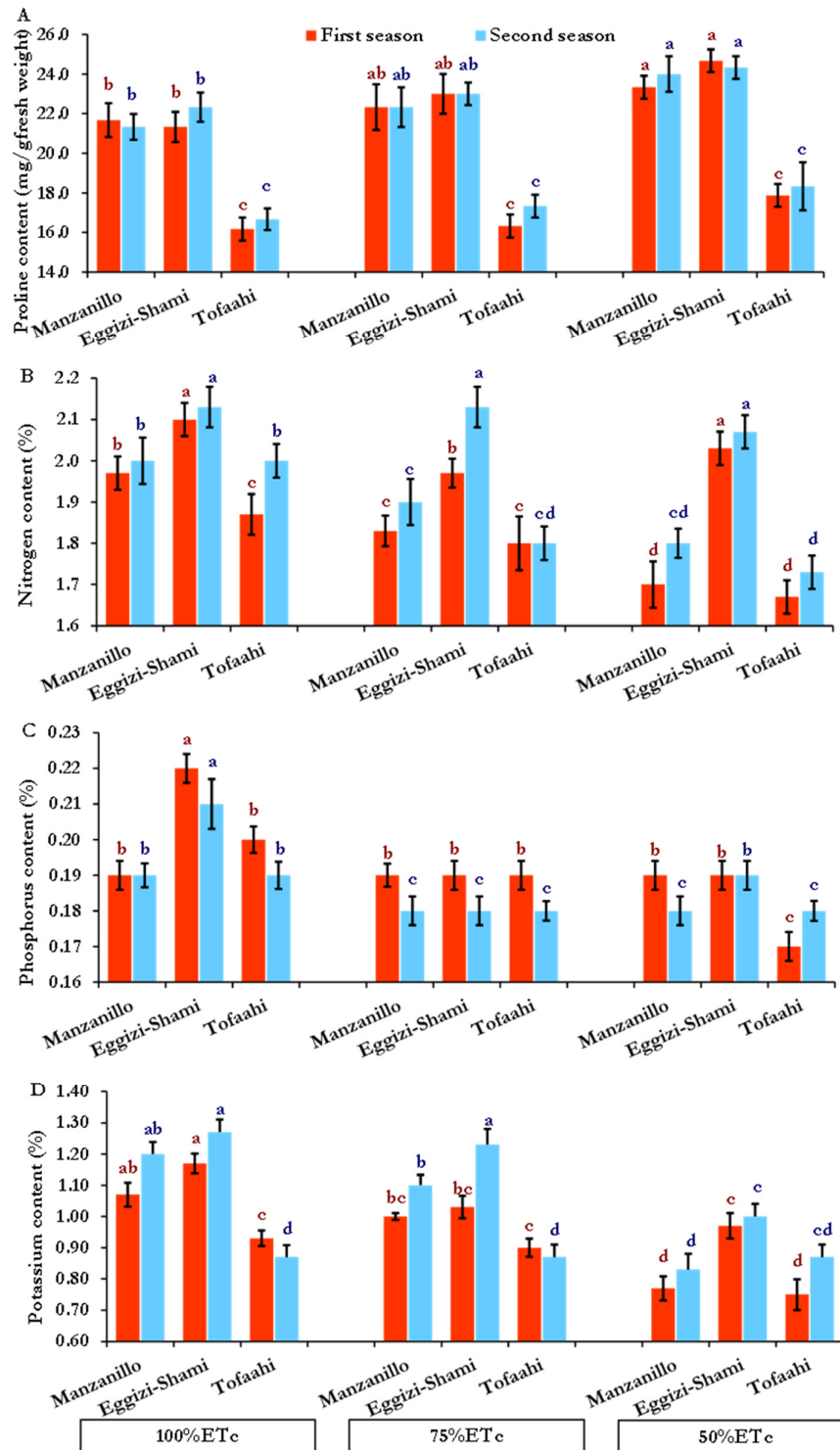


Figure 1. Leaf physiochemical characteristics of the evaluated cultivars under different irrigation levels in both seasons

The bars above the columns represent the standard deviation (SD), while different letters on the columns within each irrigation regime indicate significant differences as determined by Tukey's HSD test ($P \leq 0.05$).

