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Effect of drought stress on physiological traits and antioxidant activities in some olive cultivars

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Abstract: Drought is important abiotic stress that negatively influences the growth and development of plants. Strong efforts are currently ongoing worldwide to improve olive production under adverse environmental conditions by extending genetic diversity to improve key agro-physiological and biochemical features through various breeding programs. This research was performed to evaluate the effect of drought stress on the changes of some physiological and biochemical traits in 20 commercial and promising olive genotypes under field conditions during 2015-2017. Fruit oil content as well as some of physiological traits and antioxidant activities under control and drought stress conditions were evaluated. The results of combined analysis of variance (ANOVA) for fruit yield and other measured traits showed that year, irrigation treatments, genotype main effects and their interactions were highly significant. In general, fruit yield, relative water content (RWC), oil content and total soluble proteins (TPs) showed a decreasing trend, whereas the electrolyte leakage, H_2O_2 content and activity of catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POX) displayed an increasing trend in the tested olive genotypes during drought stress. A Principal component analysis (PCA)-based biplot demonstrated that stress tolerance index (STI) positively correlated with POX and TPs. Results also revealed a high level of genetic diversity in the tested olive genotypes, and among them, two commercial (Abou-satl) and promising genotypes (T2) responded better to drought by marinating a good balance for fruit yield and some of the antioxidant activities. These genotypes could be used in future programs to develop new olive cultivars with beneficial stress-adaptive traits.

Key words: Biochemical activities**;** Drought; Olive; Stress tolerance index.

Introduction

Olive (*Olea europaea*) is an evergreen tree and it had been known as one of the oldest cultivated species in the world. The olive tree is known for its tolerance to prolonged drought periods (1). The olives worldwide production is persistently increasing in recent years by 20.8 million tons in 2017, the second highest production level ever accomplished (2).

Among environmental stresses, drought or water deficit is one of the main edaphic stresses for reduction of olive yield and this is particularly true in the Mediterranean basin where the climate is typically characterized by low rainfall and high evaporation (3). Similar to other plants, growth of olive tree is affected by drought stress through a change in anatomical, morpho-physiological and biochemical mechanisms. Under drought conditions, a higher photosynthetic rate is an important factor for better drought tolerance in olive cultivars, and differences in drought tolerance among cultivars can be related to various physiological factors (4). Hence, the development of drought-tolerant varieties of olive is sought through plant breeding programs with better potential to access water in the deeper layer of soil and improved water use efficiency. Fernandez *et al.* (5) stated that knowledge about the mechanisms implied in drought tolerance can help to optimize the water supply in olive orchards. In olive trees, many morpho-physiological and biochemical traits have been found to be associated with drought tolerance through higher water potential gradient between root system and canopy (6), development of osmotic adjustment (7), limitation of water loss through modulation of stomatal closure (8), decrease of leaf area and increase of stomatal density (9). It has been shown the tolerant olive cultivars revealed lower stem growth (10), smaller leaf area and lower stomatal conductance (9), lower leaf water content (11) under drought condition than optimal conditions. However, response to drought stress is different among olive cultivars (11).

During the drought stress, plants have employed various defence systems that allow them to confront with drought stress for continued growth and survival (12). One of the key defence systems occurring during drought stress is an increase in the accumulation of reactive oxygen species (ROS) (13). Various types of

ROS including singlet oxygen ($^1O_2$), superoxide (O_2^-), hydroxyl radical (OH) and hydrogen peroxide (H_2O_2) affect cell membranes, proteins and DNA so that finally leading to cell death (14). To defend against the adverse effects of ROSs, plants have some defence mechanisms to detoxify ROS by producing different types of antioxidants. Antioxidants can be divided into two main groups: [1] non-enzymatic including glutathione (GSH), carotenoids, tocopherols and ascorbate acid (AsA), which together alleviate the effects of oxidative stress and [2] enzymatic including monodehydroascorbate reductase (MDHAR), peroxidase (POX), catalase (CAT), glutathione reductase (GR), ascorbate peroxidase (APX), superoxide dismutase (SOD) and dehydroascorbate reductase (DHAR) (15). It has been reported that the degree of drought tolerance in the olive tree is correlated with the antioxidant capacity (16). For instance, Bacelar *et al.* (4) indicated that drought stress increased lipid peroxidation and this process led to the improvement of drought tolerance in some of the olive cultivars. In a study conducted by Ben Abdallah *et al.* (17), drought stress increased the activity of CAT, GPX and SOD antioxidant enzymes in olive.

So far, several works have focused on olive tree responses to drought stress, however, little information is known about the association between drought tolerance and morphological and biochemical traits in the olive tree. Hence, the main goals of this work were: (i) to assess the response of 20 commercial and promising olive genotypes under drought stress condition in terms of fruit yield and some of the physiological and biochemical traits, and (ii) to study the relationships between fruit yield and measured traits.

