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Physiological and biochemical responses of some olive cultivars (*Olea europaea* L.) to water stress

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Abstract: Water stress is one of the important abiotic environmental stresses that threaten the agricultural -products in the world. This experiment was carried out to determine the effect of water stress on physiological and biochemical characteristics of three commercial olive cultivars. A factorial pot experiment was conducted in the field conditions using completely randomized design in Gilan-Gharb city, Kermanshah province during 2015. One-year-old rooted cuttings of Zard, Amigdalolia and Konservolia olive cultivars were planted in 12-liter pots and subjected to three irrigation treatments. Irrigation treatments included control (100% of field capacity), 75% and 50% field capacity. Physiological and biochemical characteristics such as relative water content (RWC), electrolyte leakage (EL), calcium, potassium and sodium content, total phenol, malondialdehyde, peroxidases, catalase, a, b and total chlorophyll, proline and total carbohydrate were measured. Results showed that relative water content, K & Ca was reduced while sodium content increased by increasing water stress. Chlorophyll content was higher in Konservolia cultivar under water stress in comparison to the others. Water stress induced increasing in proline, total phenol and soluble carbohydrate in all cultivars. The highest total phenol and proline was recorded in Zard cultivar under water stress. Total carbohydrate increased significantly ($P<0.05$) in Konservolia in comparison to the others. Malondialdehyde content was increased as an index of oxidative stress by drought. The highest peroxidases and catalase activity were recorded under drought stress of 50% irrigation in Konservolia olive cultivar. Generally based on results Konservolia, Zard and Amigdalolia were more tolerant respectively.

Key words: Olive; Drought stress; Enzymes; Variety; Osmotic adjustment.

Introduction

Plant growth and productivity limited by environmental factors to varying degrees, depending upon stress severity (1). Water is one of the main limiting factors that affect crop production in arid and semi-arid regions around the world. In these regions, water has economically attracted attention to itself and its deficit poses a threat to the world. At present, an increasing demand for water is observed in the agricultural sections especially horticulture (2). It seems that different plant species employ a wide range of tolerance mechanisms to cope with drought stress in order to reach physiological and biochemical adaptations. Osmotic adjustment is one of the most important adaptive mechanisms of water deficit that previously reported in olive (3, 4), pistachio (5) and almond (6). Water stress also can cause a variety of physiological and biochemical responses at the molecular level. Reactive oxygen species (ROS) production is enhanced by water stress (1) in a varying level and leads to considerable damage to the cell membrane via membrane lipid peroxidation (7, 8). Olive (*Olea europaea* L.) is an evergreen and water-tolerant plant (9). Olive cultivation increased during two last decades in Iran. As water stress is able to impose a negative impact on plant's growth and productivity, it is regarded as one of the main issues affecting olive plant during its growth.

In this regard, many types of research have been conducted on olive in different physiological and biochemical responses under water stress. Olive trees respond to water stress by some adaptive mechanisms like up-regulation of oxidative stress protectors (10) and protective solutes accumulation (11). Antioxidant defence in plants evolved some enzymes including peroxidase (POX), catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR) and monodehydroascorbate reductase (MDAR) to cope with oxidative stress (12). Many of the enzymatic responses to water stress are well documented in olive trees. Zarabi *et al.* (13) found that POX activity of six two-year-old olive cultivars (Zard, Roghani, Fishomi, Nabali, Arbeqina, and Gordal) significantly increased under water stress in which Zard and Gordal had the highest rate of POX activity. Aganchich *et al.* (14) stated that the highest activity of POX was observed in Picholine Marocaine olive variety under partial root zone drying (PRD50) in comparison to full irrigated ones. Amini *et al.* (15) reported that POX acti-

vity increased under drought stress in Dezfoli, T2, and Koroneiki olive cultivars. Many types of research have been reported that CAT activity increased in olive under water stress condition (10, 16-19).