Growth characteristics

The analysis of variance revealed that the effects of irrigation level, assessed cultivars and their interaction were significant for all evaluated growth characteristics (Table 5). The growth characteristics displayed significant variation among irrigation levels. Drought stress treatments, both mild and extreme, caused a gradual decreased all studied growth characteristics compared to well-watered conditions in both seasons. Likewise, the evaluated cultivars displayed significant variation in all studied growth characteristics. The cultivar 'Eggizi-Shami' followed by 'Tofaahi' exhibited the highest values of root length, shoot length, number of roots, number of leaves, number of branches, leaf area, and relative water content (Table 5). The significant interaction displayed that the cultivars 'Eggizi-Shami' displayed the highest values of root length, shoot length, number of roots, number of branches, number of leaves, leaf area, and relative water content under moderate and extreme drought stress conditions in comparison with the other cultivars (Figures 2 and 3).

Table 5. Impact of different irrigation levels on growth characteristics of the assessed olive cultivars

Studied factor	Root length (cm)		Shoot length (cm)		Seedling length (cm)		Relative water content (%)		
	2020	2021	2020	2021	2020	2021	2020	2021	
Irrigation level									
Full irrigation (100% ETc)	30.78 a	33.00 a	26.89 a	30.22 a	57.66 a	63.23 a	56.52 a	0.743 a	
Moderate drought (75% ETc)	28.56 b	30.56 b	21.16 b	23.22 b	49.71 b	53.78 b	27.23 b	0.543 b	
Extreme drought (50% ETc)	18.67 c	21.89 c	16.58 c	18.44 c	35.24 c	40.33 c	18.07 c	0.510 b	
Cultivar									
Manzanillo	21.22 c	23.00 b	20.29 b	22.56 b	41.51 c	45.56 c	40.70 a	0.663 a	
Eggizi-Shami	30.78 a	33.00 a	23.47 a	26.22 a	53.80 a	59.00 a	35.75 b	0.670 a	
Tofaahi	26.00 b	29.44 a	20.87 b	23.11 b	47.31 b	52.78 b	25.37 c	0.463 b	
ANOVA	df	P-value							
Applied Irrigation (AI)	2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cultivar (C)	2	<0.001	0.002	<0.001	0.004	0.001	0.006	<0.001	<0.001
AI×C	4	<0.001	<0.001	<0.001	0.036	0.008	0.012	0.006	0.006
		Number of roots		Number of branches		Leaf area (cm²)		Number of leaves	
		2020	2021	2020	2021	2020	2021	2020	2021
Irrigation level									
Full irrigation (100% ETc)		24.27 a	26.33 a	14.93 a	17.78 a	4.163 b	4.233 a	84.67 a	88.56 a
Moderate drought (75% ETc)		16.00 b	21.11 b	11.67 b	13.89 b	4.133 ab	4.110 ab	58.22 b	63.44 b
Extreme drought (50% ETc)		6.40 c	10.00 c	9.465 c	11.78 c	4.067 b	4.067 b	42.89 c	46.33 c
Cultivar									
Manzanillo		14.40 b	18.11 b	11.07 b	13.67 b	4.087 b	4.133 b	58.89 c	62.67 c
Eggizi-Shami		15.07 b	19.11 ab	13.26 a	15.56 a	4.153 a	4.147 a	66.22 a	69.78 a
Tofaahi		17.20 a	20.22 a	11.73 b	14.22 ab	4.123 a	4.130 b	60.66 b	65.89 b
ANOVA	df	P value							
Applied Irrigation (AI)	2	<0.001	0.004	0.003	<0.001	0.049	0.035	<0.001	<0.001
Cultivar (C)	2	<0.001	0.002	0.046	0.038	0.026	0.039	0.009	0.004
AI×C	4	<0.001	<0.001	<0.001	<0.001	0.043	0.042	0.004	<0.001

