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Effect of Drought Stress on Growth Characteristics, Osmolyte Accumulation, and Nutrient Uptake of Several Olive (*Olea europaea* L.) Cultivars

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ABSTRACT

The identification of suitable cultivars to mitigate environmental stress is crucial for effective olive orchard management. This study aimed to identify olive cultivars with enhanced tolerance to drought stress, facilitating the establishment of new drought-resilient orchards. Six cultivars were analyzed, i.e., Zard, Amygdalolia, Conservolia, Abou-Salt, Arbequina, and Manzanilla. Olive plants were cultivated in 10 L pots filled with an equal mixture of sand, field soil, and cocopeat, and were subjected to four levels of drought stress over a three-month period. Key osmolytes, growth characteristics, and leaf nutrient concentrations were measured. The findings revealed that Abou-Salt and Conservolia experienced a smaller decline in root and shoot dry weight under severe drought stress compared to other cultivars. Notably, dry biomass production in these two cultivars increased under the 0.25 field capacity treatment. Additionally, Arbequina, Conservolia, and Abou-Salt displayed enhanced shoot growth under stress conditions. Among the cultivars, Zard had the lowest proline concentration under extreme drought, whereas Abou-Salt exhibited the highest. In terms of nutrient response, Abou-Salt showed the greatest increase in leaf calcium concentration, while both Conservolia and Abou-Salt had minimal reductions in leaf potassium levels. Overall, Abou-Salt and Conservolia demonstrated superior drought resistance by maintaining root biomass, minimizing phenol accumulation, and exhibiting higher concentrations of proline, carbohydrates, and calcium in their leaves. These findings suggest that Abou-Salt and Conservolia are promising candidates for initiatives focused on developing drought-resistant olive orchards.

Introduction

Olive trees, as perennials indigenous to the semi-arid Mediterranean, frequently endure prolonged droughts during summer (Chartzoulakis et al., 1999; Khoshzaman et al., 2018). To survive such extreme environmental conditions, they have evolved numerous adaptive strategies. Structural adaptations in leaves, including increased cuticle thickness, stomatal density, and cell density in the epidermis and mesophyll, are among the most notable responses to drought (Ennajeh et al., 2010; Bosabalidis and Kofidis, 2002). These modifications, along with stomatal closure, help minimize water loss and conserve internal moisture. However, these changes also reduce photosynthetic activity due to the diminished water content in leaves.

Research has consistently shown that olive trees mitigate water loss by closing their stomata in response to elevated temperatures (Brito et al., 2019; Sofo et al., 2008; Moriana et al., 2002). Stomatal control plays a crucial role in reducing dehydration during drought stress (Kapoor et al., 2020; Fernández et al., 1997). The drought resilience of olive trees largely depends on their ability to regulate gas exchange and biochemical activities efficiently (Ben Abdallah et al., 2018). This efficiency is highlighted by the strong correlation between stomatal conductance and leaf water potential (Tugendhaft et al., 2016; Ben Ahmed et al., 2009).

Osmotic regulation is another essential adaptation mechanism, as noted by Tognetti et al. (2006). During drought stress, olive trees synthesize substances like proline and sugars that serve as osmotic regulators (Sofo et al., 2004). In drought-tolerant cultivars, proline accumulation occurs more gradually but eventually reaches higher concentrations compared to sensitive cultivars (Ennajeh et al., 2006). Additionally, increased levels of soluble carbohydrates, potassium, and calcium in leaves are strongly associated with drought tolerance in olive trees (Karimi et al., 2018).

Water deficits negatively impact vegetative growth, fresh biomass, and dry biomass in olive trees (Gholami and Gholami, 2019). Drought stress influences several vegetative parameters, including root length, leaf area and number, and shoot growth (Ahmadipour et al., 2019; Calvo-Polanco et al., 2019). The degree of drought tolerance varies across cultivars, with some exhibiting thicker epidermal layers and higher hyphal density in leaves (Ennajeh et al., 2010). These variations highlight the differing capacities of cultivars to withstand drought, with some experiencing significantly reduced growth under water stress (Karimi et al., 2018).

The impacts of climate change, including reduced rainfall, higher temperatures, and longer growing seasons, are becoming increasingly evident in various regions of Iran, including the Tarom area of Zanjan province. With approximately 20,000 hectares dedicated to olive cultivation (Anonymous, 2018), Zard is the predominant cultivar in Tarom (Zeinanloo et al., 2015). Other cultivars, such as Amygdalolia, Conservolia, Abou-Salt, Arbequina, and Manzanilla, have also been identified as suitable options for this region (Azimi et al., 2016a, b). Abou-Salt is highly adaptable to the Tarom climate and is recognized for its precocity (Azimi et al., 2016a; Azimi et al., 2018). Arbequina, a highly productive and relatively dwarf cultivar, has been successfully cultivated in Iran and is commonly used in establishing semi-dense orchards (Arji et al., 2012; Azimi et al., 2016b).

This study aims to evaluate the effects of drought stress on the leaf nutrient content as well as the morphological and physiological traits of six olive cultivars. The findings will provide valuable insights into their resilience and potential adaptability to the increasing environmental stresses associated with climate change.

Material and methods

Plant materials

The experiment was carried out in 2020 at the Tarom Olive Research Station in Zanjan Province, Iran, situated at 49°E longitude and 36°N latitude, at an elevation of 350 m above sea level. One-year-old seedlings from six olive cultivars—Zard, Amygdalolia, Conservolia, Abou-Salt, Arbequina, and Manzanilla—were planted in 10 L pots filled with a substrate consisting of equal proportions of sand, field soil, and cocopeat (Table 1). The seedlings were initially cultivated in a greenhouse under optimal irrigation and nutrient conditions for six months to establish healthy growth. Subsequently, they were subjected to varying levels of drought stress over a three-month period.

