Climate change due to heat and drought stress can alter the physiology of Maratheftiko local Cyprian grapevine variety

Antonios Chrysargyris, Panayiota Xylia, Omiros Antoniou and Nikos Tzortzakis

ABSTRACT

The effect of climate change (CC) on viticulture in Europe is of great concern and several international and indigenous grapevine cultivars have been examined for their adaptation to CC. In this study we focused on the short-term effects of light and moderate drought stress (DS) as well as heat stress (HS) on physiological and biochemical stress markers in Maratheftiko cultivar. We showed that leaf photosynthetic rate was decreased with DS and HS after 8 and 20 days. The leaf stomatal conductance was decreased in the case of DS after 8 days, while no differences could be found due to HS. Total phenols and flavonoids content and antioxidant capacity (FRAP and ABTS) were increased and seemed to be dependent on the relevant DS, HS and the period of stress exposure. Chlorophyll fluorescence was decreased in 50% volumetric water content (VWC) after 8 days of DS compared with the 100% VWC (control treatment). Leaf K and P content increased in moderate (50% VWC) irrigation stress and HS. Leaf hydrogen peroxide and lipid peroxidation increased after 8 days of DS, and this resulted in the increase of antioxidant enzymes activity. Overall, Maratheftiko performance against environmental stresses is related more to short-term DS than HS.

Key words | antioxidants, climate change, drought stress, enzymes activity, heat stress

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INTRODUCTION

Climate change (CC) nowadays is a fact, with countless effects on the environment and living organisms. CC includes global warming and lengthening of dry periods, while the decreased stratospheric ozone layer and expansion of the ozone hole (Muller 2009) is affecting plants' physiology and productivity. On the other hand, drought stress (DS) is considered one of the major abiotic factors that impede the metabolism of the higher plants (Chaves *et al.* 2009). Plants' physiological parameters are altered in response to abiotic stresses, such as heat stress (HS) and DS, with decrease in stomatal conductance and changes in leaf structure to sustain the positive turgor pressure, affecting the chlorophyll binding proteins and photosynthetic responses (Chaves *et al.* 2003). Adaptation to various abiotic stresses, including water deficiency, is related to changes in leaf anatomical traits and plant developed mechanisms, by lowering leaf surface/volume ratio, leaf rolling, higher density of stomata, decreasing the stomata size, and presence of smaller size cuticle on the leaf surface (Bosabalidis & Kofidis 2002; Kulkarni *et al.* 2007).

Grapevines (*Vitis vinifera* L.) are well expanded crops of great economic importance. Grapevines grown in the Mediterranean zone are often exposed to drought conditions because of the high evaporative demand and low soil water availability (Patakas *et al.* 2005). During the summer period, grapevines cultivated in the Mediterranean basin

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are often subjected to various environmental stresses including strong winds, high air temperature (heat waves), and soil and atmospheric water deficits (Beis & Patakas 2012). Usually, plants grown under field conditions experience more than one stress simultaneously.

In the future, water demands are expected to increase sharply and it is obvious that irrigated agriculture will become a primary user of water, especially in drought situations. Studies on mechanisms of grapevines' responses to irrigation management techniques (i.e., regulated deficit irrigation, partial root drying and/or low-quality irrigation water by using treated waste water) at morphological, physiological and molecular level have been conducted over the past decades (Mendoza-Espinosa *et al.* 2008; Chaves *et al.* 2009). The strategy of deficit irrigation is based on the fact that imposing water stress during plant growth has minimal effects on yield while it could also lead to an improvement in the quality of the product (Goodwin & Jerie 1992). The effective use of irrigation water is considered as a key component of preventing DS and maintaining plant productivity. However, several studies seem to confirm grapevine defense mechanisms against drought (Beis & Patakas 2012).

Plant sensitivity or tolerance to combined stresses remains a highly complicated issue and is not easily understood (Alexieva *et al.* 2001). Under stress conditions, plants accumulate reactive oxygen species (ROS), such as the superoxide anion ($O_2^{-\cdot}$), hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2), and singlet oxygen (O_2^1), which are normally produced by metabolism in all cellular compartments (Bolisetty & Jaimes 2013). The limitation of CO_2 assimilation in water-stressed plants causes the over-reduction of the photosynthetic electron chain. Since leaf cannot dissipate the excess light energy, there is a redirection of photon energy into processes that favor the production of ROS (Doupis *et al.* 2013). ROS can destroy normal metabolism through oxidative damage of lipids, proteins, and nucleic acids when they are produced in excess owing to oxidative stress (Gill & Tuteja 2010; Chrysargyris *et al.* 2018). An increased capacity to scavenge or detoxify ROS is a well-known mechanism of plant adaptation to environmental stresses and occurs through a plant's ROS defense network, which consists of enzymatic and non-enzymatic components (Chrysargyris *et al.* 2018). Therefore, in order to overcome oxidative-related stress, together with non-enzymatic antioxidant molecules (ascorbate, glutathione, α-tocopherol, proline, etc.), plants detoxify ROS by upregulating antioxidative enzymes like superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), glutathione peroxidase (GPX), glutathione *S*-transferases (GST), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), glutathione reductase (GR), and monodehydroascorbate reductase (MDHAR) (Turkan & Demiral 2009). Thus, an increase in the activities of these enzymes is believed to closely correspond to the ability of plants to withstand a variety of abiotic stressors (Costa *et al.* 2002).

Hence, the aim of the present study was to elucidate and compare the effects of drought and HS on grapevine growth parameters and biochemical stress markers as well as to define grapevine adaptation mechanisms in response to the above stressors.