Means followed by different letters under the same factor differ significantly based on Tukey's HSD test ($P \leq 0.05$)

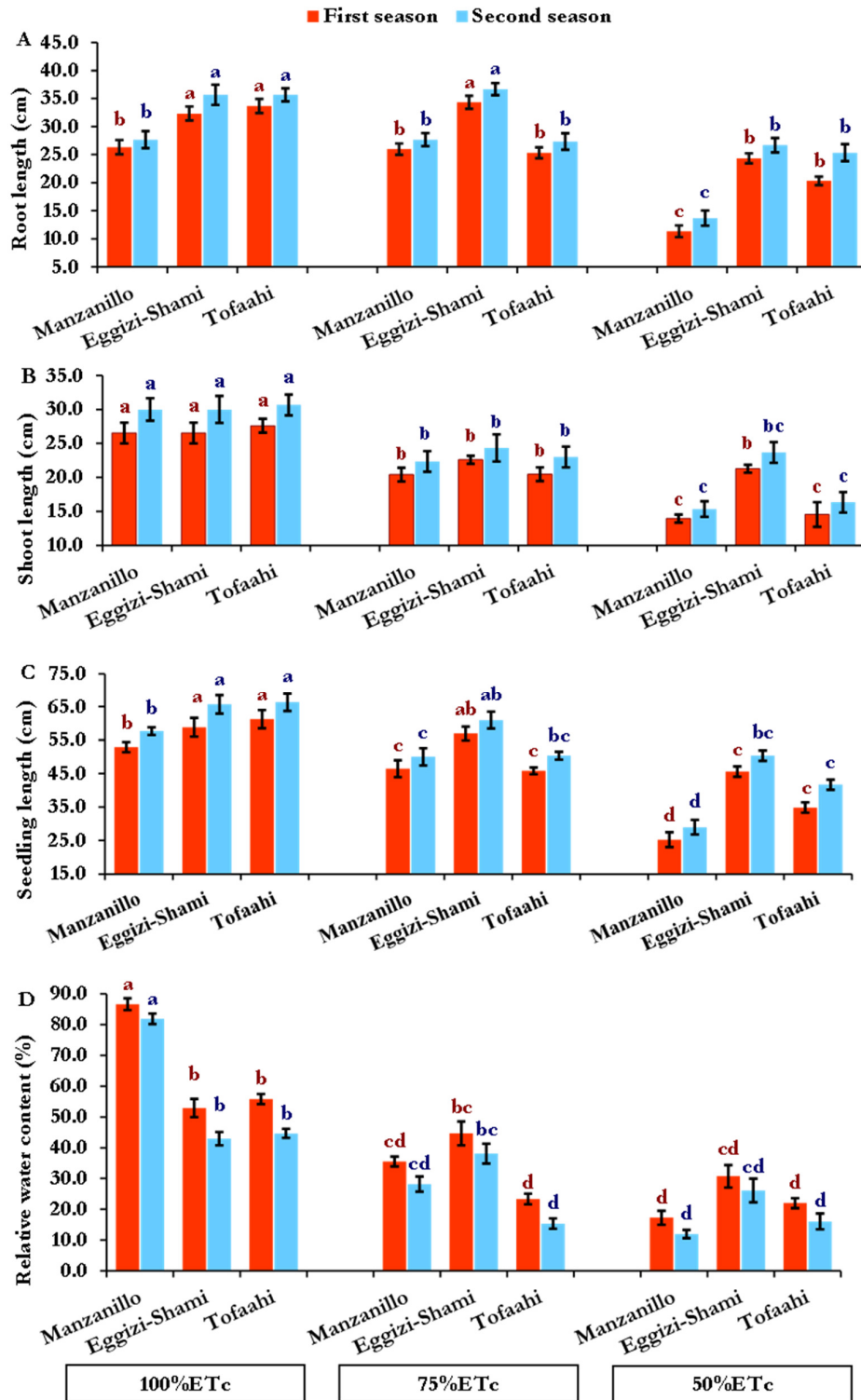


Figure 2. Influence of different irrigation levels on root length (A), shoot length (B), seedling length (C), and relative water content (D) of the assessed three cultivars in both seasons. The bars above the columns represent the standard deviation (SD), while different letters on the columns within each irrigation regime indicate significant differences as determined by Tukey's HSD test ($P \leq 0.05$).