Table 1. The physical and chemical properties of the culture media used in the experiment.

OC	Texture	Clay	Silt	Sand	pH	Lime	EC	K	P
(%)	(-)		%		(-)	(%)	(dS m^{-1})	(mg kg^{-1})	
6.4	Sandy Loam	8	23	69	7.2	7.9	0.9	215	116

To evaluate the effects of drought stress on olive plants, varying levels of drought stress were applied by adjusting the field capacity (FC) to 75%, 50%, and 25% using the water balance method and gravimetric water content. Before initiating the treatments, a measured amount of water was added to the control pot, and the volume of drained water was collected and measured over a two-day period. The difference between the amount of water added and the volume drained was used to calculate the water consumed by the control treatment. This procedure was repeated three times to ensure accuracy. The remaining treatment levels were then calculated as percentages of the control, with the 25% FC treatment containing only 0.25 of the gravimetric water content of the control, and similarly for the 75% and 50% FC treatments. Additionally, the volume of water required to flush out accumulated salts in the stress treatments was estimated following a method described by Nikbakht et al. (2013).

Growth parameters

At the end of the experiment, shoot length was measured in cm. Each plant was dissected to separate roots, new leaves, and stems, and the fresh biomass of each component was recorded. The shoots and roots were then individually washed with distilled water and weighed. Then, the dissected plant parts were dried at 60 °C for 72 h, and the dry biomass of the shoots, roots, and whole plants was measured in grams.

Determination of carbohydrates, total phenol, and proline content

At the end of the experiment, leaf samples were collected and weighed to assess carbohydrate content. The leaves were then dried at 65 °C for 48 h, and their dry mass was recorded. Leaf samples were ground into a fine powder, and 100 mg was selected for carbohydrate analysis. Soluble sugars were extracted three times using hot 80% ethanol, and their concentration was determined using the anthrone reagent, with glucose as the standard (Schaffer et al., 1985). Total phenol content in the leaves was determined using the Folin reagent. Fresh leaves (0.5 g) were macerated in 80% methanol, followed by centrifugation at 10,000 rpm for 5 min. Subsequently, 50 µL of the leaf extract was mixed with 450 µL of distilled water, and 2.5 mL of 10% Folin reagent was added. After 10 min, 2 mL of saturated sodium carbonate was added to neutralize the reaction. The absorbance of the solution was measured at 765 nm after 2 h incubation in the dark at room temperature. Total phenol concentration was determined using a standard curve based on gallic acid (Talhaoui et al., 2015). Proline concentration in leaf samples was determined according to a method described by Bates et al. (1973). Fresh leaf tissue (0.1 g) was homogenized in 10 mL of 3% sulfosalicylic acid. The extract was clarified by centrifugation at 10,000 rpm for 5 min. Two mL of the supernatant was mixed with ninhydrin reagent and pure acetic acid. It was then heated in a bain-marie at 100 °C for one h. After cooling in an ice bath, toluene was added, forming two layers. The upper colored layer, containing toluene and proline, was used for measuring proline concentration. The absorbance was read at 520 nm, and proline concentration was determined using a standard curve.

Determination of nutrient uptake

At the end of the experiment, leaf samples from the different cultivars were collected to analyze nitrogen, potassium, calcium, and phosphorus concentrations. The leaves were rinsed with deionized water and dried at 65 °C for 48 h. Calcium concentration was determined by atomic absorption spectroscopy (GBC-Avanta, Australia), while potassium concentration was measured using a flame photometer. Phosphorus content was analyzed by calorimetry using the ammonium phosphomolybdate method. Nitrogen concentration was determined using the Kjeldahl method. Nutrient concentrations were expressed as a percentage of leaf dry weight (DW).

Statistical analysis

The experiment followed a factorial arrangement based on a completely randomized design, with six cultivars and four drought stress levels, each with three replications and two plants per replication. The total sample population comprised 144 plants. Data were analyzed using SAS (version 9.4), and advanced statistical analyses were performed with JMP version 13 (SAS Institute Inc., 2017).

Results

Morphological characteristics

The morphological characteristics, including root and shoot dry weights, total biomass, and shoot growth length, were significantly influenced by both drought stress and genotype, as indicated by analysis of variance, which showed statistical significance at the $P < 0.001$ level. The combined effects of these traits were also significant, with the exception of root dry weight. Across all drought stress levels, both root and shoot dry weights decreased compared to plants maintained under normal watering conditions. Among the cultivars, Abou-Salt and Conservolia exhibited smaller reductions in root and shoot dry weights under 0.25 field capacity drought stress (Table 2). Specifically, reductions in root dry weight for Abou-Salt and Conservolia were

36.87% and 28.28%, respectively, compared to the control treatment. In contrast, the reductions in root dry weight for Amygdalolia, Arbequina, Manzanilla, and Zard were 55.71%, 38.22%, 43.06%, and 49.05%, respectively.

Table 2. Measures of morphological traits (dry weight of root, dry weight of shoot, biomass and shoot growth) of olive cultivars in drought stress treatments, field capacity (FC), 75% field capacity (0.75 FC), 50% field capacity (0.50 FC), and 25% field capacity (0.25 FC).