Materials and Methods

Plant materials and experimental setup

The study was carried out during two consecutive years (2015-2017) in the Tarom Research Orchard (Latitude 36°47, Longitude 49°6, Altitude 335 m a.l.s.), located in the Zanjan province, from the 100 Km Zanjan City, Iran. The climate was characterized by mean annual precipitation of 145 mm per year, annual minimum temperature of 12.5 °C, annual maximum temperature of 19.5 °C, and mean of annual evaporations 1229.4 mm. The experiment was a two-way factorial arranged in a randomized complete blocks design with three replications. Two levels of irrigation including full irrigation (100% FC) field capacity (control) and drought stress conditions (50% FC) were selected as the first factor. Duration of each irrigation, the second factor was 18-year-old trees of twenty olive cultivars (including 10 new promising genotypes, 3 local cultivars and 7 commercial cultivars). Detailed information on the tested cultivars is in Table 1. During the growth period of the trees until the endocarp stage (until August 3), drought treatments were applied based on 50% of the field capacity (FC = 50%) for each tree. The FC for each plot was estimated according to Gholami et al., (18). Each plot consisted of six tresses, and distance among them was 8 m. From each olive cultivar, two trees were selected to sampling and record parameters as detailed below.

Determination of electrolyte leakage (EL) and relative water content (RWC)

To estimation of electrolyte leakage (EL), 200 mg of fresh leaves were sliced into small discs and kept in 10 mL of double distilled water for 3 h at 37 °C. After incubation, the conductivity of the solution was measured. The solution was then incubated at 95 °C for 30 min, and the conductivity was measured again (value B). Finally, ion leakage was calculated according to the following equation (17);

$$EL\ (\%) = \frac{\text{Conductivity of the initial solution}}{\text{Conductivity of the second solution}} \times 100 \quad (1)$$

The leaf samples of each tested olive tree were used for RWC assay according to Smart and Bingham (19). After fresh weight (FW) determination, leaves were floated on distilled water in the dark for 24 h. Then the turgid weights (TW) were recorded and the samples were transferred into oven drying at 70 °C for 48 h. After determination of dry weights (DW), the RWC for each genotype was estimated by the following equation:

$$RWC\% = \frac{FW\text{-}DW}{TW\text{-}DW} \times 100 \quad (2)$$

Total protein and antioxidant enzymes assays

Leaf total soluble protein content was determined spectrophotometrically based on the method described by Bradford (20) using bovine serum albumin (BSA) as a standard. Crude enzymes were extracted from olive leaves as described by Beauchamp and Fridovich (21). First, 0.5 g of fresh leaves were accurately measured and homogenized at 4°C in 1 mL of 50 mM potassium phosphate buffer (pH 7.0) containing 2% polyvinyl pyr-

Table 1. List of the studied olive genotypes in the present work.

Genotype code	Genotype	Origin	Genotype code	Genotype	Origin
G1	T2	Iran	G11	Zard	Iran
G2	T6	Iran	G12	Roghani	Iran
G3	T7	Iran	G13	Mari	Iran
G4	T10	Iran	G14	Beladi	Lebanon
G5	T17	Iran	G15	Mission	USA
G6	T19	Iran	G16	Manzanilla	Spain
G7	T20	Iran	G17	Koroneiki	Greece
G8	T21	Iran	G18	Kalamata	Greece
G9	T18	Iran	G19	Corfolia	Spain
G10	T24	Iran	G20	Abou-satl	Syria

rolidone (PVP) and 1 mM ethylenediaminetetraacetic acid (EDTA) and the homogenate was centrifuged at 15,000 g for 15 min at 4°C. The supernatant was used as the crude enzyme extract for estimation of hydrogen peroxide (H_2O_2) content and activity of three antioxidant enzymes including catalase (CAT), peroxidase (POX) and ascorbate peroxidase (APX) according to Sergiev *et al.*, (22), Beers and Sizer (23) , Chance and Maehly (24) and Nakano and Asada (25), respectively.

Determination of fruit yield and oil content

Fruits were harvested by hand and the total yield (kg tree $^{-1}$) was determined at the maturity stage. Olive oil extraction was performed following the procedures of the IOOC protocol (26). Briefly, the fresh fruit (without stone) samples were dried at 70 ºC for 48 h. Then 2 g of dried samples were charged to Soxhlet extractor with 500 ml of diethyl ether for 5 hours. In the next step, each sample transferred into the oven for 2 h and dried at 70 ºC. The oil content was estimated through the difference between the two last dried samples.

Statistical analysis

Combined analysis of variance (ANOVA) was performed using SAS software (SAS Institute, Cary, NC, USA). Significant differences among the means of treatments were determined by Duncan's test at $P < 0.05$. To identify the genotype belonging to Fernandez's Groups A, B, C and D (27), a three-dimensional plot was created based on stress tolerance index (STI) and both fruit yields under control and drought stress conditions. An online toolkit, *i*PASTIC (28) was used to calculate STI and illustrate the three-dimensional plot. Principal component analysis (PCA) was used to discover interrelationships among fruit yield and different measured physiological and biochemical traits using the XLSTAT package (Addisonsoft XLSTAT, Paris).

Results

Effect of drought stress on physiological traits and fruit yield

The results of combined analysis of variance (ANOVA) for relative water content (RWC), electrolyte leakage (EL), fruit yield and oil content traits across two years are shown in Table 2. Drought stress affected all of these traits with significant differences ($P \leq 0.01$) among years and tested genotypes. The overall mean of the 20 tested genotypes for EL increased by 34.48%. Under control condition, LE ranged from 18.72 (G9) to 27.03 (G4) with an average of 24.87%, while under drought condition it varied between 29.75 (G9) and 36.95 (G14) with an average of 33.45% (Table 3). Drought stress decreased RWC in the stressed olive trees by 15.44% (Table 2). Under control condition RWC varied between 64.25 and 86.95% with a mean of 72.66% and the highest values recorded in G20 followed by G18 and G10, whereas under drought condition it ranged from 43.82 to 71.58% with a mean of 62.23%. Three genotypes (G20, G15 and G1) showed the highest RWC than among the studied genotypes (Table 3).