MATERIALS AND METHODS

Plant material and treatments

The research was carried out at the Cyprus University of Technology, Limassol, Cyprus during the spring–summer period (2017). The local red grapevine (*Vitis vinifera* L.) variety Maratheftiko was used. Maratheftiko is a red grapevine variety, in which does not have hermaphrodite flowers like many cultivated grape varieties and requires co-planting with other varieties to achieve fertilization and fruit development. A total of 60, one-year-old grapevine cuttings were grown in 8 L pots containing soil, transferred from the original viticulture area of Maratheftiko cultivation, to avoid soil variability and to ensure uniform plant growth. The soil had a clay-loam texture: 2.21% organic matter; available $CaCO_3$ 67.3%; pH 7.46; EC 0.27 mS/cm. The climate of the region is dry with less than 30 mm of summer rainfall (June to August). Outdoors and indoors (greenhouse) average day and night temperature and relative humidity during the stress study are presented in Table 1.

Rooted cuttings were pruned during spring, to a two-bud spur. After bud break, two shoots were allowed to grow. During the first 3.5 months, plants were uniformly irrigated through a drip irrigation system, on a daily basis, to soil

Table 1 | Temperature and relative humidity for outdoors and indoors (greenhouse) during the drought and HS experiment

			Mean	± SE	Max	Min
Temperature (°C)	Day	Outdoors	30.85	0.37	33.50	28.88
		Greenhouse	39.31	0.48	42.79	37.43
	Night	Outdoors	22.84	0.48	27.04	19.75
		Greenhouse	24.88	0.37	27.37	21.86
Relative humidity (%)	Day	Outdoors	45.90	1.50	50.85	29.18
		Greenhouse	35.88	1.21	41.04	24.20
	Night	Outdoors	63.73	3.46	75.87	36.49
		Greenhouse	61.94	3.13	72.85	35.65

Values are mean of records taken every 30 min during a 2.5-week period.

capacity. Thereafter, they were divided into four treatment groups, each one consisting of 15 plants of similar leaf area. The experiment was divided into two sub-experiments, namely drought stress (DS: i, ii, and iii treatments) and heat stress (HS: i and iv treatments) as follows: (i) well-watered plants with full irrigation regime (FI) control treatment, in which the plants were irrigated every day to soil capacity; (ii) light water-stressed (LS) treatment, in which the plants were receiving during the week 80% of the amount of irrigation water provided to well-watered plants; (iii) moderate water-stressed (MS) treatment, in which the plants were receiving during the week 50% of the amount of irrigation water provided to full irrigation plants; (iv) full irrigation plants under HS treatment, in which the plants were grown in a greenhouse (with increased temperature of +8–9 °C) and irrigated every day to soil capacity. Plants grown under DS and HS were measured/sampled at 4, 8, and 20 days. Volumetric water content (VWC) in soil was measured every 2–3 days by field-scout TDR300 with 20 cm rods (Spectrum Technologies Inc., Aurora, IL, USA), as presented in Figure 1.

Physiological parameters and photosynthetic pigment content

Leaf tissue (four replications/treatment; each replicate consisting of two measurements and each measurement of a pool of plant tissue; 0.1 g) was incubated in a heat bath at 65 °C for 30 min, in the dark, with 10 mL dimethyl sulfoxide (DMSO) for chlorophyll extraction. Photosynthetic pigments, i.e., chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll (t-Chl) contents were calculated (as described by Chrysargyris *et al.* (2017)). Maximum F_v/F_m photochemical quantum yields of PSII were measured with an OptiSci OS-30p Chlorophyll Fluorometer (Opti-Sciences). Leaves were incubated in the dark for

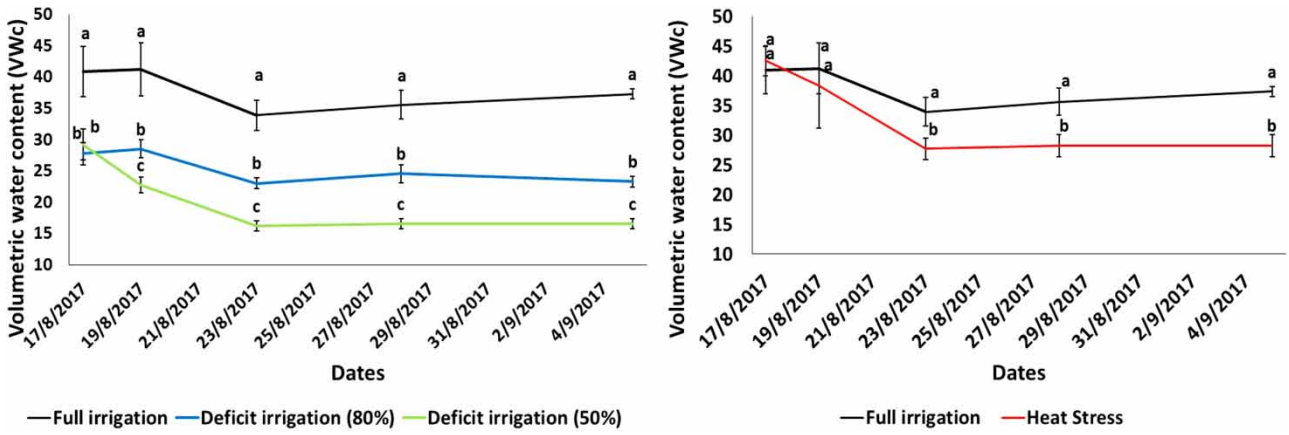

Figure 1 | Impact of short-term drought stress [full irrigation (FI); light water-stressed (LS) with 80% of the FI; and moderate water-stressed (MS) with 50% of the FI] and heat stress (HS) in soil volumetric water content in Maratheftiko cuttings grown in a pot culture.