Cultivar	**Drought**	**DWr (g)**	**DWs (g)**	**Biomass (g)**	**Shoot growth(cm)**
Abou-Salt					
	FC	10.93^{a}	35.77^{b}	65.78^{a}	136.00^{ab}
	0.75FC	9.32^{b}	30.94^{d}	53.17^{b}	120.67^{d}
	0.50 FC	8.43^{b}	27.82^{e}	43.75^{e}	$115.67^{d\text{-}g}$
	0.25 FC	6.90^{c}	$23.52^{g\text{-}i}$	36.05^{gh}	110.67^{gh}
Amygdalolia					
	FC	6.39^{cd}	36.62^{b}	47.57^{d}	$113.67^{e\text{-}g}$
	0.75FC	$4.45^{f\text{-}h}$	24.13^{gh}	34.01^{h}	106.00^{hi}
	0.50 FC	$4.06^{g\text{-}i}$	17.68^{l}	25.10^{kl}	$101.00^{i\text{-}k}$
	0.25 FC	2.83^{jk}	10.92^{n}	14.64^{n}	81.33^{l}
Arbequina					
	FC	$5.18^{e\text{-}g}$	39.64^{a}	50.70^{bc}	140.00^{a}
	0.75FC	$4.40^{f\text{-}h}$	25.89^{f}	34.47^{h}	121.67^{d}
	0.50 FC	$4.18^{g\text{-}i}$	20.38^{jk}	26.61^{jk}	110.67^{gh}
	0.25 FC	$3.20^{i\text{-}k}$	15.34^{m}	21.78^{m}	$103.33^{i\text{-}k}$
Conservolia					
	FC	6.93^{c}	37.43^{b}	49.74^{cd}	128.67^{c}
	0.75FC	6.41^{cd}	28.11^{e}	40.45^{f}	119.00^{de}
	0.50 FC	$5.58^{d\text{-}f}$	25.00^{fg}	34.10^{h}	$114.00^{e\text{-}g}$
	0.25 FC	4.97^{fg}	19.47^{k}	28.38^{ij}	$101.00^{i\text{-}k}$
Manzanilla					
	FC	$6.20^{c\text{-}e}$	33.59^{c}	43.78^{e}	119.33^{de}
	0.75FC	4.90^{fg}	28.68^{e}	37.60^{g}	112.67^{fg}
	0.50 FC	4.39^{gh}	22.93^{hi}	29.81^{i}	98.67^{jk}
	0.25 FC	$3.53^{h\text{-}j}$	17.17^{l}	22.81^{lm}	84.67^{l}
Zard					
	FC	$4.20^{g\text{-}i}$	24.92^{fg}	35.12^{gh}	132.67^{bc}
	0.75FC	$3.39^{h\text{-}j}$	21.92^{ij}	25.36^{kl}	$117.00^{d\text{-}g}$
	0.50 FC	$3.10^{i\text{-}k}$	16.58^{lm}	21.74^{m}	$104.33^{h\text{-}j}$
	0.25 FC	2.14^{k}	12.29^{n}	16.43^{n}	97.33^{k}
Cultivar		***	***	***	***
Drought		***	***	***	***
C×D		ns	***	***	**

Means within the column followed by the same letter are not significantly different at $P = 5\%$ level, using LSD test. ns, *, **, *** significant at the 0.05, 0.01, and 0.001 probability level, respectively. DWr = root dry weight, DWs = shoot dry weight.

For shoot dry weight, Abou-Salt experienced a 34.25% reduction, while Conservolia showed a 47.98% reduction under 0.25 field capacity drought stress (Table 2). As drought severity increased, the total biomass of all cultivars declined. However, Abou-Salt and Conservolia maintained higher dry biomass under these conditions. Specifically, the reductions in dry biomass for Conservolia, Abou-Salt, and Manzanilla were 42.94%, 45.19%, and 47.90%, respectively. In contrast, Amygdalolia, Arbequina, and Zard exhibited greater reductions of 69.24%, 57.04%, and 53.22%, respectively, under the same drought stress level.

Shoot growth was also significantly affected by both drought stress and genotype, with noticeable reductions observed in all treatments. Abou-Salt, Arbequina, and Conservolia demonstrated higher shoot growth under 0.25 field capacity, whereas Amygdalolia, Manzanilla, and Zard exhibited lower growth. Shoot growth reductions for Abou-Salt and Conservolia were 18.63% and 21.50%, respectively, while reductions for the other cultivars exceeded 26% (Table 2). A high degree of genetic variation was evident among the cultivars in terms of the root-to-shoot dry weight ratio, with statistically significant differences observed at the $P < 0.001$ level. Under drought conditions, Abou-Salt and

Conservolia displayed the highest root-to-shoot dry weight ratios, while Zard, Manzanilla, and Arbequina exhibited the lowest ratios (Fig. 1).

Total phenol, proline, and soluble sugars

Significant variations in total phenol content were observed among the cultivars and in response to different levels of drought stress. As drought severity increased, total phenol content in the leaves also increased. The largest percentages of increase were recorded under the 0.25 field capacity treatment, with Arbequina and Conservolia cultivars showing increases of 51.80 and 46.79%, respectively. In contrast, the lowest total phenol levels were found in the Abou-Salt, Zard, and Amygdalolia cultivars (Fig. 2).