Average fruit yield of olive genotypes was signi-

Table 2. Combined analysis of variance, mean values and percentage change due to drought stress in measured traits in the 20 different olive genotypes.

Source of variation	df	Mean square								
		EL	RWC	TPs	POD	APX	POX	CAT	FY	DFO
Year (Y)	1	484.67**	317.56*	3270.60**	5.95**	15280.10*	59.53**	556.62**	5782.02**	43.94**
R/Y	4	24.41	188.182	560.73	0.04	37681	6.76	1016.66	80.60	645.26
Irrigation (I)	1	4417.67**	7553.192**	4.41^{ns}	21.34**	6085530**	30.06**	23631.40**	2088.60**	383.29**
Y×I	1	5.21^{ns}	1423.94**	578.98**	1.46*	14.50^{ns}	4.44**	6176.28^{ns}	66.15^{ns}	7.6^{ns}
Genotype (G)	19	44.80**	338.58**	4641.89**	0.39**	198560**	9.36**	4931.65**	1800.26**	472.19**
Y×G	19	54.94**	177.93**	1042.70**	0.59**	81316.40**	5.84**	1026.30^{ns}	1520.86**	72.03**
I×G	19	10.62^{ns}	118.88**	283.98**	0.32**	55361.30**	6.04**	2907.86**	110.94*	10.89**
Y×I×G	19	11.71^{ns}	79.86**	351.47**	0.23**	36869.50**	4.96**	1373.07**	70.22^{ns}	15.86^{ns}
Error	156	12.42	51.65	29.68	0.01	3044.66	0.57	376.04	61.34	13.05
Coefficient of variance (%)		12.08	10.72	3.62	11.86	21.42	48.02	33.71	18.79	6.42
†Mean for optimal condition		24.88 ^{b}	72.66 ^{a}	152.7908	0.62 ^{b}	207.18 ^{b}	1.23 ^{b}	46.05 ^{b}	45.08 ^{a}	57.45 ^{a}
Mean for drought condition		33.46 ^{a}	61.44 ^{b}	147.8256	1.22 ^{a}	307.88 ^{a}	1.94 ^{a}	69.00 ^{a}	39.18 ^{b}	54.93 ^{b}
††Percentage of change due to drought		-34.48	15.44	3.24	-96.77	-48.61	-57.72	-49.83	13.08	4.38

ns, * and ** Non-significant, significant at 0.05 and 0.01 probability levels, respectively. EL, RWC, TPs, POD, APX, POX, CAT, FY and DFO indicate electrolyte leakage, relative water content, total soluble protein (mg g^{-1} FW), H_2O_2 content (m mol), ascorbate peroxidase activity (U g^{-1} FW.min), peroxidase activity (U g^{-1} FW), catalase activity (U g^{-1} FW), fruit yield (kg tree^{-1}), dried fruit oil content (%) and oil content fresh fruit (%), respectively. † The different letters indicate significant difference by Duncan's test. ††Negative numbers show value higher than the control condition.

Table 3. Mean values of measured traits in the 20 olive genotypes under control and drought stress conditions over two years.