Olive employs non-enzymatic components to cope with water stress. Polyphenol is one of the non-enzymatic components that act as antioxidant reagent against water stress in olive (19). Polyunsaturated lipids are oxidized by oxidative damages in the cell membrane and lead to accumulation of malondialdehyde (MDA) (20). Sofo *et al.* (21) stated that MDA is a useful biomarker of lipid peroxidation. Many reports have been documented on variation between olive cultivars lipid peroxidation based on Malondialdehyde content under drought stress (19, 22, 23). Compatible solutes like carbohydrate (9, 24) and proline accumulate in olive trees and act as osmolytes against drought stress (11*)*. Accumulation of proline and soluble carbohydrate was reported by Arzani and Yazdani, (25); Arji and Arzani, (26); Shaheen *et al.* (27); Boussadia *et al.,* (11) in young olive trees under water stress condition. Drought tolerance could be the result of production or concentration of compatible osmotic solutes. By lowering cells' osmotic potential, they reinforce cells to absorb more water from the environment in order to mitigate the harmful effect of water stress in plants' cells (28). Some plants like olive, through lowering the level of water potential in their leaves, improve their tolerance to drought stress (29) and similar findings were also reported by Charttzoulakis *et al.,* (30). The results of an experiment on olive demonstrated that the relative water content (RWC) and chlorophyll content of leaf were associated with irrigation regimes and low irrigation significantly reduced the content of chlorophyll (31). Arji and Arzani, (26) stated that chlorophyll a, b and carotenoid significantly decreased under water stress conditions of five young potted olive cultivars.

Under the drought-stress condition, irrigation regime had a significant effect on cell mineral nutrient contents. In this regard, some researchers reports showed that drought stress led to decrease in the amount of calcium and potassium and conversely increased the rate of sodium in the leaves (9, 32). Shaheen *et al.* (27) evaluate the effect of four levels of water 100, 75, 50 and 25% field capacity on nutrient uptake of five young olive cultivars (i.e. Picual, Koroneiki, Manzanillo, Coratina and Eggizi Shami). Results showed that the amount of K, Ca and Na was decreased by increasing water stress.

Water stress is one of the environmental factors that limit agricultural crop production (33). Olive is one of the fruit trees that can be cultivated in water-restricted areas. Selection of tolerant olive cultivars to combat drought stress is of great importance. Drought tolerant cultivars can be found by understanding the mechanisms involved in drought stress (17). In this regard, physiological and biochemical evaluation of commercial olive cultivars are needed. Olive cultivars responded differentially to arid and semi-arid environmental conditions. Arji *et al.* (34) reported that Zard, Amigdalolia and Konservolia olive cultivars were superior in fruit yield in arid environmental of Sarpole Zehab, Kermanshah, Iran. Understanding of drought stress responses of mentioned olive cultivars is very important for olive cultivation in such conditions.

The supply of water for olive groves is one of the most important factors in the development of olive cultivation. Considering the serious risk of drought and water shortages, especially during the last few years, it is necessary to adopt appropriate methods for optimum utilization of water resources, including using resistant cultivars (35), determining critical irrigation times (36), using mulch (37), and using plant growth regulators (32). The water is an important limiting factor for agricultural producers in arid and semi-arid regions in the world (38).

The main purpose behind doing this research was to investigate and screen the tolerance of young olive cultivars of Zard, Amigdalolia and Konservolia to drought stress condition and scrutinize the physiological and biochemical mechanisms involved in plant tolerance to drought stress.

Materials and Methods

Experimental site and plant material

This experiment was carried out to evaluate the effect of water stress on three young potted olive cultivars under field environmental condition in Gilane Gharb region (longitude: 45º 56′ E, latitude: 34º 08′ N, altitude: 816 m and 20.1°C annual mean temperature) of Kermanshah province during 2015 growing season. Gilane Gharb is located in the west of Iran with semi-arid environmental conditions. Annual, maximum and minimum mean temperature was 21.1, 14.98 and 27.18 ^{o}C respectively. Summation of annual evaporations was 2215.3 mm. Mean relative humidity during the experiment period was 23.8%. The experimental period began on May 10, immediately after the last spring rainfall in this region and ended up on September 21, 2015, for about 120 days. One- year- old rooted cuttings of Zard, Amigdalolia and Konservolia olive cultivars with a similar canopy and height were selected and planted in 12 litres pots containing a mixture of soil, sand and animal manure (1:1:1) on March 2015.

Experimental design and treatments application

A factorial experiment was used based on a completely randomized design with three replications. Treatments were 3 olive cultivars (Zard, Amigdalolia and Konservolia) and 3 levels of irrigation (100%) field capacity (FC) (control), (75%) FC, and (50%) FC. A total of 9 treatments with three replications were used. Each experimental unit contains 5 pots, yielding a total of 135 pots. Pots were irrigated at 3-day intervals based on soil sampling and periodic pots weighting method. Treatments were applied based on pot capacity. Pot capacity was calculated by weighting the pot at full water saturated and dried soil pot at 105^{o}c in a constant weight. The weight difference was calculated as pot capacity and the other percentages were calculated accordingly (39). The total applied water was measured during the experimental by using a measuring cylinder.