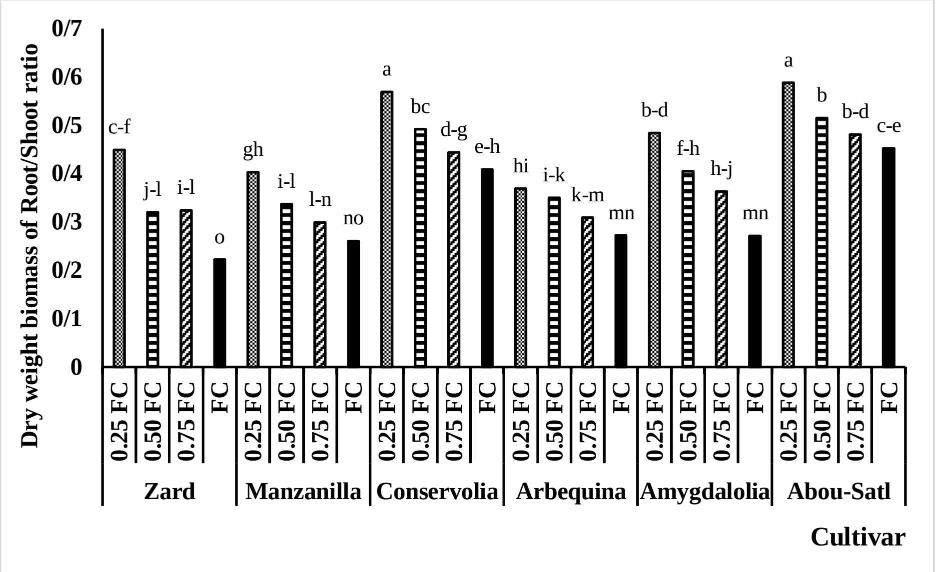

Fig. 1. Mean comparison of the interaction effects between roots to shoot dry weight ratio in six olive cultivars under drought stress conditions, field capacity (FC), 75% field capacity (0.75 FC), 50% field capacity (0.50 FC) and 25% field capacity (0.25 FC).

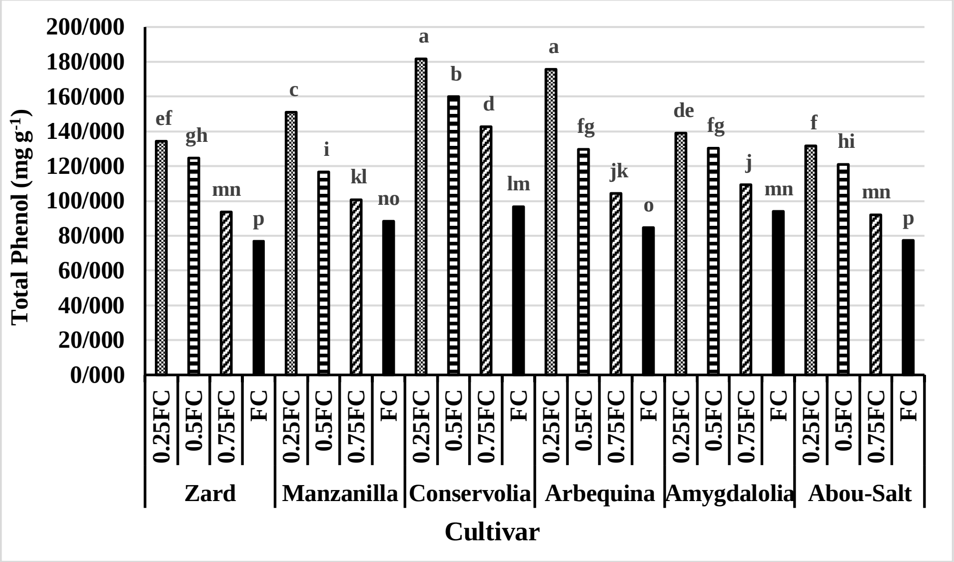

Fig. 2. Mean comparison interaction effects of leaf total phenol content (mg g^{-1}) in six olive cultivars exposed to drought stress conditions, field capacity (FC), 75% field capacity (0.75 FC), 50% field capacity (0.50 FC), and 25% field capacity (0.25 FC).

The main effects of cultivar and drought stress levels revealed significant differences in proline accumulation ($P < 0.0001$). Proline concentrations increased across all drought stress conditions, with the highest concentration observed in the Abou-Salt cultivar under 0.25 field capacity, representing a 38.56% increase compared to the control. Conversely, the lowest proline accumulation was recorded in the Zard cultivar. No significant differences in proline concentrations were noted among the Conservolia, Arbequina, and Amygdalolia cultivars (Fig. 3).

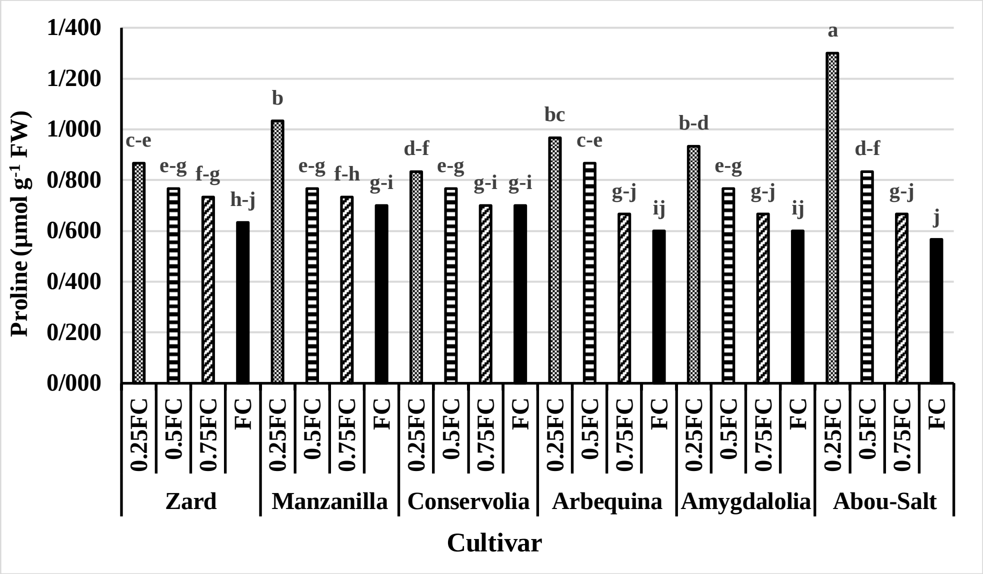

Fig. 3. Mean comparison interaction effects of leaf proline content (μmol g^{-1} FW) among six olive cultivars subjected to drought stress conditions, field capacity (FC), 75% field capacity (0.75 FC), 50% field capacity (0.50 FC), and 25% field capacity (0.25 FC).