20 min prior to F_v/F_m measurements. Leaf photosynthetic rate (*p*n), stomatal conductance (*g*s), and internal leaf concentration of CO_2 (*C*i) were measured using a portable infra-red gas analyzer (model Li-6400-40, Li-Cor, Biosciences, Lincoln, Nebraska, USA). Measurements of *p*n, *g*s, and *C*i were carried out between 9:00 and 11:10 a.m., when the leaf temperature within the chamber was 28 ± 2 °C, with photon flux density of 1,200 μmol $m^{-2} s^{-1}$ at ambient CO_2 concentration. The Li-6400 was equipped with a leaf chamber with constant area inserts (6.0 cm^2). All gas-exchange measurements started 3 h after the onset of the photoperiod and were replicated with four plants for each treatment and two fully expanded, healthy, sun-exposed leaves per plant.

Polyphenol content and antioxidant activity

The total phenolic content was determined with the Folin–Ciocalteu method at 755 nm according to Tzortzakis & Economakis (2007) and results were expressed as equivalents of gallic acid (Scharlau, Spain) per g of fresh weight (mg of GAE g^{-1} Fw). The antioxidant capacity using the ferric reducing antioxidant power (FRAP) and 2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) methods as well as total flavonoids content were performed as previously described (Chrysargyris *et al.* 2016, 2017). The results for antioxidant activities were expressed as equivalents of trolox per g of fresh weight (mg of Trolox g^{-1} Fw) and for the content of total flavonoids as rutin equivalents (mg Rutin g^{-1} Fw).

Determination of content of H_2O_2 and lipid peroxidation

The content of H_2O_2 in leaves was determined using the method of Loreto & Velikova (2001). Leaf tissue (four replicates/treatment; 0.2 g) was homogenized in ice cold 0.1% trichloroacetic acid (TCA) and centrifuged at 15,000 *g* for 15 min, and an aliquot of the supernatant was used for the reaction mixture. The H_2O_2 concentration was evaluated using standards prepared from dilutions of H_2O_2. The absorbance was measured at 390 nm and results were expressed as μmol H_2O_2 g^{-1} fresh weight.

Lipid peroxidation was assessed according to Azevedo Neto *et al.* (2006) and measured in terms of the malondialdehyde content (MDA). Absorbance of the reaction mixture was measured at 532 nm and corrected at 600 nm. The amount of MDA was determined using the extinction coefficient of 155 $mM^{-1} cm^{-1}$. Results were expressed as nmol of MDA g^{-1} fresh weight.

Activities of antioxidant enzymes in leaves

Fresh leaf tissue (four replicates/treatment) was homogenized using an ice cold extraction buffer containing 1 mM ethylenediaminetetraacetic acid (EDTA), 1% (w/v) polyvinylpyrrolidone (PVPP), 1 mM phenylmethylsulfonyl fluoride (PMSF), and 0.05% Triton X-100 in 50 mM potassium-phosphate buffer (pH 7.0). Protein content was determined using bovine serum albumin (BSA) as a standard (Chrysargyris *et al.* 2018).

Catalase activity (CAT) (EC 1.11.1.6) and SOD (EC 1.15.1.1) were assayed following the methods described previously (Chrysargyris *et al.* 2018). Catalase activity was assayed in a reaction mixture (1.5 mL) containing 50 mM K-phosphate buffer (pH 7.0), 10 mM H_2O_2, and an enzyme aliquot. The decomposition of H_2O_2 was measured at 240 nm. The results were expressed as CAT units mg^{-1} of protein (1 unit = 1 mM of H_2O_2 reduction min^{-1}). SOD was assayed using a photochemical method; a reaction mixture (1.5 mL) containing 50 mM K-phosphate buffer (pH 7.5), 13 mM methionine, 75 μM nitro blue tetrazolium (NBT), 0.1 mM EDTA, 2 μM riboflavin, and an enzyme aliquot. The reaction began by exposing the mixture to a light source of two 15 watt fluorescent lamps for 15 min, and it was stopped by placing the tubes in the dark. Absorbance was determined at 560 nm and activity was expressed as units mg^{-1} of protein.

Plant nutrient content analysis

Leaf tissue (four replicates/treatment) were dried at 65 °C for 4 days, weighed, and ground in a Wiley mill to pass through 40 mesh screens. Sub-samples (0.2 g) were digested using hydrochloric acid (2N HCl). The determination of K was made using a flame photometer (Lasany Model 1832, Lasany International, India), P (spectrophotometric; Multiskan GO, Thermo Fischer Scientific, USA), Mg by the Atomic Absorption Spectrophotometer (PG Instruments

AA500FG, Leicestershire, UK), and N by the Kjeldahl method (BUCHI, Digest automat K-439 and Distillation Kjelflex K-360, Switzerland). Data were expressed in g kg^{-1} of dry weight.

Statistical methods

Statistical analysis was performed using IBM SPSS version 22 comparing data means (±SE) with one-way analysis of variance (ANOVA) and Duncan's multiple range tests were calculated for the significant data at $P < 0.05$. Measurements were done in four biological replications/treatments (each replication consisted of a pool of 2–3 individual measures/samples).

RESULTS

Microclimate and soil water content

The temperature and relative humidity during the DS and HS study are presented in Table 1. During the day time, temperature inside the greenhouse was ca. 8.5 °C higher than outdoors, while during the night, temperature was only 1.9 °C higher than outdoors. During the HS study, plants were subjected up to 42.8 °C. In general, the higher temperatures during the day were reflected in a decrease in relative humidity (varying from 24.2 to 50.8%), and the opposite was evident during the night hours.

The VWC in soil was kept almost under the desirable levels according to the application of full irrigation (FI), light water-stressed (LS) with 80% of the FI, and moderate water-stressed (MS), as presented in Figure 1. Grapevine cuttings grown under HS with full irrigation, similar to the control treatment (plants were grown outdoors), had less available water, as the VWC decreased up to 24%.