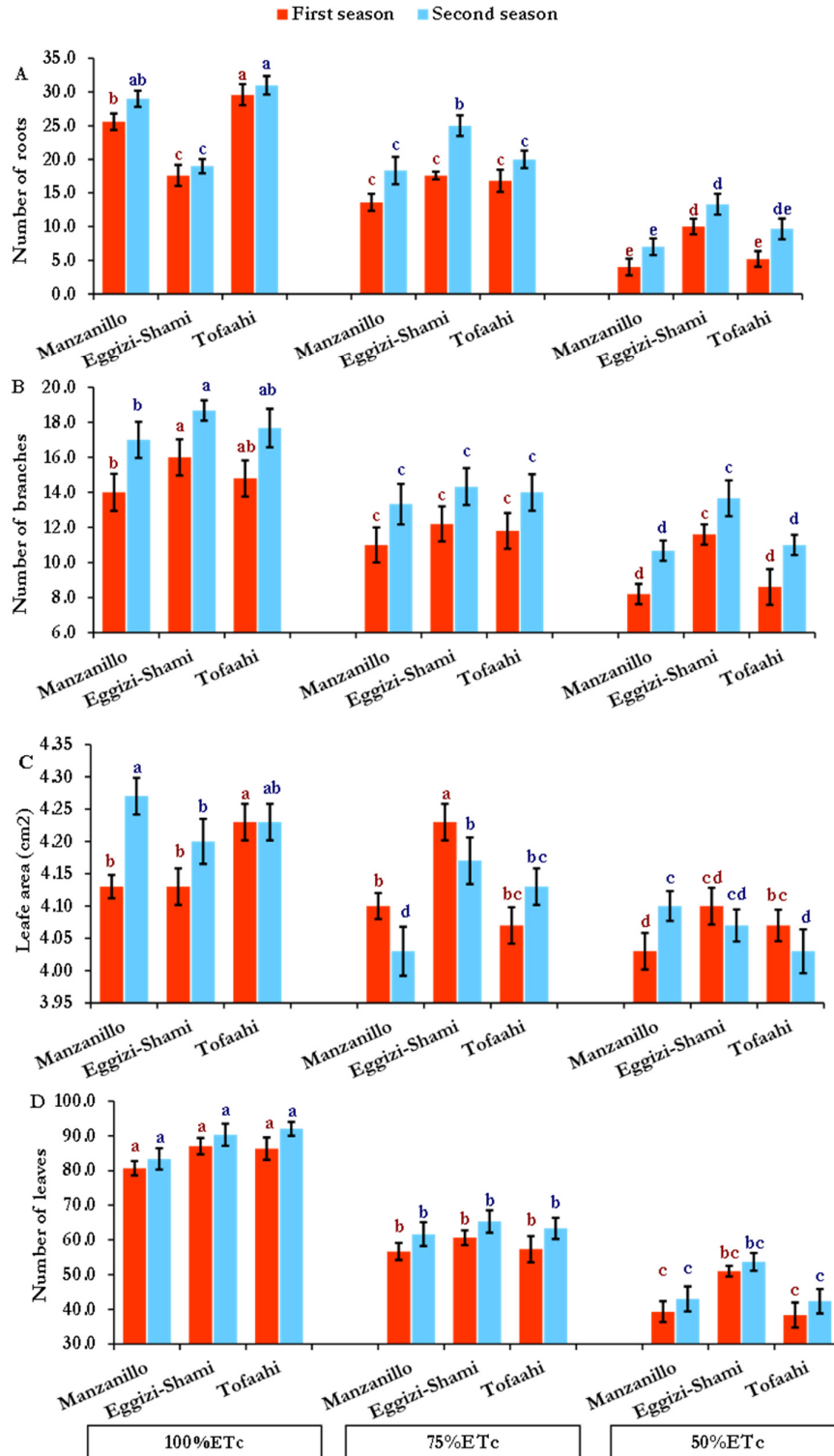


Figure 3. Influence of different irrigation levels on number of roots (A), number of branches (B), leaf area (C), and number of leaves (D) of the assessed three cultivars in both seasons. The bars above the columns represent the standard deviation (SD), while different letters on the columns within each irrigation regime indicate significant differences as determined by Tukey's HSD test ($P \leq 0.05$).