Measured traits

Some physiological and biochemical traits were measured at the end of the experiment. Leaf samples prepared from different treatments and transferred to the laboratory to measure corresponding traits. Traits were

measured included of relative water content (RWC), electrolyte leakage (EL), calcium, potassium and sodium content, total phenol, malondialdehyde (MDA), peroxidase (POX), catalase (CAT), chlorophyll a, chlorophyll b, total chlorophyll, proline and total carbohydrate.

Determination of total phenolic

Singleton and Rossi, (40) method was used to measure the amount of total phenol. For this purpose, 100 mg leaf samples were completely pulverized with 3 ml methanol 85% in a mortar and then extractions were filtered (Whatman No. 1). 300 microliters of the filtrated extract were added to test tubes containing 1500 µL of Folin-Ciocalteu and then kept for 5 minutes at room temperature. After adding 1200 µL of Na_2CO_3 (7%) to test tubes, test tubes properly shook for 2 hours at room temperature. The absorbance was recorded at 760 nm. Gallic acid was used as a standard.

Peroxidase and catalase activity

Enzymes extraction was performed at 4°C. Frozen leaf samples (0.5 g) were homogenized in 0.05 M sodium phosphate buffer (pH 7.8) containing 1 mM EDTA. Na_2 and 2% (w/v) polyvinylpolypyrrolidone (PVPP). Homogenized samples were centrifuged at 14,000 g for 30 min at 4°C (41). The peroxidase enzyme activity was determined according to (42) method. The supernatant (0.1 ml) was added to the reaction mixture containing 0.05 ml guaiacol solution and 0.03 ml hydrogen peroxide solution in 3ml of phosphate buffer solution (pH 7.0). The absorbance was recorded at 465 nm for 180 seconds.

The catalase enzymes activity was measured according to Aebi (43) method. Enzyme extract of 0.1 ml with 13.2 mM H2O2 in 50 mM phosphate buffer (pH 7.0) was used to determine CAT activity. The decomposition of H_2O_2 was monitored by the decline in absorbance (A) at 240 nm for 3 min.

Malondialdehyde measurement

Malondialdehyde was measured as a lipid peroxidation index according to Stewart and Bewley) 44) method. 0.5 gram of fresh leaves were homogenized with liquid nitrogen in a porcelain mortar. Five ml of 50 mM phosphate buffer (7 = pH) was added to prepared powder in a 15 ml test tube. Then the samples were centrifuged at 14000 *g* for 30 min at a temperature of four degrees. The supernatant was mixed with an equal volume of 0.5% TBA (2-thiobarbituric acid) in 20% trichloroacetic acid then incubated at 95 C for 30 min. Test tubes were put in an ice bucket for stopping the reaction immediately. Samples were centrifuged at 14000 *g* for 30 min at a temperature of four degrees. Absorbance was recorded at 532 and 600 nm. MDA concentration calculated using the extinction coefficient of 155 mM^{-1} cm^{-1} (45).

Electrolyte leakage determination

Electrolyte leakage was assessed based on methods introduced by Lutts *et al.(*46). Olive leaves were washed twice with distilled water. Leaf discs with 1 cm in diameter prepared and dipped in 20 mL of distilled water then kept for 24h on a rotary shaker (150 rpm) at room temperature. The electrical conductivity (EC) of the bathing solution (EC1) was measured as the first conductivity. Second conductivity (EC2) was determined after autoclaved solution at 120 °C for 20 min and then cooled at room temperature. The electrolyte leakage was determined by equation (1) and expressed as a percent.

$$E = \frac{E_1}{E_2} \times 100 \quad (1)$$

Determination of proline

Proline content was measured by Bates et al., (47) method. Three grams of leaf tissue was homogenized in 3% sulfosalicylic acid and then filtered and the residue was removed by centrifugation at 3500 g for 10 minutes. Two ml of the centrifuged extract reacts with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid in the test tube for 45 minutes at 100°C in a boiling water bath and then cooled in an ice bath to stop the reaction. Four ml of toluene was added to the mixture then shacked for unique mixing then kept for 30 minutes at room temperature to separate into two divided phases. The optical density of upper phase was measured at 520 nm using spectrophotometer Varian Cary 100. Toluene was used as a blank. The proline concentration was determined by using D-Proline as a standard curve.

Soluble carbohydrate determination

Extracting and measuring of soluble carbohydrate was performed according to the method used by Buysse and Merckx (48). For this purpose, 0.3g of fresh leaf tissue was triturated with five ml of 95% ethanol in a porcelain mortar. The pulverized sample was poured into the test tube and shaken vigorously for two minutes. Thus, the samples were carefully separated into two solid and liquid phases. Again, five ml of 70% ethanol was added to the solid phase and shaken vigorously until the liquid phase was detected. Total liquid phase was centrifuged at 3500 × g for 10 min. Three (ml) freshly prepared anthrone reagent was added to 0.1ml of the extract and the mixture was incubated for 10 min in boiling water bath with 100 ℃. After cooling, absorbance was recorded at 625 nm by using a spectrophotometer Varian Cary 100.