Code	EL		RWC		FY		DFO		TPs		POD		APX		CAT		POX	
	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D
G1	26.26	34.22	71.3 $^{b-h}$	67.2 $^{c-k}$	57 $^{a-e}$	52.66 $^{b-g}$	66.8 ^{a}	60.1 $^{b-g}$	174.0 ^{bc}	156.0 ^{ef}	0.61 $^{k-n}$	1.05 ^{fg}	0.61 $^{k-n}$	1.05 ^{fg}	38.25 $^{j-o}$	69.25 $^{d-l}$	1.85 $^{c-i}$	4.27 ^{a}
G2	26.53	33.838	73.0 $^{b-g}$	55.0 $^{j-l}$	33.66 $^{i-p}$	27.66 $^{m-p}$	61.4 $^{a-e}$	60.7 $^{b-g}$	153.2 ^{f}	151.6 ^{f}	0.47 $^{n-p}$	1.02 ^{ge}	0.47 $^{n-p}$	1.02 ^{ge}	54.25 $^{f-n}$	108.25 ^{b}	2.85 $^{b-d}$	0.68 $^{h-j}$
G3	26.94	35.113	67.3 $^{c-k}$	54.5 ^{kl}	64.66 ^{ab}	54 $^{b-g}$	60.3 $^{b-g}$	57.8 $^{b-g}$	167.3 ^{cd}	165.1 ^{cd}	0.53 $^{l-o}$	1.27 ^{e}	0.53 $^{l-o}$	1.27 ^{e}	34.50 $^{l-o}$	62.00 $^{f-m}$	0.90 $^{f-g}$	1.56 $^{d-j}$
G4	27.03	32.308	74.8 $^{a-f}$	58.4 $^{h-k}$	32.66 $^{j-p}$	31 $^{k-p}$	58.0 $^{d-j}$	57.1 $^{e-k}$	137.2 $^{g-i}$	125.7 ^{k}	0.56 $^{l-o}$	1.10 ^{fg}	0.56 $^{l-o}$	1.10 ^{fg}	77.50 $^{b-i}$	101.00 $^{b-d}$	0.66 $^{h-j}$	0.44 ^{j}
G5	25.38	34.707	79.8 $^{a-d}$	71.4 $^{b-h}$	40 $^{g-n}$	36.66 $^{h-o}$	64.0 $^{a-d}$	61.1 $^{a-f}$	162.7 ^{de}	152.9 ^{f}	0.55 $^{l-o}$	0.84 ^{hi}	0.55 $^{l-o}$	0.84 ^{hi}	63.50 $^{e-m}$	51.25 $^{f-o}$	0.36 ^{j}	4.65 ^{a}
G6	26.33	35.332	73.5 $^{b-g}$	61.6 $^{e-k}$	31.16 $^{k-p}$	24.5 ^{op}	65.4 ^{ab}	64.9 $^{a-c}$	124.7 ^{k}	124.5 ^{k}	0.52 $^{l-o}$	1.30 ^{e}	0.52 $^{l-o}$	1.30 ^{e}	64.50 $^{e-l}$	104.00 ^{bc}	0.51 $^{h-j}$	0.73 $^{h-j}$
G7	22.41	32.018	67.9 $^{c-k}$	59.5 $^{e-k}$	50.16 $^{c-g}$	42.5 $^{f-l}$	61.5 $^{a-e}$	54.8 $^{g-l}$	168.4 $^{b-d}$	163.1 ^{de}	0.69 $^{i-l}$	1.20 ^{ef}	0.69 $^{i-l}$	1.20 ^{ef}	21.50 ^{no}	36.75 $^{k-o}$	0.86 $^{g-j}$	1.89 $^{c-h}$
G8	22.30	31.162	72.1 $^{b-h}$	59.9 $^{g-k}$	35.16 $^{h-o}$	32 $^{j-p}$	52.2 $^{j-m}$	50.0 $^{l-n}$	135.1 $^{g-j}$	124.6 ^{k}	0.81 $^{h-j}$	1.50 ^{cd}	0.81 $^{h-j}$	1.50 ^{cd}	29.00 $^{m-o}$	72.25 $^{c-j}$	0.63 $^{h-j}$	0.49 ^{ij}
G9	18.72	29.757	72.2 $^{b-h}$	57.4 $^{i-k}$	26.33 $^{n-p}$	24.83 ^{op}	48.5 $^{m-o}$	48.4 $^{m-o}$	170.7 $^{b-d}$	166.7 ^{cd}	0.75 $^{i-k}$	1.30 ^{e}	0.75 $^{i-k}$	1.30 ^{e}	47.50 $^{f-o}$	55.75 $^{f-n}$	3.74 ^{ab}	2.95 ^{bc}
G10	26.99	35.623	80.8 $^{a-c}$	60.8 $^{f-k}$	29 $^{o-p}$	20.33 ^{p}	58.6 $^{d-i}$	56.4 $^{e-k}$	123.5 ^{k}	140.8 ^{g}	0.65 $^{j-m}$	1.58 ^{c}	0.65 $^{j-m}$	1.58 ^{c}	50.00 $^{f-o}$	64.00 $^{e-m}$	2.87 $^{b-d}$	2.43 $^{c-e}$
G11	25.52	36.66	70.8 $^{b-i}$	43.8 ^{l}	69.83 ^{a}	49.66 $^{c-g}$	58.4 $^{d-j}$	58.1 $^{d-i}$	177.0 ^{ab}	164.8 ^{cd}	0.43 ^{op}	1.10 ^{fg}	0.43 ^{op}	1.10 ^{fg}	16.75 ^{o}	48.00 $^{f-o}$	0.98 $^{f-g}$	2.75 $^{b-d}$
G12	24.43	34.182	68.3 $^{c-k}$	68.1 $^{c-k}$	51.5 $^{b-g}$	49.5 $^{c-g}$	62.1 $^{a-e}$	59.4 $^{b-h}$	128.4 $^{i-k}$	127.9 $^{i-k}$	0.48 $^{m-p}$	1.96 ^{a}	0.48 $^{m-p}$	1.96 ^{a}	45.75 $^{h-o}$	81.00 $^{b-g}$	0.74 $^{h-j}$	0.58 $^{h-j}$
G13	25.86	30.893	70.1 $^{b-i}$	65.1 $^{e-k}$	43.83 $^{d-k}$	31.33 $^{k-p}$	53.4 $^{h-m}$	52.7 $^{i-m}$	183.7 ^{a}	167.0 ^{cd}	0.77 $^{i-k}$	1.05 ^{fg}	0.77 $^{i-k}$	1.05 ^{fg}	25.50 ^{no}	46.00 $^{g-o}$	0.69 $^{h-j}$	0.88 $^{f-j}$
G14	24.50	36.95	67.0 $^{d-k}$	61.8 $^{e-k}$	49 $^{c-h}$	40.66 $^{f-m}$	60.7 $^{b-g}$	56.6 $^{e-k}$	137.9 ^{gh}	124.2 ^{k}	0.67 $^{i-l}$	1.36 ^{de}	0.67 $^{i-l}$	1.36 ^{de}	63.00 $^{k-o}$	78.75 $^{b-h}$	0.84 $^{h-j}$	1.06 $^{f-g}$
G15	24.49	33.04	68.7 $^{b-j}$	65.3 $^{c-k}$	45.83 $^{d-j}$	40.33 $^{g-n}$	60.8 $^{b-g}$	56.5 $^{e-k}$	169.1 $^{b-d}$	176.8 ^{ab}	0.33 ^{p}	0.73 $^{i-k}$	0.33 ^{p}	0.73 $^{i-k}$	22.50 ^{no}	35.00 $^{l-o}$	0.94 $^{f-j}$	2.21 $^{c-g}$
G16	25.577	33.558	64.2 $^{e-k}$	55.2 $^{j-l}$	45.5 $^{d-j}$	43.33 $^{g-n}$	53.1 $^{i-m}$	51.3 $^{k-m}$	135.3 $^{g-j}$	131.1 $^{h-k}$	0.73 $^{i-k}$	1.07 ^{fg}	0.73 $^{i-k}$	1.07 ^{fg}	36.50 $^{k-o}$	67.50 $^{d-l}$	1.24 $^{e-j}$	4.39 ^{a}
G17	26.292	36.385	67.4 $^{c-k}$	62.8 $^{e-k}$	47.16 $^{c-i}$	43 $^{e-l}$	59.3 $^{c-h}$	57.7 $^{e-j}$	171.9 $^{b-d}$	170.4 $^{b-d}$	0.84 ^{hi}	0.94 ^{gh}	0.84 ^{hi}	0.94 ^{gh}	81.25 $^{b-f}$	25.50 ^{no}	0.85 $^{h-j}$	1.75 $^{c-g}$
G18	24.81	31.268	82.0 ^{ab}	68.3 $^{c-k}$	27.66 $^{m-p}$	24.83 ^{op}	55.2 $^{f-l}$	52.9 $^{i-m}$	128.8 $^{h-k}$	127.9 ^{jk}	0.74 $^{i-k}$	0.83 ^{hi}	0.74 $^{i-k}$	0.83 ^{hi}	71.00 $^{c-k}$	97.42 $^{b-e}$	0.55 $^{h-j}$	1.19 $^{e-j}$
G19	21.892	30.3	75 $^{a-e}$	63.7 $^{e-k}$	57.66 $^{a-d}$	54.66 $^{b-f}$	45.5 $^{n-p}$	41.8 ^{pq}	171.5 $^{b-d}$	169.6 $^{b-d}$	0.49 $^{m-p}$	1.35 ^{de}	0.49 $^{m-p}$	1.35 ^{de}	34.75 $^{l-o}$	36.75 $^{k-o}$	0.91 $^{f-j}$	1.69 $^{c-j}$
G20	25.242	31.875	86.9 ^{a}	71.6 $^{b-h}$	63.83 ^{ab}	60.16 $^{a-c}$	43.2 $^{o-q}$	39.5 ^{q}	135.3 $^{g-j}$	125.8 ^{k}	0.76 $^{i-k}$	1.77 ^{b}	0.76 $^{i-k}$	1.77 ^{b}	43.50 $^{i-o}$	139.50 ^{a}	1.67 $^{c-j}$	2.22 $^{c-f}$