Gene expression analysis

The analysis of qRT-PCR displayed diverse levels of expression among assessed olive cultivars under moderate and extreme drought stress conditions. The profile expression of analysis was different among the three cultivars, which gave a significant result for all analysed genes. A Melting Curve test was performed to ensure quantitative amplification success, which showed only one band for the target genes and one clear peak for one gene. One clear peak was observed on the Melting Curve test (measuring the success of quantitative amplification), and every cultivar had at least one transcript whose expression ranged within a 2-fold range. The current study used polyubiquitin and UBI (AF429430) as housekeeping control. Based on the expression analysis of the CAT gene related to ROS-scavenging systems was weakly modulated by cultivars Manzanillo and 'Tofaahi' at the ETc of 75% and 50% (Figure 4). However, in the case of the cultivar, 'Eggizi-Shami' at the same conditions was overexpressed. Water deficit conditions caused a restricted effect on the expression of the dehydration-responsive genes (DREB and DHN) for cultivars 'Manzanillo' and 'Tofaahi' at the ETc levels of 75% and 50%. A noted increase in the expression was for cultivar 'Eggizi-Shami' which recorded the highest fold change values compared to the other two cultivars. Each target gene, DREB, DHN, and CAT, was represented by a single band. This finding reveals the specialization of the applied primers, and accordingly, the amplification curves were employed to record values (Ct). By estimating ΔCT , the rate of alteration was calculated for each cultivar to determine how gene expression changes over time (fold change).

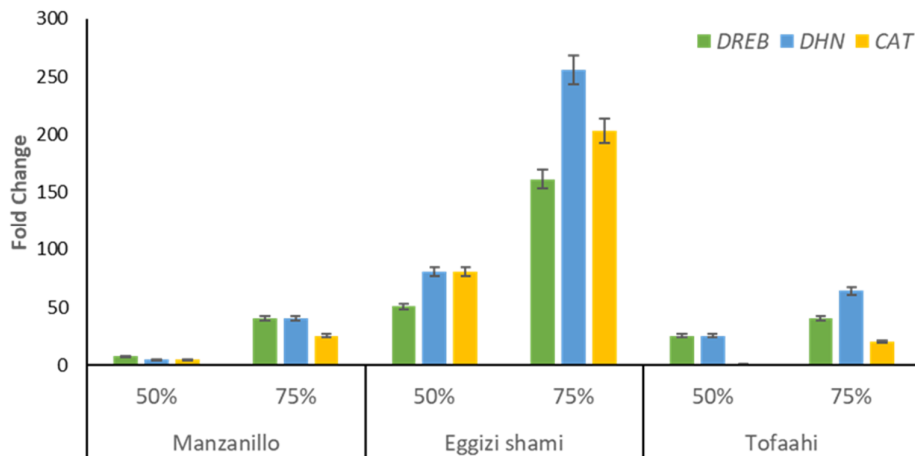


Figure 4. Expression analysis of stress-responsive genes (*DREB*, *DHN*, and *CAT*) in the three olive cultivars ('Manzanillo', 'Eggizi-Shami', and 'Tofaahi'), subjected to drought stresses of ETc 75, and 50% relative to 100% (used as Control)

Association among evaluated treatments and studied traits

Principal component analysis (PCA) was applied to explore the association among the evaluated cultivars under different irrigation regimes and studied parameters (Figure 5). The first two PCAs displayed most of the variability; PCA1 explained 68.52%, and PCA2 explained 15.82% (Figure 5). PCA1 was associated with raising the irrigation level from 50%ETc to 100%ETc. The full irrigation treatment (100% ETc) was situated on the positive right side of PCA1 and positively associated with all growth characteristics. Otherwise, extreme drought conditions were situated on the negative left side, while mild drought conditions were positioned in the central points. Otherwise, PCA2 was associated with assessed genotypes from the bottom, with the cultivar 'Tofaahi' to 'Eggizi-Shami' on the top, while Manzanillo was located in the middle. The cultivar 'Eggizi-Shami' was mainly situated on the positive side of PC1 under mild drought, indicating higher performance compared to the other cultivars.