Physiological parameters

The content of chlorophylls was mainly affected after 20 days of DS as Chl a, Chl b, and total Chl content was maintained in light water-stress (LS) compared to full irrigation (FI) but decreased in moderate-water stress (MS) (Figure 2). HS reduced the content of Chl a and, as a consequence, the total chlorophyll content only after 20 days of stress.

Physiological parameters of Maratheftiko, such as chlorophyll fluorescence, leaf photosynthetic rate, and stomatal conductance were affected mainly by the DS rather than the HS treatments (Figure 3). Chlorophyll fluorescence, leaf photosynthetic rate, and stomatal conductance were significantly decreased in plants subjected to DS (LS and MS) in a period longer than 8 days. The effects of HS were mainly observed in leaf photosynthetic rates and, to a lesser extent, in chlorophyll fluorescence. Therefore, HS for more than 8 days decreased the rate of photosynthesis by up to 69%. No differences were found in leaf stomatal conductance and internal CO_2 concentration in plants subjected to HS (greenhouse) or control (outdoors) conditions.

Polyphenols, flavonoids and antioxidant activity

The effect of DS on the content of polyphenols is presented in Figure 4. Following 20 days of DS, total phenolics were increased (almost doubled) compared to the control treatment (FI). The content of flavonoids and the antioxidant activity of leaves, measured by FRAP and ABTS methods, were increased in plants subjected to DS after 8 days. The content of polyphenols and flavonoids and the FRAP antioxidant activity were increased after 4 and 20 days of HS when compared to the control treatment (outdoors growing plants). The ABTS activity was increased only at the 20th day of HS.

H_2O_2 production and lipid peroxidation

The effect of short-term DS and HS on Maratheftiko on the damage index (H_2O_2 production and lipid peroxidation (MDA)) and enzymes activity (SOD, CAT) is presented in Figure 5. Leaf H_2O_2 and MDA production increased after 8 days of DS, and this increase was followed by the SOD increase. CAT activity did not increase due to the DS.

HS did not have a strong effect on lipid peroxidation and damage index, and as a consequence, SOD remained unchanged. Interestingly, CAT activity decreased due to the early stage of short-term HS.

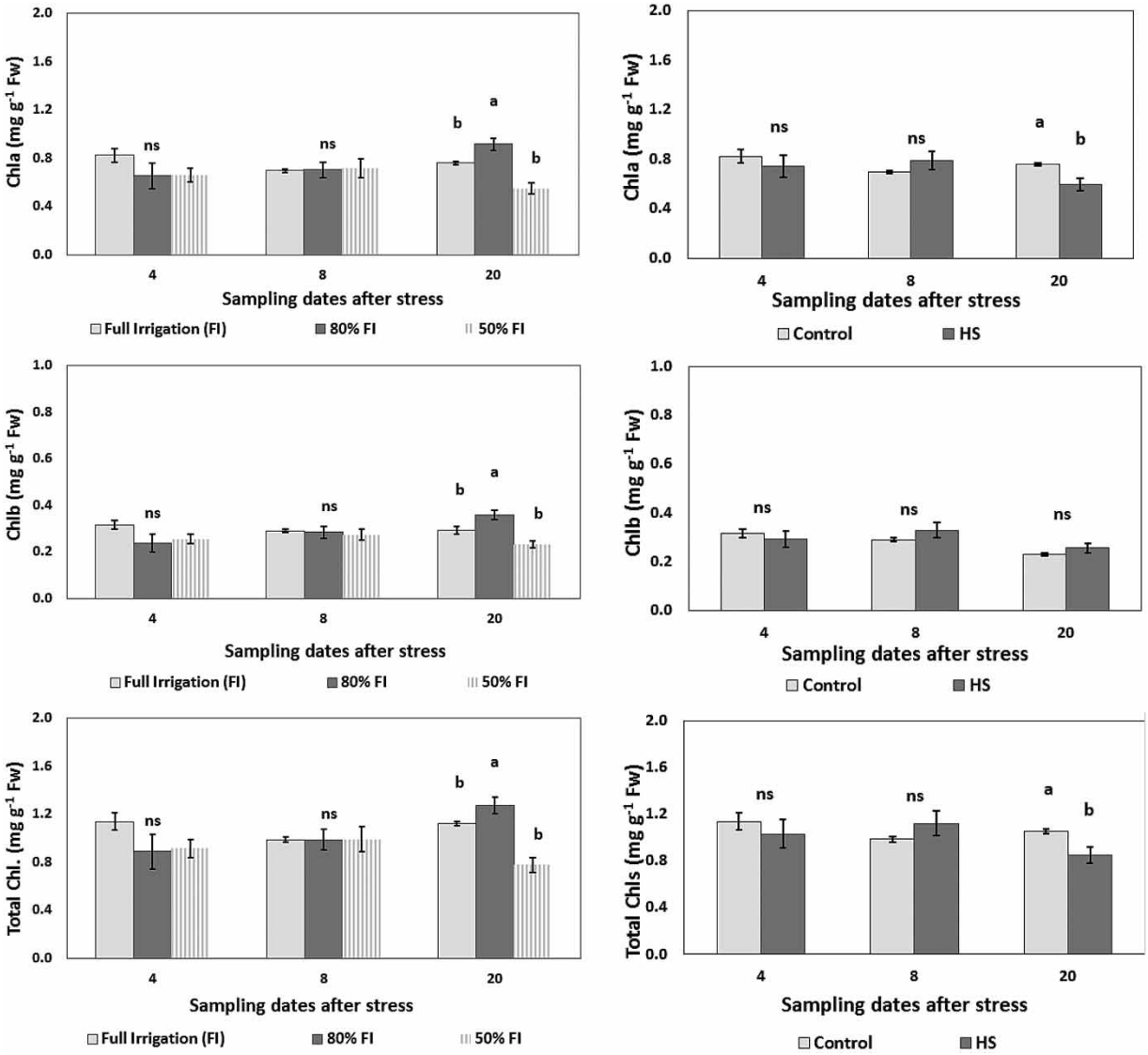

Figure 2 | Impact of short-term drought stress [full irrigation (FI); light water-stressed (LS) with 80% of the FI; and moderate water-stressed (MS) with 50% of the FI] and heat stress (HS) in Maratheftiko cuttings on chlorophyll a (Chla), chlorophyll b (Chlb), and total chlorophylls (total Chl). Sampling took place after 4, 8, and 20 days of stress. Significant differences ($P < 0.05$) among treatments and dates are indicated by different letters. ns: no significant. Error bars show SE ($n = 4$).