C and D indicate control and drought stress conditions, respectively. The different letters indicate significant difference by Duncan's test.

ficantly different ($P \leq 0.01$) between two years and growth conditions (Table 2). As shown in Table 2, drought stress reduced average fruit yield by 13%. Under control condition, fruit yield ranged from 29 to 69.83 kg tree^{-1} and genotypes G3, G11 and G20 produced the highest yield, whereas G9, G10 and G18 produced the lowest yield compared with other genotypes. Under drought condition, a wide range of variability was showed for fruit yield among the tested genotypes so that it varied between 20.33 and 60.16 kg tree^{-1}. Three genotypes G20 (60.16 kg tree^{-1}), G19 (54.66 kg tree^{-1}) and G1 (52.66 kg tree^{-1}) were identified as the high-yielding genotype, while G7 (24.5 kg tree^{-1}), G10 (24.83 kg tree^{-1}) and G10 (20.33 kg tree^{-1}) produced the lowest yield. Our results showed that oil content across all tested genotypes is not closely related to the year and water supply. In other words, no significant difference was observed between a year and drought treatments. However, there was a significant difference ($P \leq 0.01$) among genotypes for this trait and interaction effects between a year with genotype and stress with genotypes were significant. Drought stress declined the average of the oil content (~5%) compared to control condition. Under control condition, oil content ranged from 43.28 to 66.81% and genotypes G1, G6 and G5 with 66.81, 65.45 and 64.01% showed the highest oil content, whereas under drought stress the range of this trait varied between 39.51 and 64.90% and genotypes G6, G5 and G2 were identified as the best genotypes compared with others by 64.9, 61.16 and 60.78% oil content, respectively.

Effect of drought stress on biochemical traits

The results of combined ANOVA showed that significant differences ($P \leq 0.01$) occurred in the total soluble protein (TPs) and the activity of (CAT), peroxidase (POX), ascorbate peroxidase (APX) and hydrogen peroxide (H_2O_2) between years (except for CAT), drought conditions (except for TPs) and genotypes. Also, the interaction between the main factors was significant ($P \leq 0.01$) for all biochemical traits (Table 2). Drought stress decreased TPs by 3.25% in relative to the control condition. At control condition, TPs ranged from 123.45 to 183.74 with an average of 152.79 mg g^{-1} FW, whereas under drought condition it ranged from 124.45 to 176.79 with a mean of 147.83 mg g^{-1} FW. All genotypes had higher TPs content at control than drought stress condition. However, as a result, two groups of tested genotypes including G1, G13 and G17 under control and G13, G15 and G19 under drought stress condition accumulated more TPs than the other genotypes (Table 3). This result reveals that increasing protein content during drought stress is an adaptive mechanism in these genotypes. On the contrary, drought stress treatment significantly increased hydrogen peroxidase content (POD) by 96.44% compared with the control condition (Table 2). With respect to mean comparisons, all tested genotypes produced a high level of H_2O_2 due to drought stress and among the G9, G10, G12 and G20 produced the highest H_2O_2 content, whereas G17, G15 and G18 produced the lower content than others (Table 3).