On the contrary, 'Tofaahi' under extreme drought was located on the extremely negative side of PC1 and negatively associated with evaluated traits indicating lower performance. Likewise, hierarchical clustering

based on the studied characteristics divided the cultivars under different irrigation regimes into distinct main clusters (Figure 6). The irrigation treatments were the primary separating factor. The full irrigation treatment exhibited the highest values for all studied characteristics (represented in blue). Otherwise, the extreme drought had minimal values (red values). The cultivar ‘Eggizi-Shami’ displayed high values for most characteristics under extreme drought stress conditions.

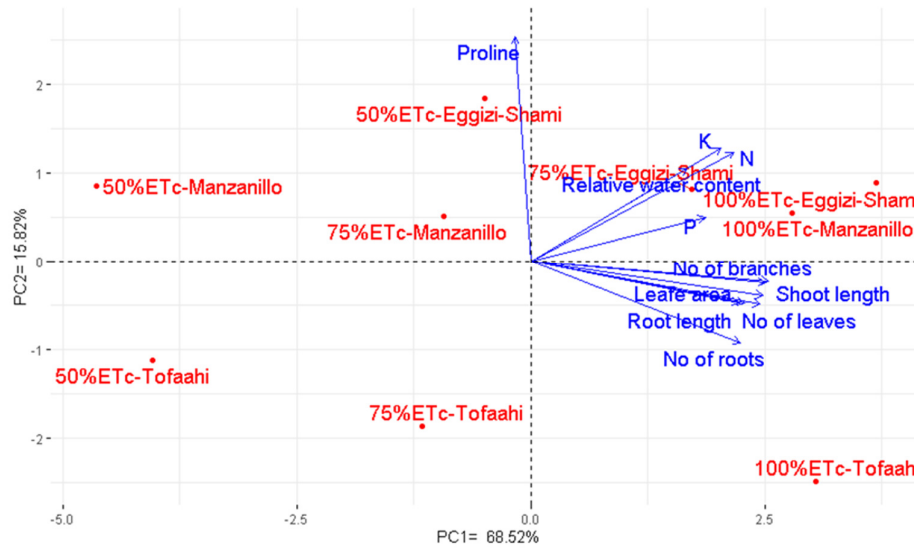


Figure 5. PC-biplot for the evaluated traits of the assessed three olive cultivars (‘Manzanillo’, ‘Eggizi-Shami’, and ‘Tofaahi’) under three irrigation regimes (50, 75, and 100% ETc).