Relative water content (RWC)

Relative water content (RWC) was measured according to Gucci *et al.,* (49) method. Three to four fully expanded leaves with similar age were used. Punched leaves were weighed to determine the fresh weight (FW) and placed in a petri dish for 20 h in the dark condition to fully rehydrate and then fully rehydrated leaf disks were dried at 80 °C for 48 h. Leaves disks were weighed at fully rehydrate and oven dried stage. The RWC was measured using equation (2):

$$RWC = \frac{Fresh\ \ weight - Dry\ \ weight}{Turgid\ \ weight - Dry\ \ weight} \times 100 \quad (2)$$

Chlorophyll content measurement

Chlorophyll content was extracted according to Dere *et al.,* (50) with a little change. For this purpose, 0.125 g of fresh leaf tissue was pulverized with 10 ml acetone

80% and 0.1 g of calcium carbonate "CaCO3" (to neutralize the acidic condition of the cell and preventing the degradation of chlorophyll) in a porcelain mortar. The operation was performed in low light and cool room environment. The extract was centrifuged at 10000 rpm for 10 minutes. The supernatant was removed and used to determine chlorophyll a, b and total. Absorption was recorded using spectrophotometer Varian Cary 100 at 663 and 645 nm. A blank of acetone 80% was used. Chlorophyll concentrations (mg /g fresh weight) were calculated by using Arnon's (51) equations as below:

Chlorophyll a (mg/g F.W) = $(12.7\ A_{663} - 2.69\ A_{645}) \times V/1000 \times n$ (3)

Chlorophyll b (mg/g F.W) = $(22.9\ A_{645} - 4.68\ A_{663}) \times V/1000 \times n$ (4)

Total chlorophyll (mg/g F.W) = $(20.2\ A_{645} + 8.02\ A_{663}) \times V/1000 \times n$ (5)

Where: A_{645} = absorption value at 645 nm, A_{663}= absorption value at 663 nm, V = total volume of filtrate, n = tissue weight.

Mineral measurements

In order to measure mineral nutrients including calcium, potassium, and sodium, leaves sample was collected at the end of the experiment, washed with distilled water and dried in 80°C to a constant weight. Samples were grounded by a mill and then 1 g of grounded leaves prepared by dry ashing method at 500°C. The ash was dissolved in hydrochloric (HCL) (52). Potassium, Calcium and sodium were measured by using Flame photometer (Model, PSP 7 Genoy, UK).

Statistical analysis

The collected data were analyzed using SAS (Ver. 9.1) North Carolina software. The data on different parameters were evaluated using ANOVA and mean differences were separated Duncan's multiple range test at the 5 % level of significance.

Results

Biochemical and physiological characteristics

Total phenol

Leaf total phenol content was significantly influenced by cultivar and water stress at 5% statistical level. Total phenol content increased by increasing water stress level in all cultivars and hence the highest level was observed at 50% FC irrigation. Under 50% FC treatment, the highest (31.33 mg/100g FW) and lowest (21.73 mg/100g FW) amount of total phenol content was recorded in Zard and Konservolia cultivars, respectively (Table 1).

Peroxidase (POX)

The interaction effect of cultivar and drought stress on leaf peroxidase activity was significantly observed ($p<0.05$) and the highest (2.2 units/mg) and lowest (1.31 units/mg) rate of leaf peroxidase activity were recorded in Konservolia and Amigadalolia under 50% FC treatment, respectively (Table 1). The results of this experiment cleared that the peroxidase (POX) activity was less at full irrigation (100% FC) whereas it was intensified at 50% FC irrigation (Table 1).

Catalase (CAT)

Depending on the cultivar and degree of water stress, the activity of CAT was significantly varied ($p<0.05$). The results also showed that irrigation at 50% FC led to increasing in CAT activity (Table 1).The highest (3.13 units/mg) and lowest (1.78 units/mg) extent of CAT activity was observed in Konservolia and Amigdalolia under 50% FC treatment, respectively (Table 1).

Malondialdehyde (MDA)

The MDA content in leaves of all cultivars was significantly affected by water stress ($p<0.05$). The highest (13.58 nmol/g FW) amount of MDA was obtained in Amigdalolia whereas the lowest (10.57 nmol/g FW) was observed in Konservolia under severe water stress (Table 1). There was a negative relationship between MDA and level of irrigation so that the highest and lowest MDA contents were observed at 100% FC (full irrigation) and 50% FC irrigation, respectively (Table 1).