According to our results, drought stress significantly increased CAT activity by 49.83% as compared with the control condition. Under control and drought stress conditions, the tested olive genotypes exhibited a wide range of variability for CAT activity. Under control condition, CAT varied between 16.75 and 81.25 Ug^{-1} FW and genotypes G4, G7, G17 and G18 showed a significant increase in CAT than other genotypes. In contrast, under drought stress, CAT activity showed variability between 25.50 and 139.50 U g^{-1} FW. At this condition, the highest activity occurred in G2, G4, G7 and G20 genotypes. Among studied genotypes, G4 and G7 showed the higher activity of CAT in both conditions, hence these genotypes can be a candidate as the most tolerant in relative to other genotypes (Table 3). Drought stress significantly increased APX activity by 48.61% relative to the control condition. Under both conditions, there was a high level of variability among tested genotypes so that it ranged from 77.17 to 430 U g^{-1} FW, and 80 to 597.83 U g^{-1} FW under control and drought stress conditions, respectively. Based on mean comparisons, genotypes G7, G9 and G12 for control condition and G12, G14 and G19 for drought stress condition were selected as the best genotypes (Table 3). Peroxidase (POX) as another antioxidant enzyme is widely found in plants, and oxidizes a vast array of compounds in the presence of hydrogen peroxide. Our results showed that POX activity significantly affected by drought stress (57.47%) (Table 2). The activity of POX under control/stress conditions significantly varied between 0.36/0.44 and 3.74/4.65 U g^{-1} FW. Among the tested olive genotypes, two genotypes G1 and G10 showed a high level of activity of POX under both growth conditions (Table 3).

Stress tolerance index (STI) and grouping olive genotypes

Among drought tolerance indices, STI has been suggested as an important selection criterion because it identifies genotypes with high yield and stress tolerance potentials. Our results indicated that genotypes with STI > 1 had a relative tolerance to drought stress. The STI results demonstrated that following genotypes showed a high value of STI (in brackets); G1 (1.70), G5 (1.68), G2 (1.49), G17 (1.27), G19 (1.26), G9 (1.15) and G13 (1.13) (Figure 1A). To identify the genotype belonging to Fernandez's groups A, B, C and D, a three-dimensional plot was created based on STI and both fruit yield under control and drought stress conditions (27). According to this theory; group A consist the genotypes with relatively uniform performance in both control and stress conditions, B comprise the genotypes with high performance in control condition, group C includes the genotypes with high performance in stress condition and group D consist the genotypes with low performance in both control and stress conditions. As shown in Figure 1B, genotypes G1, G3, G11, G12, G19 and G20 with the high fruit yield in both control and drought stress conditions were identified as the most tolerant genotypes compared with other genotypes. Group B consisted of genotypes G7, G14, G15 and G16. The rest of genotypes (G2, G4, G5, G6, G8, G9, G10, G13, G17 and G18) placed into Group D.

Principal component analysis (PCA)

Principal component analysis (PCA) was computed to determine the interrelationships between different

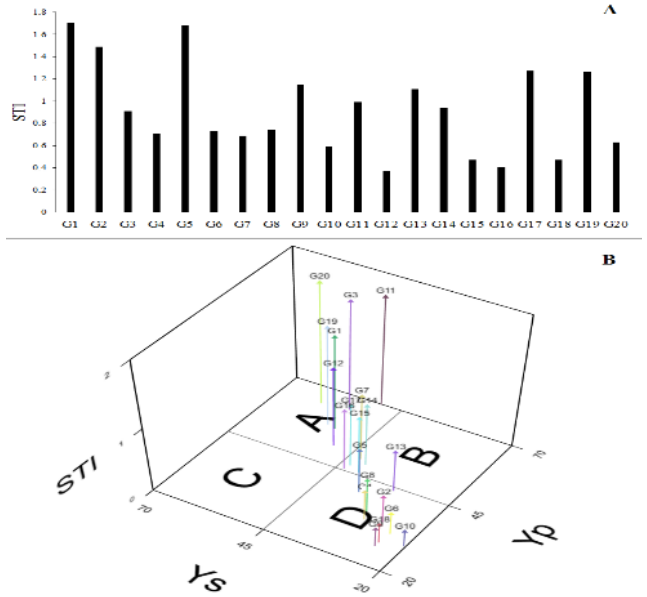

Figure 1. STI values in 20 olive genotypes tested under two non-stress and drought stress conditions (A) and three-dimensional plots rendered based on STI and fruit yields under non-stress (Yp) and stress (Ys) conditions over two years (B). See Table 1 for definitions of genotypes.