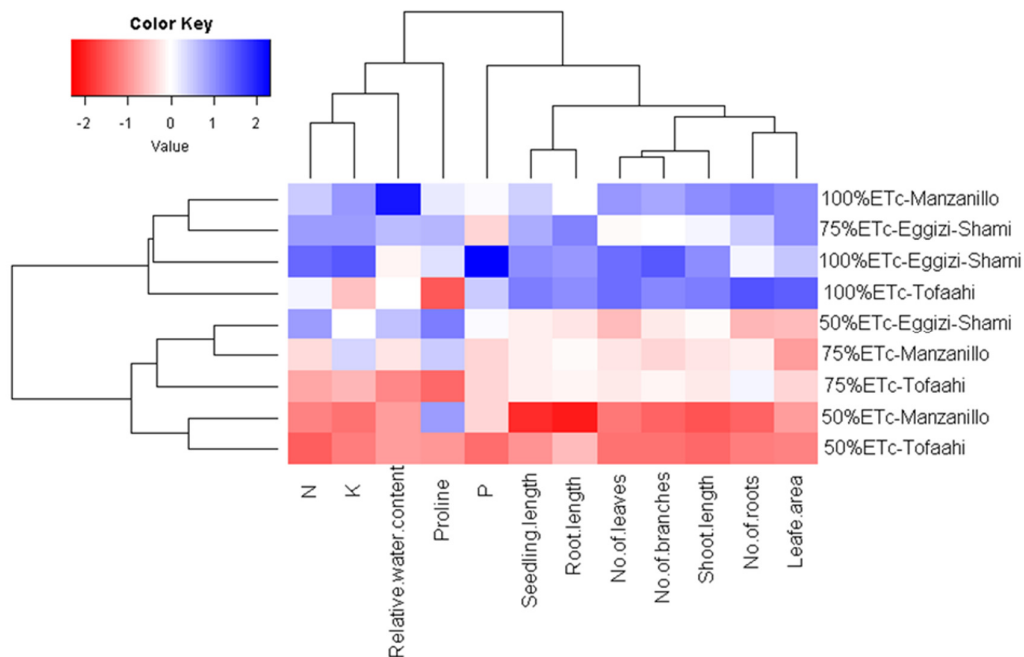


Figure 6. Heatmap and hierarchical clustering divide the assessed olive cultivars (‘Manzanillo’, ‘Eggizi-Shami’, and ‘Tofaahi’) under three irrigation regimes (50, 75, and 100 %ETc) into different clusters based on the evaluated traits. Blue and red colors denote low and high values for the studied trait, in the same order

Discussion

Drought tolerance is a complex trait caused by interconnected physiological, biochemical, and morphological parameters (Calvo-Polanco *et al.*, 2019; Abd El-Moneim *et al.*, 2008 and 2010). Hence, a better understanding of how olive plants respond to water deficit conditions is necessary for developing drought-tolerant genotypes. In the present study, diverse olive cultivars were evaluated under different irrigation regimes; 100, 75 and 50% ETc. The volumetric soil water content was assessed to determine drought stress levels. The results indicated a considerable reduction in the volumetric soil water content with applied regimes 50 and 75% ETc. The reduction in soil water content was ascribed to the shrinking available water for grown seedlings. Accordingly, these findings unveiled that the olive plants under 75 and 50% ETc regimes were subjected to drought stress compared to 100% ETc conditions. Under drought conditions, olive plants develop various morphological and physiological adaptations (Trabelsi *et al.*, 2019). The obtained results of leaf nutrient content (nitrogen, phosphorus, and potassium) were significantly different under the stressed irrigation with 50% ETc compared to control irrigated conditions at 100% ETc. In this regard, Hosseini *et al.* (2013) disclosed that the phosphorus, nitrogen, and potassium content in leaves was substantially influenced by water stress. Moreover, according to the present experiment, drought-stressed olive cultivars accumulated proline in their leaves which is a major osmoprotectant that accumulates in plants under water scarcity (Rahemi *et al.*, 2017). The amino acid proline is mainly responsible for osmotic adjustment by creating and maintaining osmotic pressure (Liu *et al.*, 2011; Ali *et al.*, 2021). However, the highest proline accumulation in leaves was at 50% ETc in comparison to the full irrigation (100 ETc) and mild drought stress conditions (75% ETc). Abiotic stresses increase proline accumulation in all plant parts, especially the leaves (Morsi *et al.*, 2023). It was reported that proline accumulation was observed when transplanted olive cultivars were exposed to different levels of drought (Shaheen *et al.*, 2011). Also, proline accumulation is associated with tolerance to various abiotic stresses in different plant species (Karimi *et al.*, 2012 ; Gowayed and Abd El-Moneim, 2021). Growth inhibition of plants (root length, seedling length, shoot length, number of leaves, number of branches, number of roots, moisture content, and leaf area) has been attributed to the drought stress of the evaluated cultivars. The decrease in soil water availability led to a significant progressive reduction in shoot height as soil moisture stress increased (Mohammed and Noori, 2008; Kamara *et al.*, 2021). In the current study, number of leaves changed under drought stress of 50% ETc in the evaluated cultivars 'Manzanillo', 'Eggizi-Shami', and 'Tofaahi' cultivars. As described by Hanin *et al.* (2011) the most sensitive process to water stress is the loss of turgor. Therefore, water deficit decreased stem elongation, growth rate, stomatal aperture, and leaf expansion.