Significant differences in soluble carbohydrate concentrations were observed among the cultivars and in response to drought stress treatments. Drought stress induced a notable increase in soluble carbohydrate concentrations compared to the control treatment. Among the cultivars, Arbequina and Abou-Salt exhibited the highest concentrations at 0.25 field capacity, with increases of 31.86% and 31.44%, respectively. In contrast, the Zard cultivar showed the smallest increase, with soluble carbohydrate levels rising by only 16.11% under the same conditions (Fig. 4).

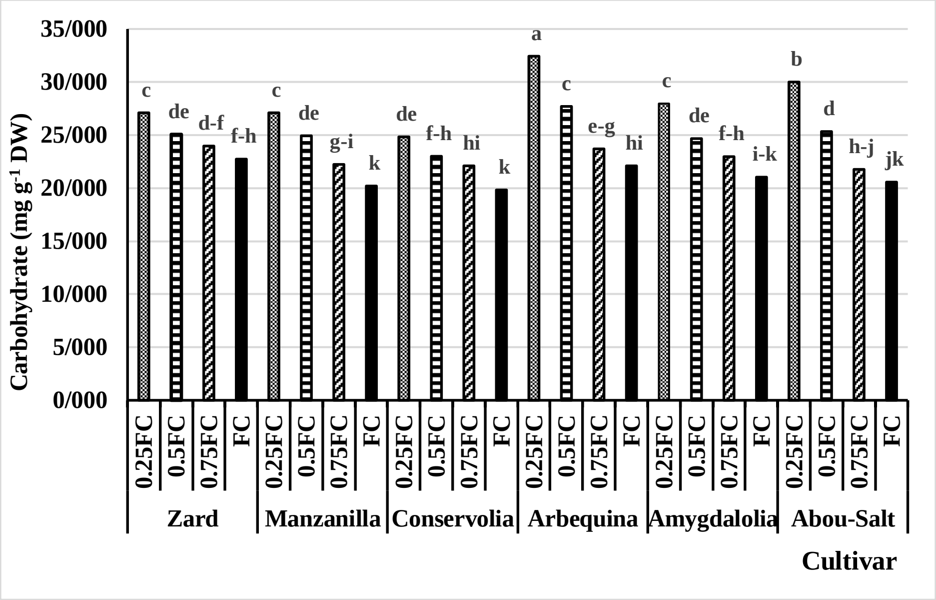

Fig. 4. Mean comparison interaction effects of leaf carbohydrate content (mg g^{-1} DW) in different olive cultivars under drought stress conditions, field capacity (FC), 75% field capacity (0.75 FC), 50% field capacity (0.50 FC), and 25% field capacity (0.25 FC).

Concentration of nutrients

Significant differences ($P < 0.0001$) occurred in the concentrations of calcium, potassium, phosphorus, and nitrogen in the leaves of various cultivars subjected to drought stress treatments. Under drought conditions, both calcium and potassium concentrations in the leaves of olive cultivars increased significantly (Table 3). This increase was especially pronounced under severe stress conditions at 0.25 and 0.50 field capacity, with the Conservolia cultivar showing the highest concentrations of these nutrients. In contrast, the calcium concentrations in the leaves of the Arbequina and Manzanilla cultivars were lower than those of the other cultivars.

Table 3. Mean comparison of nutrient uptake (calcium, potassium, phosphorus, and nitrogen percentages) of olive cultivars in drought stress treatments, field capacity (FC), 75% field capacity (0.75 FC), 50% field capacity (0.50 FC), and 25% field capacity (0.25 FC).

Cultivar	Drought	Ca	K	P	N
		%			
Abou-Salt					
	FC	1.21^{o}	$0.73^{f\text{-}h}$	$0.214^{a\text{-}d}$	1.34^{p}
	0.75FC	1.52^{l}	$0.78^{e\text{-}g}$	$0.187^{d\text{-}f}$	1.56^{n}
	0.50 FC	$2.22^{d\text{-}f}$	1.03^{cd}	$0.170^{f\text{-}h}$	1.66^{m}
	0.25 FC	2.69^{b}	1.14^{bc}	0.108^{m}	1.73^{lm}
Amygdalolia					
	FC	1.85^{i}	0.79e-g	$0.204^{a\text{-}e}$	1.43^{o}
	0.75FC	2.15^{fg}	1.10^{bc}	$0.171^{f\text{-}h}$	1.89^{j}
	0.50 FC	2.30^{d}	1.21^{bc}	$0.147^{h\text{-}k}$	2.13^{hi}
	0.25 FC	2.52^{c}	1.45^{a}	$0.143^{i\text{-}l}$	2.29^{ef}
Arbequina					
	FC	1.33^{n}	0.55^{h}	0.228^{a}	2.20^{gh}
	0.75FC	1.43^{m}	0.67^{gh}	$0.215^{a\text{-}c}$	2.56^{d}
	0.50 FC	1.62^{k}	$0.72^{f\text{-}h}$	$0.193^{b\text{-}f}$	2.64^{c}
	0.25 FC	1.89^{i}	$0.85^{d\text{-}g}$	$0.168^{f\text{-}i}$	2.85^{a}
Conservolia					
	FC	2.09^{gh}	$0.74^{e\text{-}g}$	0.229^{a}	1.25^{q}
	0.75FC	2.21^{ef}	$0.84^{e\text{-}g}$	$0.182^{e\text{-}g}$	1.58^{n}
	0.50 FC	2.59^{c}	$0.90^{d\text{-}f}$	$0.135^{j\text{-}m}$	1.78^{kl}
	0.25 FC	2.88^{a}	1.11^{bc}	$0.127^{k\text{-}m}$	1.96^{j}
Manzanilla					
	FC	1.35^{mn}	$0.75^{e\text{-}g}$	0.217^{ab}	1.81^{k}
	0.75FC	1.52^{l}	$0.80^{e\text{-}g}$	$0.207^{a\text{-}e}$	2.11^{i}
	0.50 FC	1.63^{k}	0.91^{de}	$0.188^{c\text{-}f}$	2.20^{gh}
	0.25 FC	1.86^{i}	1.28^{ab}	$0.152^{h\text{-}k}$	2.23^{fg}
Zard					
	FC	1.41^{mn}	$0.72^{f\text{-}h}$	$0.166^{f\text{-}i}$	2.19^{gh}
	0.75FC	1.72^{j}	$0.76^{e\text{-}g}$	$0.160^{g\text{-}j}$	2.36^{e}
	0.50 FC	2.02^{h}	$0.89^{d\text{-}f}$	$0.151^{h\text{-}k}$	2.58^{cd}
	0.25 FC	2.28^{de}	1.42^{a}	0.119^{lm}	2.76^{b}
Cultivar		***	***	***	***
Drought		***	***	***	***
C×D		***	*	*	***