Leaf mineral content

Mineral content accumulated in Maratheftiko leaves was changed in plants subjected to short-term DS and HS (Figure 6). K accumulation increased in 4 days but decreased in 20 days of DS (i.e., 50% FI). However, the P content increased (up to 38%) in plants subjected to moderate irrigation stress (50% FI) after 20 days. No main differences were found for the N content in leaves.

HS had less profound effects than DS on mineral content in leaves. The levels of P were increased after 4 and 20 days of HS while the K content increased only after 20 days of HS (Figure 6).

DISCUSSION

Our results indicated that Maratheftiko's performance against environmental stresses is mainly affected by short-term DS

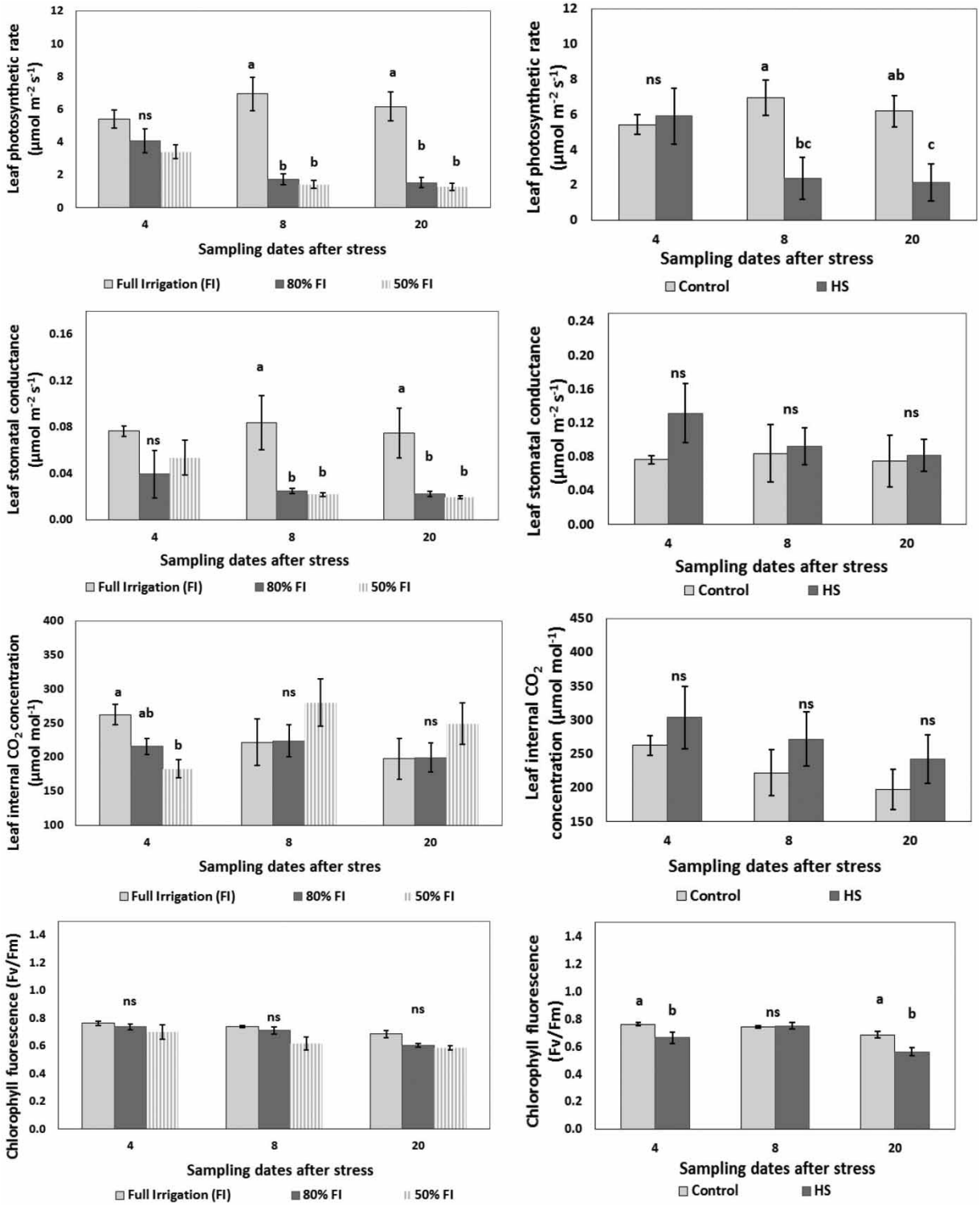

Figure 3 | Impact of short-term drought stress [full irrigation (FI); light water-stressed (LS) with 80% of the FI; and moderate water-stressed (MS) with 50% of the FI] and heat stress (HS) in Maratheftiko cuttings on leaf photosynthetic rate (*Pn*), stomatal conductance (*gs*), internal CO_2 concentration (*Ci*), and chlorophyll fluorescence (F_v/F_m). Sampling took place after 4, 8, and 20 days of stress. Significant differences ($P < 0.05$) among treatments and dates are indicated by different letters. ns: no significant. Error bars show SE ($n = 4$).