The assessed olive cultivars displayed significant variation in their tolerance mechanisms to drought stress. The cultivar 'Eggizi-Shami' possessed promising physiological and morphological characteristics attributed to its tolerance under moderate and extreme drought stress compared to the other cultivars. Likewise, previously published reports depicted significant variation among olive cultivars in their tolerance to drought stress. Previously published studies by Ennajeh *et al.* (2006), Ennajeh *et al.* (2008) and Ennajeh *et al.* (2010) elucidated that the cultivars 'Chemlali' and 'Meski' provided sustain higher photosynthetic rates under water scarcity conditions. Other olive cultivars have shown similar adaptations such as the cultivars 'Madural', and 'Cobrançosa' (Bacelar *et al.*, 2004; Alowaiesh, 2007), which displayed high performance under drought stress conditions. In addition, real-time quantitative PCR (RT-qPCR) analysis was applied due to its precise sequence-specificity and ability to increase sample throughput (Tian *et al.*, 2015; Safhi *et al.*, 2022; Mesfer *et al.*, 2022). The evaluated cultivars displayed significant variations in the gene expressions of *CAT*, *DHN*, and *DERB*. It was found that the expression levels of these genes in vegetative tissues were higher in the cultivar Eggizi-Shami than in the other two cultivars. In this respect, Zhou *et al.* (2021) disclosed an association between drought stress tolerance and the accumulation of *DHN* and *DREB* in plants. Besides, Kidokoro *et al.* (2015) revealed that increasing levels of *DREB* expression increases the expression of downstream target genes

including dehydrins (*DHNs*). Accordingly, tolerant genotypes initiated protective mechanisms such as alterations in gene expression permitting adaptation of the physiological, biochemical, and morphological responses to drought stress. Moreover, integrating morphological and physiological assessment with gene expression analysis can provide highly confirmed information regarding the drought-tolerant olive cultivar. Accordingly, this approach can be employed as an innovative methodology to detect and validate genes involved in abiotic stress.

Conclusions

It is decisive to explore different mechanisms responsible for olive plants against drought stress. Significant variations were detected among evaluated olive cultivars for all studied physiological, morphological, and gene expression parameters. Highlighting morphological, physiological, and molecular adaptation to water scarcity explained the variation in drought tolerance among the evaluated olive cultivars. The positive physiological, morphological, and molecular responses of Eggizi-Shami under water deficit conditions led to relative tolerance compared to the other assessed olive cultivars. Furthermore, combining the results of morphological and physiological parameters with gene expression analysis of drought-related genes can provide highly confirmed information regarding the drought-tolerant olive cultivar. Consequently, this approach can be employed as an innovative methodology to detect and validate genes involved in abiotic stress.

Authors' Contributions

Conceptualization: B.F.A., A.B.E., D.A.E., E.A.R. and D.A.E.; Data curation: B.F.A., A.B.E., D.A.E., E.A.R. and D.A.E.; Formal analysis: E.A.R. and D.A.E.; Funding acquisition: B.F.A. and A.B.E.; Investigation: B.F.A., A.B.E., D.A.E., E.A.R. and D.A.E.; Methodology: B.F.A., A.B.E., D.A.E., E.A.R. and D.A.E.; Project administration: B.F.A. and D.A.E.; Resources: B.F.A. and D.A.E.; Software: A.B.E., D.A.E. and E.A.R.; Validation: B.F.A., A.B.E., D.A.E., E.A.R., D.A.E.; Visualization: B.F.A., A.B.E., D.A.E., E.A.R., D.A.E.; Writing – original draft: B.F.A. and D.A.E.; Writing - review and editing: B.F.A., A.B.E., D.A.E., E.A.R., D.A.E. 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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