Means within the column followed by the same letter are not significantly different at $P = 5\%$ level, using LSD test. *, **, *** significant at the 0.05, 0.01, and 0.001 probability level, respectively.

Under the drought treatment at 0.25 field capacity, the Abou-Salt cultivar exhibited a remarkable 122.31% increase in calcium concentration compared to the control treatment, while the Amygdalolia cultivar showed the lowest increase at 36.22%. Among the cultivars, the potassium concentrations in the leaves of Amygdalolia, Zard, and Manzanilla were the highest under the 0.25 field capacity treatment. The Abou-Salt and Conservolia cultivars also showed significant potassium concentrations. The percentage increases in potassium concentration at 0.25 field capacity, compared to the control treatment, were 83.54%, 97.22%, and 70.67% for the Amygdalolia, Zard, and Manzanilla cultivars, respectively. In contrast, the increases in Abou-Salt, Arbequina, and Conservolia cultivars were 56.16%, 54.55%, and 50.00%, respectively (Table 3).

Phosphorus concentrations in the leaves of olive cultivars decreased as the intensity of drought stress increased. The Abou-Salt and Conservolia cultivars exhibited the smallest reductions in phosphorus concentration, with decreases of 49.53% and 44.54%, respectively. In contrast, the Amygdalolia, Arbequina, Manzanilla, and Zard cultivars showed reductions of less than 30% in phosphorus levels (Table 3).

Advanced statistical analyses

Principal component analysis identified five major factors: morphological characteristics, nutrient concentrations, phenol concentrations, shoot growth, and carbohydrate content. The first and second components accounted for 48.67% and 27.96% of the total variance, respectively, together explaining 76.63% of the variance (Table 4). The first component, labeled "morphological characteristics," was primarily influenced by variables such as root and shoot fresh and dry weights, as well as the root-to-shoot fresh and dry weight ratios. The second component, which focused on nutrient concentrations, was positively impacted by calcium, potassium, and sodium, while phosphorus, nitrogen, and soluble carbohydrates had a negative influence (Fig. 5).

Table 4. Initial Eigenvalue and total variance explained.

Number	Eigenvalue	Percent of Variation	Cumulative Percent
1	7.7874	48.671	48.671
2	4.4733	27.958	76.629
3	1.8685	11.678	88.308
4	1.3539	8.462	96.769
5	0.5169	3.231	100.000

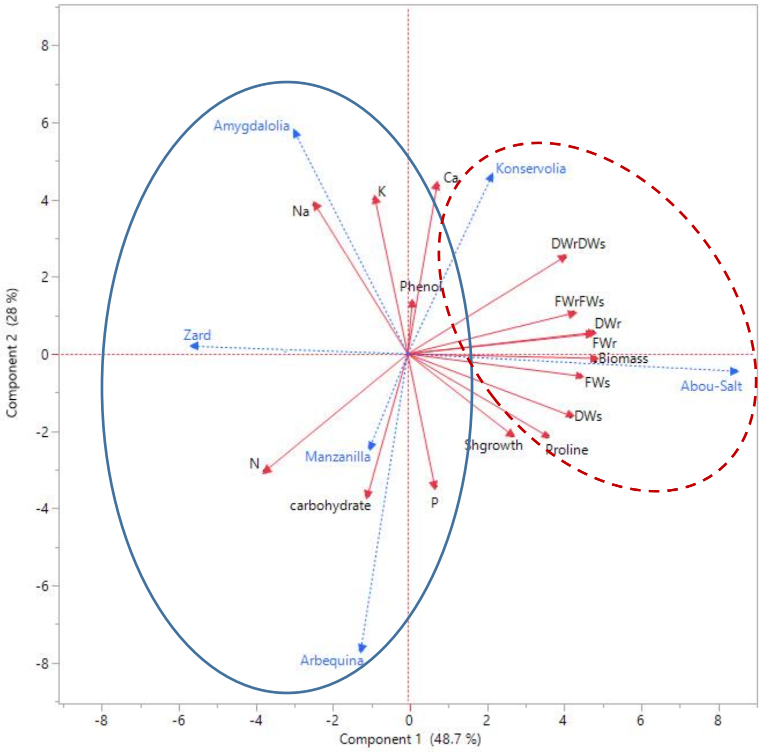

Fig. 5. Impact of various characteristics such as growth parameters, carbohydrates, total phenol, proline content, and nutrient uptakes (calcium, potassium, phosphorus and nitrogen percentages) on the categorization of six olive cultivars under drought stress conditions, field capacity (FC), 75% field capacity (0.75 FC), 50% field capacity (0.50 FC), and 25% field capacity (0.25 FC).