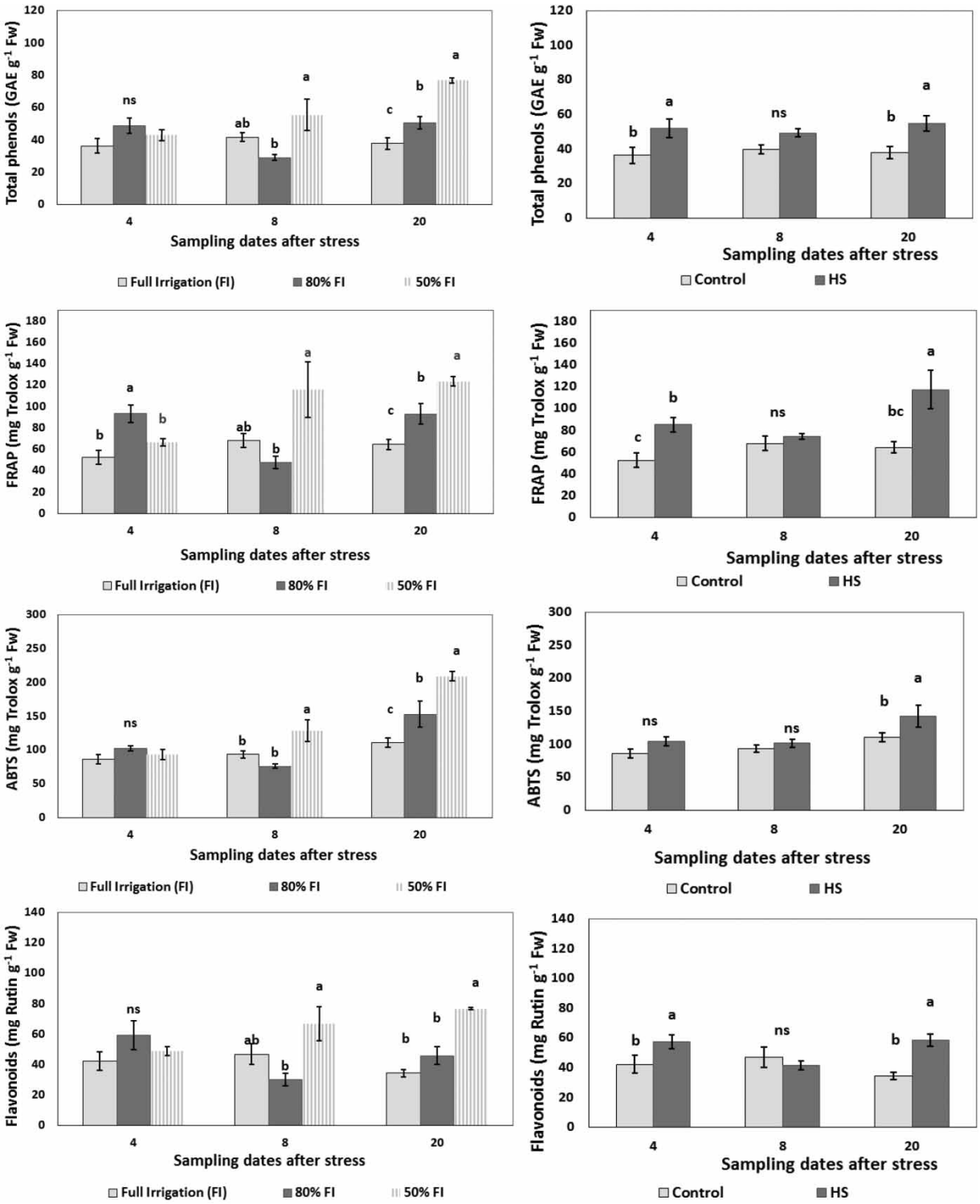

Figure 4 | Impact of short-term drought stress [full irrigation (FI); light water-stressed (LS) with 80% of the FI; and moderate water-stressed (MS) with 50% of the FI] and heat stress (HS) in Maratheftiko cuttings on the content of total phenols, total flavonoids and antioxidant activity (FRAP, ABTS). Sampling took place after 4, 8, and 20 days of stress. Significant differences ($P < 0.05$) among treatments and dates are indicated by different letters. ns: no significant. Error bars show SE ($n = 4$).