Electrolyte leakage

The results of the table (2) represent that electrolyte leakage (EL) was affected by water stress. Electrolyte leakage was increased with water stress progress in all cultivars. In regard to cultivars, Amigdalolia had the highest (35.27 %) increase in electrolyte leakage in comparison to control and others cultivars. However, in Zard cultivar leaves (50% FC), a lesser of electrolytes leakage was recorded (Table 2).

Table 1. Total phenol, peroxidase, catalase and malondialdehyde of olives under water stress.

Cultivars	Water regimes (% FC)	Total Phenol (mg/100g FW)	Peroxidase (unit/mg)	Catalase (unit/mg)	Malondialdehyde (nmol/g FW)
	100 %	25.50^{c}	1.197^{e}	$1.187^{b\,c}$	8.74^{d}
Zard	75%	28.80^{b}	1.690^{bc}	2.190^{b}	10.39^{c}
	50%	31.33^{a}	1.890^{b}	2.447^{b}	11.88^{b}
	100 %	20.27^{def}	0.860^{f}	1.260^{d}	11.64^{b}
Amigdalolia	75 %	22.87^{cd}	0.960^{f}	1.430^{d}	12.41^{b}
	50%	24.43^{c}	1.310^{de}	1.780^{c}	13.58^{a}
	100%	18.20^{f}	1.500^{cd}	2.220^{b}	7.09^{e}
Konservolia	75%	19.23^{ef}	1.750^{b}	2.523^{b}	7.90^{de}
	50%	21.73^{de}	2.200^{a}	3.130^{a}	10.57^{c}

Different letters indicate significant differences at ($P \leq 0.05$) by Duncan's test.

Table 2. Electrolyte leakage, proline content, soluble carbohydrate, relative water content and chlorophyll a of olive cultivars under water stress.

Cultivars	Water regimes	Electrolyte Leakage %	Proline content (µg/g FW)	Total Soluble Sugar (mg/g FW)	(%)Relative Water Content	Chlorophyll a (mg/g FW)
	100%	22.29^{d}	17.76^{bcd}	15.03^{f}	86.40^{ab}	3.120^{b}
	75%	24.96^{d}	19.30^{a}	17.41^{f}	73.90^{cd}	2.560^{cd}
Zard	50%	30.12^{bc}	22.76^{a}	21.60^{de}	59.39^{e}	2.353^{d}
	100%	24.38^{d}	15.39^{de}	20.83^{e}	90.57^{a}	3.863^{a}
Amigdalolia	75%	27.74^{c}	18.49$^{b\,c}$	22.05^{cde}	81.23^{bc}	2.920^{bc}
	50%	35.27^{a}	20.11^{b}	25.10^{bc}	57.74^{e}	2.450^{d}
Konservolia	100%	28.28^{c}	11.21^{d}	24.27^{bcd}	86.56^{ab}	4.073^{a}
	75 %	30.36^{bc}	13.38^{ef}	26.97^{b}	70.41^{d}	3.793^{a}
	50%	32.62^{b}	16.27^{cd}	30.29^{a}	45.81^{f}	3.177^{b}

Different letters indicate significant differences at (P ≤0.05) by Duncan's test.

Proline content

Presented data in Table (2) indicated that proline content gradually increased significantly ($p<0.05$) by increasing water stress in all cultivars. Proline content was higher in Zard, Amigdalolia and Konservolia in normal and stress condition respectively (Table 2). The amount of proline was significantly increased due to water stress. The highest and lowest amount of proline was observed in Zard (22.76 µg/g FW) and Konservolia (16.27 µg/g FW) under 50 % FC treatment, respectively.

Soluble carbohydrate

Total carbohydrate was accumulated in olive leaves significantly by increasing water stress. Total soluble sugars varied among cultivars so that Konservolia, Amigdalolia and Zard had the highest amount in control plants respectively. The highest increase rate was recorded in Konservolia, Amigdalolia and Zard respectively under water stress condition (Table 2).

Relative water content

Relative water content was significantly ($p<0.05$) different between cultivars and water stress treatments. Zard (59.39%) and Konservolia (45.81%) had the highest and lowest RWC under 50% FC respectively (Table 2). Water stress reduced RWC of all cultivars and the plants received 50% of FC irrigation had the lowest rate of RWC (Table 2).