Following the principal component analysis, the six cultivars were divided into two groups. The first group comprised the Abou-Salt and Conservolia cultivars, while the second group consisted of Amygdalolia, Arbequina, Manzanilla, and Zard cultivars. The primary distinguishing factors for the first group, particularly for the Abou-Salt and Conservolia cultivars, were their root and shoot morphological traits, as well as their proline content. These cultivars demonstrated greater drought tolerance compared to those in the second group.

Discussion

The growth and fruiting of olive trees have been adversely affected by frequent droughts driven by climate change in many regions worldwide. Understanding how different olive cultivars respond to drought stress is essential for selecting varieties suitable for future climatic conditions (Ennajeh et al., 2010; Parri et al., 2023). Several indices have been used to assess the drought resistance of olive cultivars (Brito et al., 2019; Ojaghloo et al., 2022; Petridis et al., 2012). In this study, the Abou-Salt and Conservolia cultivars exhibited less reduction in dry biomass under the 0.25 field capacity drought stress treatment compared to the Amygdalolia, Arbequina, Zard, and Manzanilla cultivars. These two cultivars also showed smaller declines in both root and shoot dry weights under the same drought stress conditions. Furthermore, Abou-Salt and Conservolia displayed the highest root-to-shoot dry weight ratios during the drought treatments.

Morphological traits, such as leaf size and number, are key indicators of how olive cultivars respond to drought. Drought stress not only reduces leaf size and limits leaf number but also affects shoot growth and leads to biomass reduction. The root system is similarly impacted, which increases the ratio of root to shoot biomass. Previous studies have shown that the Cobrançosa cultivar exhibits greater drought tolerance, attributed to its higher leaf tissue density, thicker cuticle layers, and hyphal formation (Bacelar et al., 2009). Carbohydrates, particularly soluble carbohydrates like glucose, fructose, and sucrose, are the most abundant solutes that accumulate in olive tree tissues under water deficit conditions (Rejsková et al., 2007). These soluble carbohydrates play a significant role in osmotic regulation for olive trees and other plants facing environmental stresses (Rahemi et al., 2017). In response to drought, plants synthesize and store amino acids, proteins, sugars, and organic acids (Ingram and Bartels, 1996). These physiological adaptations help olive trees lower their osmotic potential, facilitating water movement to their roots and leaves to maintain cell mass (Dichio et al., 2005). Additionally, these solutes protect proteins and cell membranes from stress (Chaves et al., 2003). In the evaluated drought stress treatments, soluble carbohydrate concentrations increased compared to the control, with the Arbequina and Abou-Salt cultivars showing higher concentrations at 0.25 field capacity than the other cultivars. Proline concentrations also increased under drought stress, with the highest levels of this amino acid found in the Abou-Salt cultivar under the 0.25 field capacity drought treatment. Proline tends to accumulate in olive leaves under drought stress. Ben Ahmed et al. (2009) demonstrated a correlation between photosynthesis and proline accumulation, highlighting the important role of this osmolyte in sustaining photosynthetic activity. Proline accumulation may serve as a valuable indicator of stress tolerance in various plant species. Additionally, similar to sugars, proline helps retain water within the cytoplasm of cells (Parida and Das, 2005) and plays a critical role in protecting cell membranes and preventing protein degradation under severe drought conditions. However, the effectiveness of proline accumulation in enhancing stress tolerance can vary depending on the genotype (Ashraf and Foolad, 2007). Research by Ben Ahmed et al. (2009) revealed significant variations in proline levels among different cultivars and irrigation practices in both leaves and roots. Specifically, the Chemlali variety exhibited the highest accumulation of proline under non-irrigated conditions compared to the Picholine and Meski cultivars. In another study, Karimi et al. (2018) observed that the Fishumi cultivar experienced greater growth inhibition in response to drought than the Dezful, Amygdalolia, and Conservolia cultivars. The latter three cultivars maintained membrane stability and higher relative leaf water content, owing to their elevated concentrations of soluble carbohydrates and proline in their leaves.

In this study, phenol concentrations in the leaves increased with the severity of drought stress, with the most significant rise observed under the 0.25 field capacity drought treatment in the Arbequina and Conservolia cultivars. Conversely, the lowest phenol concentrations were recorded in the Abou-Salt, Zard, and Amygdalolia cultivars. The findings suggest that drought stress affects various physiological and biochemical parameters in olive trees, with the extent of these effects being influenced by genotype, water stress levels, and the duration of exposure. Prolonged drought stress has been shown to overwhelm the antioxidant capacities of trees, leading to elevated levels of malondialdehyde (MDA) and resulting in oxidative damage after two months of exposure.

Environmental stress has also been observed to significantly increase the concentration of phenolic compounds in the leaves of olive trees (Melgar et al., 2009; Petridis et al., 2012). This increase is attributed to the activation of the phenylpropanoid pathway, which is responsible for the accumulation of phenols in olive leaves (Mechri et al., 2020). As a result, phenolic compounds, particularly oleuropein, accumulate in the leaves, acting as antioxidants (Petridis et al., 2012). Phenolic compounds play a crucial role in protecting olive cultivars from the harmful effects of UV-B radiation associated with water scarcity (Smirnoff, 2005). An elevation in phenol levels triggers leaf drop, suggesting that lower phenol concentrations may enhance a tree’s resilience under stressful environmental conditions.