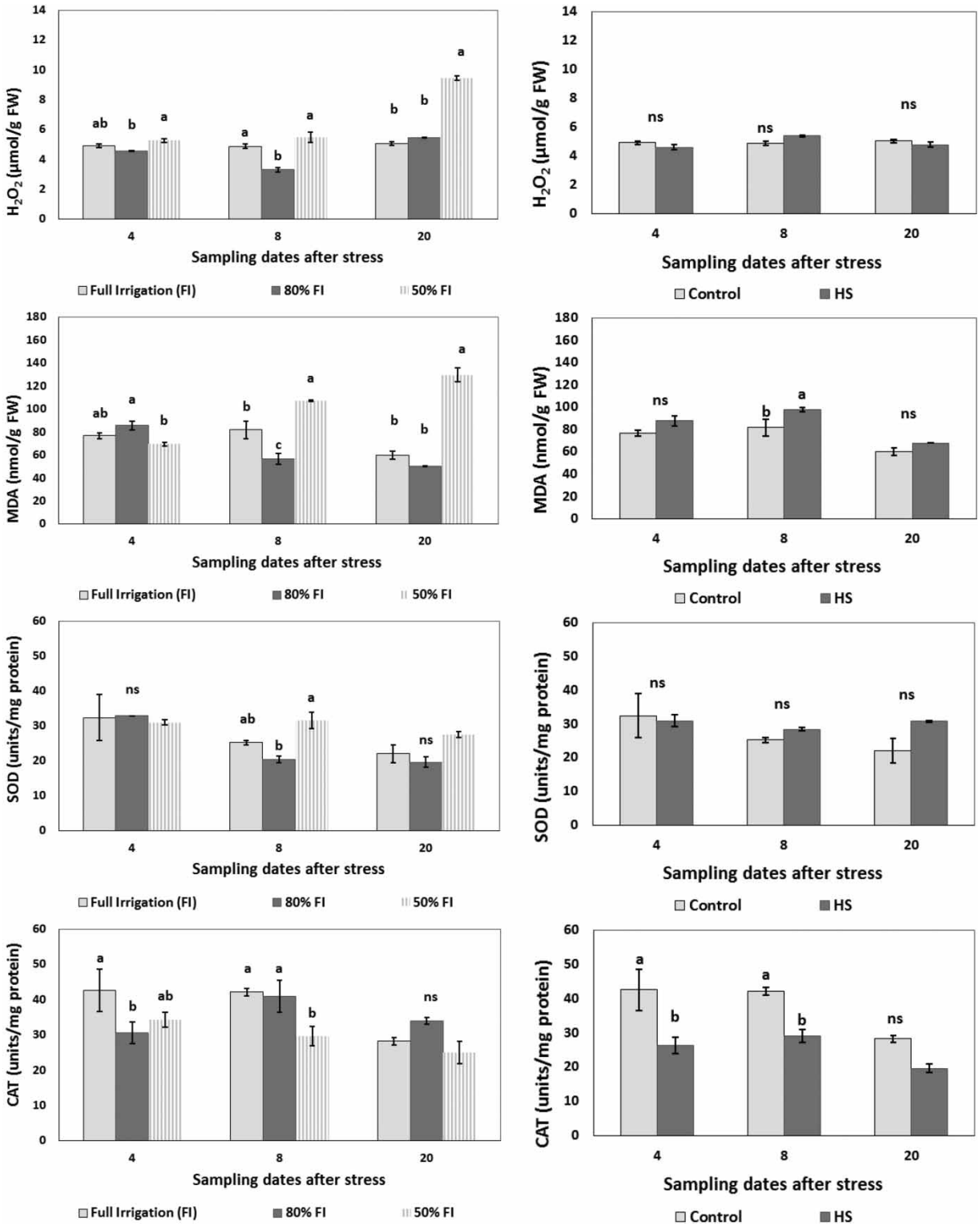

Figure 5 | Impact of short-term drought stress [full irrigation (FI); light water-stressed (LS) with 80% of the FI; and moderate water-stressed (MS) with 50% of the FI] and heat stress (HS) in Maratheftiko cuttings on the damage index [H_2O_2 production and lipid peroxidation (MDA)] and enzymes activity (SOD, CAT). Sampling took place after 4, 8, and 20 days of stress. Significant differences ($P < 0.05$) among treatments and dates are indicated by different letters. ns: no significant. Error bars show SE ($n = 4$).