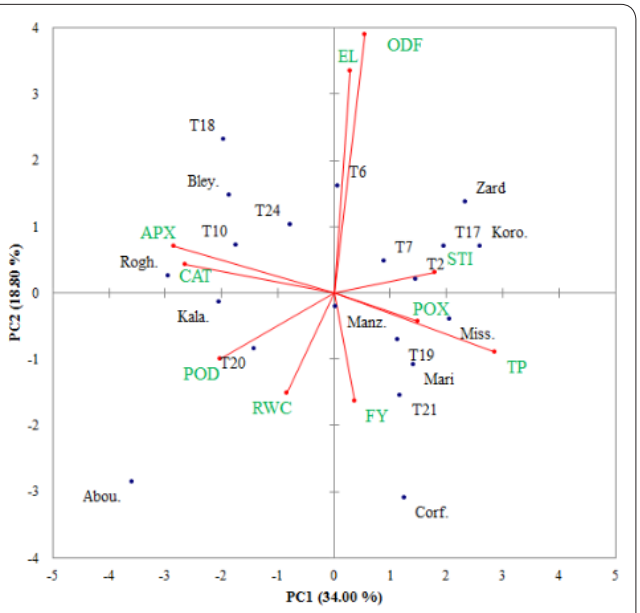

Figure 2. Principal component analysis (PCA) based on several physiological and biochemical traits in 20 olive genotypes across two years under drought stress condition. EL, RWC, TPs, POD, APX, POX, CAT, FY, DFO and STI indicate electrolyte leakage, relative water content, total soluble protein, H_2O_2 content, ascorbate peroxidase activity, peroxidase activity, catalase activity, fruit yield, oil content and stress tolerance index, respectively. See Table 1 for definitions of genotypes.

measured traits. Our results showed that the two first principal components (PCs) accounted for 52.79% of the total variation of physio-biochemical and yield traits under drought stress condition. As shown in Figure 2, PC1 justified 33.99% of the total variation, and strongly correlated with El, TP, POX activity, FY, DFO and STI. On the other hand, PC2 accounted for 18.79% of the total variation and significantly affected by TPs, APX, CAT activities and DFO. Hence, in order to the selection of genotypes using different measured traits a biplot based on the two top PCs was performed. The biplot analysis of the fruit yield and some physiological and biochemical traits of 20 olive genotypes indicated that the CAT, POD and APX activities as well as RWC were positively associated with several olive genotypes, whereas the fruit yield, TPs and STI were positively associated with a number of olive genotypes. For example, genotypes G9, G7, G16, G13 and G15 linked with fruit yield, TPs and POX activity. APX and CAT activities were associated with G18, G12, G4, G10 and G14, whereas RWC and POD activity were associated with G8 genotype. Other associations between genotypes and other traits are shown in Figure 2. In addition, the biplot rendered by PCA was used to discovery associations among different measured traits. Based on the cosine angle between trait vectors, RWC positively correlated with FY and POD activity. The correlations among POX, TP and STI were positive and significant. Furthermore, CAT, APX and POD showed a strong correlation with each other. Also, the correlation between EL and DFO found to be positive and significant.

Discussion

Drought stress is one of the most important edaphic stresses affecting plant growth and development (29). Plant cells respond to drought by inducing scavenging of reactive oxygen species (ROS) and activating antioxidant defence compounds (30, 31). In the present study, 20 olive genotypes were evaluated for their physiological and biochemical traits under drought stress conditions. Our results showed that the response to drought stress differed among the tested genotypes (Table 2). Under drought stress condition, some of the measured traits such as RWC, TP, FY and DFO decreased by varying degrees. Of these, the highest reduction was observed for RWC (15.44%) and FY (13.08%). However, two traits DFO and TP showed the lowest reduction (4.38 and 3.24%, respectively) as compared with the control condition (Table 2). RWC is a key physiological trait of the grade of tissue hydration that is crucial for optimum biochemical functioning and growth cycles in plants (32). In this study, the reduction in RWC in tested olive genotypes was agreed with previous reports in olive (17), and some of the tested genotypes such as G20, G15 and G1 exhibited the best situation for this parameter. Hence, it seems that these genotypes have a good capability in maintaining the water in their tissues and cells (12). Ennajeh *et al.*, (11), stated that late embryogenesis abundant (LEA) and dehydrin (Dhn) proteins are two important chaperons that accumulate in response to drought. However, the accumulation of soluble protein varies among plant species. Our results indicated that drought stress decreased TPs only by 3.25% in relative to control condition, suggesting that the entire tested genotypes well responded to drought stress. Besides, this result reveals that the lowest reduction in protein content during drought stress is a tolerance mechanism for tolerant genotypes.

Despite average of fruit yield across all tested genotypes reduced almost 13% less than the control condition, fruit yield in the drought condition (39.18 kg tree^{-1}) was not notability lower than the control condition (45.08 kg tree^{-1}). This result shows that this irrigation treatment could be applied in commercial orchards wit-

hout affecting yield (33). Similarly, it has been reported that fruit yield reduced 30% in olive trees (cv. Cornicabra) in resulting drought stress (34). Ghrab *et al.*, (35) reported that fruit yield decreased significantly due to drought stress in olive. However, fruit yield for olive trees irrigated when the stem water potential dropped below 2.5 MPa, was statistically similar to the control (10). As a result, three genotypes G20 (60.16 kg tree^{-1}), G19 (54.66 kg tree^{-1}) and G1 (52.66 kg tree^{-1}) can be identified as the most tolerant genotypes to use in drought-prone environments. In the present study, STI differed among the olive genotypes, which is in accordance with results of other studies where this index distinguished the tolerant genotypes (such as G1, G29 and G20) from sensitive ones (29, 36, 37, 38, 39, 40, 41).