Chlorophyll content

Chlorophyll content was affected significantly ($p<0.05$) by water stress in all cultivars (Tables 2 & 3). The results represent significant differences among irrigation treatments. Chlorophyll a, b and total reduction were occurring gradually by increasing water stress in olive leaves. The highest reduction rate of total chlorophyll was recorded in Zard, Amigdalolia and Konservolia cultivars with 24.04, 23.32 and 22.38 % respectively under 50% FC water stress in comparison to control.

Leaf nutrient

Calcium content was gradually decreased by increasing water stress in leaves of olive cultivars from 100 to 50 % of field capacity. In this regard, Amigdalolia had the highest reduction about 33.76 and 46.41 in calcium under 75 and 50 % FC in comparison to the control plants. Calcium content reduction was less in Zard cultivar when exposed to water stress in comparison to other cultivars (Table 3). The content of sodium in olive leaves also changed due to drought stress. Presented data in Table (3) indicated that sodium content gradually increased significantly ($p<0.05$) by increasing water stress in all cultivars. Amigdalolia had the highest increase of about 0.17 % in comparison to other cultivars under normal and severe water stress. Zard cultivar had the lowest increase in sodium content.

Potassium content was gradually decreased significantly ($p<0.05$) by increasing water stress in leaves of olive cultivars. Potassium content was varied among cultivars so that Konservolia and Zard had the highest amount in control plants. The highest potassium reduction was occurred in Konservolia and Zard respectively by severe water stress in comparison to control plants (Table 3). Potassium content of Amigdalolia naturally

Table3. Chlorophyll b, total chlorophyll, Ca, Na and K of olives cultivars under water stress**.**

Cultivars	Water regimes	Chlorophyll b (mg/g FW)	Total Chlorophyll (mg/g FW)	Ca(%)	Na (%)	K(%)
	100 %	1.597^{c}	4.717^{bc}	2.40^{b}	0.150^{e}	1.583^{a}
Zard	75 %	1.680^{c}	4.217^{cd}	2.08^{c}	0.143^{e}	1.323^{bc}
	50%	1.230^{d}	3.583^{d}	1.92^{d}	0.203^{de}	1.180^{cd}
	100 %	1.783^{c}	5.520^{ab}	2.37^{b}	0.320^{bcd}	1.267^{bc}
Amigdalolia	75%	1.777^{c}	4.697^{bc}	1.57^{e}	0.423^{ab}	1.167^{cd}
	50%	1.783^{c}	4.233^{cd}	1.27^{f}	0.487^{a}	0.990^{d}
	100 %	2.033^{b}	6.107^{a}	3.44^{a}	0.213^{de}	1.740^{a}
Konservolia	75%	1.837^{bc}	5.477^{ab}	3.39^{a}	0.277^{cd}	1.503^{ab}
	50%	0.9567^{e}	4.740$^{b\,c}$	2.37^{b}	0.370^{bc}	1.263^{bc}

Different letters indicate significant differences at (P ≤0.05) by Duncan's test.

was lower than other cultivars but its reduction rate was less.

Discussion

In the present study, the phenol content varied among cultivars and water stress treatments. All cultivars receiving drought treatments showed an increasing level of total phenol when the degree of water stress goes up, and hence the highest level of total phenol was recorded at 50% FC irrigation. Phenolic compound synthesis affected by biotic or abiotic environmental factors (53). One of the main roles of phenols is to take part in plant's defence mechanisms (22). Also, phenolic defences have been varied based on species genetically (54). The amount of total phenol was different among cultivars and the highest content of total phenol belonged to Zard, Amigdalolia and Konservolia, respectively. Machado *et al.* (55) reported that a reduction of phenolic content in Cobrançosa olive cultivar was observed when exposed to full irrigation in comparison to dryland and reduced irrigation. Also, an increase in total phenol of two-year-old olive cultivars (Chetoui, Chemlali, and Zalmati, respectively) was reported by Boughalleb and Mhamdi (19). In this experiment, total phenol was increased under water stress and it was in agreement with Machado *et al.,*(55) and Boughalleb and Mhamdi, (19) findings. According to Morello *et al.,* (56) findings, total phenol increasing in cells is contributed to the effect of drought stress by which the activity of phenylalanine ammonialyase (PAL) is enhanced. They also found that PAL activity is strongly related to environmental conditions modulating the rate of total phenol in plants.

Reactive oxygen species (ROS) accumulate under water stress (1) and cells will be damaged via lipid peroxides (7, 8). Under water stress condition, oxidative stresses act as a secondary stress and cause a reduction in cell membrane's stability, photosynthesis process and finally yield of plants (57). In this regard, POX and CAT enzymes counteract the adverse effect of ROS and thereby improving cell membrane's stability and enhance plant growth under such condition (58, 59). In this experiment, leaf peroxidase activity was significantly affected ($p<0.05$) by cultivar and water stress interaction. Response to water stress was cultivar dependent, so that water stress induced the higher leaf peroxidase activity in Konservolia, Zard and Amigdalolia, respectively. The results of this experiment cleared that the peroxidase (POX) activity was lessened at full irrigation (100% FC) whereas it was intensified at 50% FC irrigation.