Petridis et al. (2012) reported significant variations in total phenol content among olive cultivars subjected to drought stress treatments. Specifically, the Gaidourelia cultivar exhibited the highest total phenol content, followed by Kalamata, Megaritiki, and Koroneiki. Total phenol content increased across all olive cultivars under the 0.33 field capacity treatment. Similarly, Ahmadipour et al. (2018) found that varying drought stress levels resulted in different phenol levels among cultivars, with total phenol content rising as drought stress increased. In the current study, the Zard, Amygdalolia, and Conservolia cultivars displayed the highest total phenol levels under drought stress.

Drought stress also impacts the absorption, transport, and distribution of nutrients within plants, leading to disruptions in plant nutrition. These disruptions can significantly disturb physiological processes and the accumulation of biomass. Nutrients in plants serve critical functions, including acting as structural components in macromolecules, catalyzing enzyme reactions, functioning as osmotic solutes, and maintaining the balance of anions and cations within cells (Brito et al., 2019). According to Fernández-Escobar (2019), olive trees experiencing drought stress and potassium deficiency exhibit increased stomatal conductance compared to trees under normal conditions, resulting in reduced water use efficiency. Similarly, Restrepo-Díaz et al. (2008) found that low potassium levels hinder potassium uptake by olive trees under drought stress. Therefore, it is recommended to apply potassium fertilizer to olive trees before they reach the deficiency threshold and when they are adequately hydrated.

Karimi et al. (2018) demonstrated that potassium levels in the leaves of Fishumi olive plants decrease under drought stress, indicating greater susceptibility to drought. This reduction in potassium concentrations suggests that potassium (K^+) plays a crucial role in osmotic adjustment in olive shoots during drought stress. Similarly, the concentration of calcium (Ca^{2+}) in the leaves of Fishumi plants decreased under drought conditions. The decline in Ca^{2+} levels in drought-susceptible olive plants highlights the importance of calcium accumulation in the leaves of drought-tolerant genotypes for osmotic regulation. These findings are consistent with previous research (Chakhchar et al., 2017; Karimi et al., 2018). Furthermore, nitrogen concentrations in leaves increased with rising drought stress, likely due to the accumulation of carbohydrates in the leaves. Notably, nitrogen concentration was higher in the Arbequina and Zard cultivars under severe drought stress compared to the other cultivars.

PCA is an effective method for unraveling the complex relationships between genetic background and olive responses to salinity and drought stress. This approach helps identify the key variables driving the observed differences across these treatments (Tadić et al., 2024). In this study, PCA revealed that the six olive cultivars could be grouped into two distinct categories. The first group consisted of the Abou-Salt and Conservolia cultivars, while the other cultivars clustered in the second group. The primary distinguishing characteristics of the first group, particularly for Abou-Salt and Conservolia, included specific morphological traits related to root and shoot structure, as well as higher proline levels. These cultivars exhibited greater drought resistance compared to those in the second group.

Boussadia et al. (2023) used PCA to classify five Tunisian olive cultivars (Chetoui, Chemchali, Besbessi, Sayali, and Jarboui) based on a range of physiological parameters, including relative water content (RWC), stomatal resistance (SR), photosystem II activity, maximal photochemical efficiency (FV/FM), and performance index on an absorption basis (PI). Their analysis also revealed clustering into three distinct groups. Furthermore, Gholami et al. (2024) conducted PCA on various pomological, yield, and physiological traits of the “Shengeh” olive cultivar under varying levels of drought stress (100%, 75%, and 50% ET) during 2022 and 2023. The results indicated that the different irrigation regimes primarily influenced clustering along the first principal component (PC1), while foliar spray treatments significantly impacted the variation observed along the second principal component (PC2).

Conclusions

The Abou-Salt and Conservolia cultivars demonstrated superior total biomass, as well as higher root and shoot dry weights under severe drought conditions. These cultivars also experienced a lower reduction rate in root and shoot dry weights compared to others, indicating their enhanced tolerance to drought stress. Notably, the root-to-shoot dry weight ratio of Abou-Salt and Conservolia was significantly higher than that of the other cultivars, which likely contributes to their improved water absorption capabilities. In contrast, lower concentrations of total phenols were observed in the Abou-Salt, Zard, and Amygdalolia cultivars, suggesting that these cultivars have a greater tolerance to drought stress, which consequently results in reduced leaf shedding. Under severe drought stress, the Abou-Salt cultivar exhibited the highest proline concentration, while the Zard cultivar had the lowest. The elevation in proline concentrations is believed to contribute to the enhanced drought tolerance of Abou-Salt.

Furthermore, calcium accumulation in the leaves of the Conservolia and Abou-Salt cultivars was higher than in other cultivars, with the most significant increase observed in Abou-Salt and the lowest in Amygdalolia. Similarly, the increase in potassium concentration in the leaves of Abou-Salt, Arbequina, and Conservolia was lower than that in other cultivars. Advanced statistical analyses revealed that morphological characteristics, proline and nutrient concentrations, and carbohydrate content were the primary factors driving cultivar classification under drought stress. Based on these factors, the cultivars were divided into two groups: a drought-tolerant group (Abou-Salt and Conservolia) and a non-tolerant group. Principal component analysis identified five main factors contributing to the differences: morphological traits, nutrient concentrations, phenolic content (linked to leaf fall tolerance), shoot growth potential, and carbohydrate storage.

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Author contribution

The research conception and design were done by MA and MT. Material preparation, data collection and analysis were performed by MA and TK. MA and TK wrote the first draft of the manuscript and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Conflict of Interest

The authors indicate no conflict of interest in this work.

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