In the present study, our results showed that oil content across all tested genotypes is not closely related to the water supply. In other words, no considerable difference was observed between drought treatments (~5% in relative to control). However, there was a significant difference among genotypes for this trait and interaction effects between a year with genotype and stress with genotypes were significant. A similar result regarding the low effect of drought stress on oil content in olive was reported by Breton *et al.*, (42). Moreover, several works have shown that drought stress may increase oil content in comparison to optimal conditions. Motilva *et al.*, (43) indicated an increasing pattern for oil content in the olive tree under the rainfed condition and stated that rainfall watering probably restores the initial rate for oil accumulation. Indeed, Brescia *et al.,* (44) confirmed a strong correlation between loss of water accessibility and accumulation of oil in the olive tree. However, our results suggest that oil content is programmed in the tested genotypes and relatively dependent to both the genetic factors and environmental conditions (45) so that some of the genotypes showed a similar pattern in both growth environments. The ion leakage is a parameter of cell membrane stability and integrity, which is usually considered as one of the best indicators of drought tolerance in plants (46). The increase of EL in all tested genotypes indicated that they have a range of variability in response to drought stress. Hence, similar to RWC, these traits can commonly be used as a key physiological indicator of the water status of the plant and a useful trait for drought tolerance (17).

Under drought conditions, ROS accumulate and then cells will be damaged via lipid peroxides. At this condition, oxidative stresses induce as secondary stress and cause a induction in various changes in plant growth and development and finally yield performance (47). Plants have some defence mechanisms to scavenging ROS. One of the important mechanisms is the antioxidant defense system. This system can be divided into two enzymatic (such as glutathione reductase (GR), ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD) and peroxidase (POX)) and non-enzymatic (such as ascorbate acid (AsA), glutathione (GSH), carotenoids and tocopherols) components (15). In this regard, some of the antioxidant enzymes such as CAT, POX and APX counteract the negative effects of ROS and thereby improving plant growth under such condition (48). Owing to their ROS-scavenging activities, the levels of the key antioxidant enzymes CAT, POX and APX were examined in the different olive tree as a means to identify genotypes with enhanced antioxidant activities. These examined antioxidant enzymes exhibited a reverse trend in comparison with TP, RWC, FY and DFO traits in the tested genotypes in response to drought stress (Table 2). Drought stress significantly increased hydrogen peroxidase content (POD) by 96.77% compared with the control condition (Table 2). APX is widely dispersed in plant cells, and different isoforms are more efficient in removing H_2O_2 under stressor conditions (29, 49, 50). Drought stress significantly increased APX activity by 48.61% than the control condition. Under both conditions, there was a high level of variability among tested genotypes so that genotypes G12, G14 and G19 showed the highest APX activity (Table 3). Under drought condition, CAT turns over quickly in leaf cells and is vital for the eliminating of free oxygen radicals formed in the peroxisomes by photorespiration (50). Higher CAT activity improves membrane stability due to reducing H_2O_2 levels in cells by breaking it down directly to form oxygen and water (50). According to our results, drought stress significantly increased CAT activity (49.83%). Under control and drought stress conditions, the tested olive genotypes exhibited a wide range of variability for CAT activity. Under drought, the highest activity has occurred in G2, G4, G7 and G20 genotypes. With respect to the selected genotypes, G4 and G7 showed the higher activity of CAT in both conditions, thus these genotypes can be a candidate as most tolerant as in relative to other genotypes (Table 3). Indeed, the higher CAT activity in the aforesaid olive genotypes suggests the more effective H_2O_2 removal, which might be produced by an enhanced tolerance at drought-prone conditions (1). Peroxidase (POX) as another antioxidant enzyme is found in plants. Several important physiological functions including lignin biosynthesis, ageing, defensive responses and control of cell enlargement and protection of plants from ultraviolet radiation stress for POX have been suggested (51). Our results showed that POX activity significantly affected by drought stress (57.72%) (Table 2). Among the tested olive genotypes, two genotypes G1 and G10 indicated a higher level of activity of POX than other genotypes (Table 3). The equilibrium between the production and detoxification of ROS at the intracellular level is a key mechanism for plant stress tolerance, and the drought-induced overproduction of ROS may result in oxidative damage to membrane lipids. Changes in the activities of APX, CAT and POD in response to abiotic stresses have been reported in olive and other plant crops (52, 17, 12). Similarly, Cansev *et al.,* (51) and Hashempour *et al.*, (53) found high levels of the POD, SOD, APX and CAT activities in the olive in response to low-temperature stress relative to control conditions.

The principal component analysis revealed a positive and significant correlation between antioxidant activities and other traits. RWC is positively correlated with FY and POD activity. The correlations among POX, TP and STI were positive and significant. Correlations between CAT with APX and POD and between STI with POX and TP are noteworthy (Figure 2). These results are in agreement with previous studies, which reported that some of the antioxidant activities and physiological traits are directly correlated with final yield

and a degree of tolerance in olive and other plant crops (12, 31, 53). For instance, under cold treatment, Cansev et al. (52), reported that CAT activity is directly associated with the degree of cold-hardiness in olive leaf tissue. Hashempour *et al.,* (53) indicated a significant relation between APX, CAT and POD activities with frost tolerance of olive trees.

Changes in some of the physiological and biochemical activities may be one of the important steps for increasing olive cultivation, especially in tropical and subtropical zones suffering from limited water resources. Our results revealed that some of twenty commercial and promising genotypes like G20 and G1 responded better to drought by marinating a good balance for fruit yield and some of the antioxidant activities. These results suggest that these genotypes may cope better with drought and could be more suited to be cultivated in drought-prone zones.

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