Liu *et al.,* (60) stated that there is a direct relationship between enhancing oxidative stress and increasing antioxidant enzymes' activity in tolerant cultivars compared to sensitive ones in order to mitigate the deleterious effects of oxidative stresses. Our results were in agreement with prior reports revealing the increased POX activity of olive (10, 13-1561, 62), walnut (63); almond (64), GF rootstock (65), sweet cherry rootstock (66) and banana (67) under water stress conditions.

As plants employ some enzymes like POX and CAT to cope with drought-stress conditions, the level of their activities can be served as assessing markers for determining the extent of plants tolerance to a stress condition. In this research, the activity of CAT on evaluated olive cultivars was scrutinized and the results of this research revealed that irrigation water amount had a significant effect on the activity of CAT at drought treatments compared to control. Our results showed increased activity of CAT in Konservolia, Zard and Amigdalolia, respectively under water stress. Lima *et al.,* (68) explored that plants, possessing these types of enzymes, are able to protect themselves against oxidative damages. In the present study, Konservolia and Zard had the highest amount of mentioned enzyme activity and showed the better tolerance to water stress. The activities of both enzymes are in favour of protecting metabolite processes of plant cells playing a key role in cell survivability against oxidative stresses (69). Our results of this experiment were according to those obtained in olive (10, 16 - 19, 23), mulberry (70), sweet cherry rootstock (66), banana (67), in which the POX and CAT activities increased due to drought stress.

POX and CAT activity was significantly different between cultivar under non-stress conditions. The highest POX activity was observed in Konservolia, Zard and Amigdalolia respectively, but CAT activity was higher in Konservolia, Amigdalolia and Zard respectively. Antioxidants content and antioxidant enzymes activity will be highly variable depending on species or cultivars under drought stress (70).

The disintegration of the cell membrane is one of the effects of water deficit in plants, and there is a direct relationship between malondialdehyde (MDA) and drought stress (22). Petridis *et al.,* (22) revealed that the rates of MDA and total phenol were elevated due to drought stress and their elevation was variable depending on cultivar type and duration of imposing to drought stress. Our results revealed that MDA content was related to cultivar and water stress severity. Many studies reported that MDA increased under water stress in different olive cultivars (17, 19, 22). Our results showed that Konservolia had the lowest lipid peroxidation than the others. The MDA content was increased by increasing water stress severity. The results of this experiment were confirmed by Peterdis *et al.,* (22); Boughalleb and Mhamdi, (19); and Fouad *et al.,* (23) findings.

Cell membranes integrity and stability are one of the most important components of plant tolerance mechanisms under drought stress condition (71, 72). Our results revealed that there were significant differences of electrolyte leakages among olive cultivars under water stress. Electrolyte leakage increased with increasing water stress severity. In term of cultivar, Zard had the lowest electrolyte leakage under water stress condition. The highest electrolyte leakage recorded in Amigdalolia cultivar. Less membrane damage in our study was correlated with less accumulated malondialdehyde level in Zard and Konservolia in comparison to Amigdalolia. Our results indicated that water stress can induce membrane lipid peroxidation more in some olive cultivar and lead to increased electrolytic leakage (Table 2).

In response to drought stress, increasing soluble and active osmotic matters like proline is considered as one of the tolerance methods employed by plants under this condition. (19, 73). Due to drought stress, a high level of proline content in leaves of Meski and Chemlali (74), Bladi, Mary, Roghani, Zard and Mission (26), Chetoui, Chemlali and Zalmati (19) Konservolia (62) olive culti-

vars have been reported. Our results were in accordance with those reports. Proline content was different among olive cultivars under water stress. Zard was superior in proline accumulation than the others under water stress condition.

Depending on the type of cultivar, an increase in the amount of soluble sugars was significantly observed and the highest amount of soluble sugars was obtained in Konservolia at 50% FC irrigation, on the contrary, the lowest was attained in Zard at 100% FC irrigation. Under drought stress condition, accumulation of soluble sugars, including compatible osmotic matters, caused to reduce the level of cells' water potential in favour of remaining more water to keep cell turgor (19). Soluble sugars accumulations seemed to be related to drought tolerance in many plant species. Improving drought tolerance in woody plants contributed to soluble sugars have been reported in black poplars (75), mango (76), so that, high tolerance cultivars exhibited more active accumulation of soluble sugars in comparison to sensitive ones. There were similar findings by Boughalleb and Mhamdi (19), Arzani and Yazdani (25) and Arji and Arzani (26) on carbohydrate accumulation under water stress in olive where our results were in agreement with their findings.

Olive is one of drought-tolerant plant that keeps water up-taking under water stress conditions (77, 78). Olive cultivars vary in drought resistance (79). Drought tolerant cultivar maintains turgor and tolerates to dehydration at low plant water availability (80). Water deficit also decreases the actual water content in the olive leaves (81). Leaf relative water content (RWC) has also been proposed as a more important indicator of water status than other water potential parameters under drought stress conditions (82).

In this experiment, relative water content decreased under water stress treatment and its reduction was cultivar dependent. Zard, Amigdalolia and Konservolia maintain higher RWC under water stress respectively. The higher reduction in RWC was recorded in Konservolia under severe water stress. Relative water content is an important trait in plants under water stress. Since this property indicates the water content of leaves. Therefore, drought tolerance cultivars have the better situation in relation to this trait. Xiloyannis *et al.,* (83) stated that turgor maintenance in olive leaves is one of drought tolerance mechanisms. Drought resistant cultivar maintains higher leaf water content compared to susceptible ones under limited water. The ability of Zard variety to maintain higher RWC was more than the others. The finding of this experiment was in consistent with those obtained by Jorba *et al*., (84); Ben Ahmad *et al.,* (73); Guerfel *et al.,* (18); Ennajeh *et al.,* (85); Boussadia *et al.,* (11) in which RWC was decreased by drought stress.

Under drought stress condition, the amount of chlorophyll a and b significantly decreased in all cultivars. The lowest amount of Chl (a) was observed in Zard irrigated at 50% FC, whereas the lowest amount of Chl (b) was recorded in Konservolia at 50% FC. Also, there was a significant difference in total Chl content of cultivars. The highest rate of total chlorophyll was observed in Konservolia at 100% FC, whereas the lowest rate was recorded in Zard at 50% FC. Chlorophyll loss is a typical sign of oxidative stress (86). Guerfel *et al.,* (18) reported that Chl (a + b) content in Chemlali and Chetoui olive cultivars were reduced to lower content. Similar results were obtained in our research where loss of chlorophyll a, b and total occurred under water stress but it was cultivar dependent.

Leaf nutrients of calcium, sodium and potassium content were significantly different under various water stresses in leaves of olive cultivars. In this regard, calcium reduction was higher in Amigdalolia, Zard and Konservolea leaves respectively. Our findings were in agreement with those obtained by Shaheen *et al.,* (27) in which calcium content in olive leaves was significantly affected by water stress and cultivars. Sodium content also changed due to water stress condition, so that it was increased by increasing water stress. Amigdalioa, Konservolia and Zard showed the higher increase in sodium content respectively. Also, the effect of drought stress on potassium content of leaves in all cultivars was significant under water stress. Potassium content was varied between cultivars. Amigdalolia had the lower amount of potassium content in comparison to the others.

Potassium mainly serves as an important osmoregulator in cherry trees (87). Otoole (88) reported that accumulation of potassium (sometimes sodium and calcium) in the rye and barley leaf after exposing to a long-term drought stress indicates the importance of potassium in osmoregulation mechanism. On the other hand, water relation could be affected by potassium content in olive (89). Many studies have been reported that potassium reduction takes place in some olive cultivars under water stress (27, 90, 91). Regarding a reduction in calcium and potassium content and conversely increasing the sodium content of leaves induced by drought stress, the findings of the current research were in agreement with those obtained by Bacelar *et al.,* (9) and Gholami *et al.,* (32). Olive has been mentioned as a medicinal plant in Holly Quran (92).

Considering compatibility of olive Zard, Konservolia, and Amigdalolia cultivars in semi-tropical arid regions, it is important to determine their tolerance to drought to develop olive cultivation. The findings showed that there is a difference between cultivars in terms of drought tolerance. Konservolia and Zard (native) cultivars, fewer than 50% treatment, were more resistant to drought stress compared to Amigdalolia cultivar due to having relatively high water content, less ionic leakage, lower malondialdehyde and high potassium content. The Zard cultivar showed a decrease in some traits such as chlorophyll b and amount of peroxidase and catalase in some levels of stress compared to Konservolia cultivar; however, this decrease cannot be a sign of Zard cultivar weakness, because it was better in some vegetative traits including number of leaves, leaf area, branch length, and root length (data not shown). In water shortage conditions, therefore, the use of Konservolia and Zard cultivars is recommended.

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