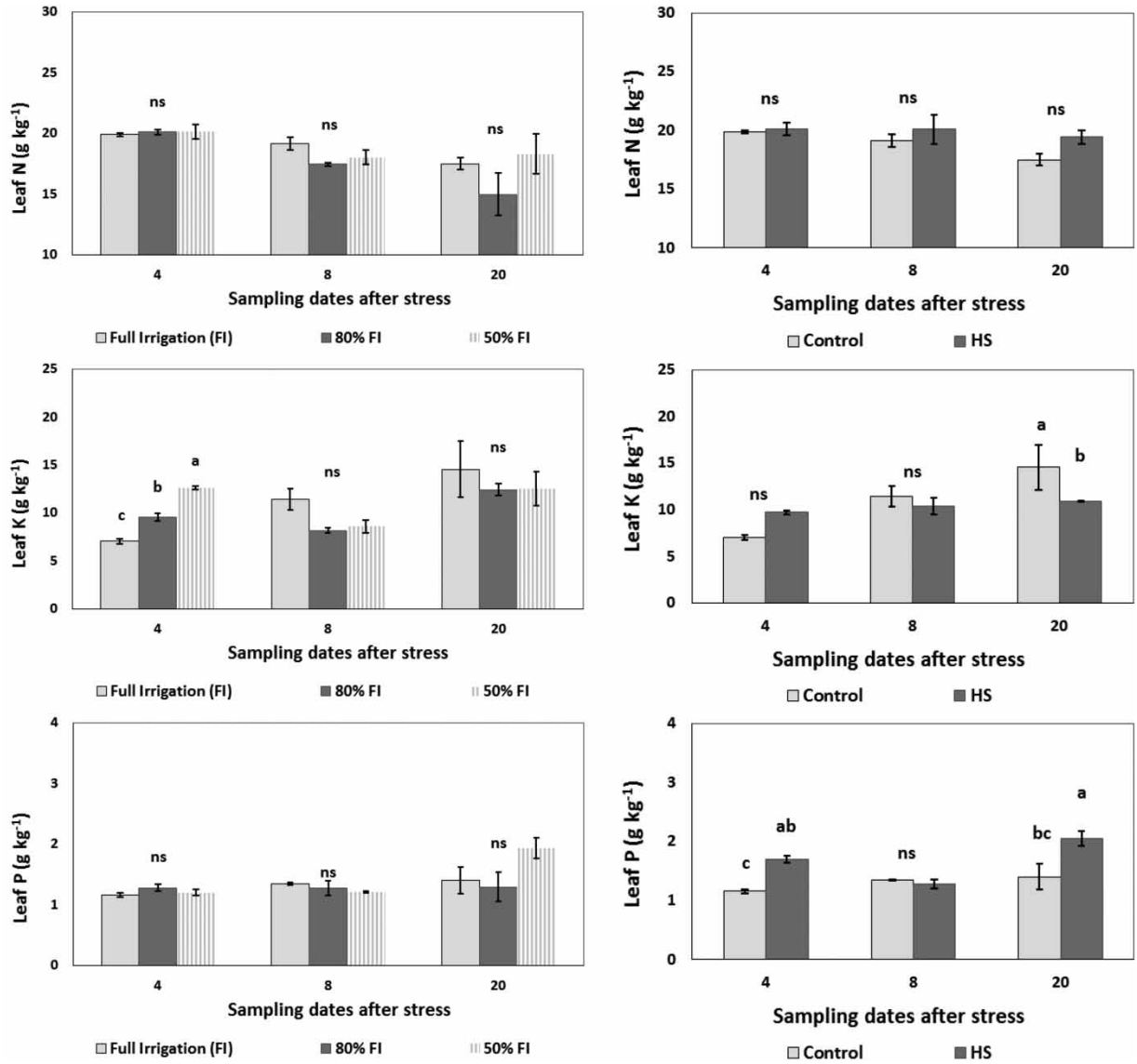

Figure 6 | Impact of short-term drought stress [full irrigation (FI); light water-stressed (LS) with 80% of the FI; and moderate water-stressed (MS) with 50% of the FI] and heat stress (HS) in Maratheftiko cuttings on the leaf content of macronutrients. Sampling took place after 4, 8, and 20 days of stress. Significant differences ($P < 0.05$) among treatments and dates are indicated by different letters. ns: no significant. Error bars show SE ($n = 4$).

than HS. The plant's age might affect the drought resistance as adult field-grown plants might be more tolerant. Plants' sensitivity to drought may be evaluated using different proxies of plant physiological status such as water potential, gas exchange characteristics, and chlorophyll bleaching (Bacelar *et al.* 2007). Frequently, large depressions in photosynthetic activity are associated with changes in water status. Under stressed conditions, the accompanying inhibition of photosynthesis is attributed mainly to stomatal closure, as reducing stomatal conductance is a major way of decreasing water loss from the leaves (Patakas *et al.* 2010). The function of stomata appears to be regulated by multiple signals (chemical, hydraulic, or even electric) in response to changes in both the soil and aerial environment (Lovisolo *et al.* 2010). Recent studies support the important role of the root-derived hormone abscisic acid (ABA) in mediating stomatal closure under drought conditions (Davies *et al.* 2002).

One of the primary processes affected by drought is photosynthesis, and due to the stomatal closure, not only the losses of water decrease but also the carbon flux to the sites of carboxylation (Escalona *et al.* 2002). Thus, monitoring of plant water status in grapevines under field conditions is considered of great interest, as it would allow diagnosis of the onset and severity of water stress so as to schedule irrigation according to the actual plant needs (Patakas *et al.* 2005). In the present study, plants subjected to DS exhibited a decrease of chlorophyll fluorescence, the rate of photosynthesis, and leaf stomatal conductance in a period longer than 8 days. According to Tardieu *et al.* (1999), even a short period of mild water stress could significantly retard leaf expansion rate, conferring to grapevines the ability to maintain leaf water content and to regulate water losses by transpiration. The effects of HS were mainly observed in leaf photosynthetic rate reduction after 8 days, while no marked differences were found in leaf stomatal conductance and internal CO_2 concentration in plants subjected to HS (greenhouse) or control (outdoor) conditions.

Under DS and HS, not only the content of polyphenols and antioxidant activity (measured by FRAP and ABTS assay) but also the flavonoid content was increased. Flavonoids are known to be synthesized by the phenylpropanoid pathway, using the amino acids phenylalanine or tyrosine as precursors (Hahlbrock & Scheel 1989). An antagonistic linkage between the flavonoids and proline-related pathways has been suggested, as grapevines subjected to environmental stresses have the capacity to alter the amino carbon flow from heterocyclic proline to the aromatic flavonoid precursors, tyrosine and phenylalanine (Hofmann *et al.* 2003). This flexible mechanism by grapevines is of great importance as they are capable of adapting better to different environmental stressors (Doupis *et al.* 2011). However, in the present study, proline content was not measured and we cannot make such a clear statement.

Chaumont *et al.* (1997) reported similar leaf chlorophyll content in both irrigated and non-irrigated grapevines while Maroco *et al.* (2002) found a significant reduction of total chlorophyll content in drought-stressed grapevines, being in accordance with our findings. Therefore, in the present study, the chlorophyll a content and as a consequence the total chlorophylls were decreased after 20 days of drought and HS. Minerals were accumulated in Maratheftiko leaves in both short-term DS and HS treatments, as K and P content increased in moderate irrigation stress and HS.

The close relationship between H_2O_2 and MDA (Figure 5) indicates that changes in hydrogen peroxide and lipid peroxidation could be used as reliable stress markers in order to quantify the intensity of the environmental stress in grapevine plants. The increased leaf damage index observed after 8 days represents the time period of tolerance that Maratheftiko can be subjected to DS, whereas the stress of the increased temperature had no consistent impact according to the present findings. Plants subjected to stress evolve both enzymatic and non-enzymatic defense systems for scavenging ROS and detoxifying them (Yildiz-Aktas *et al.* 2009). In our results, antioxidant enzyme activity significantly increased in water-stressed treatments (Figure 5). SOD provides the first line of defense against the toxic effects of ROS-elevated levels. The SODs convert $O_2^{-\cdot}$ to H_2O_2 while H_2O_2 is a strong nucleophilic oxidizing agent and the oxidation of SH-group is one of the major modes of its toxicity. The produced H_2O_2 is then scavenged by CAT and a variety of peroxidases (Tarchoune *et al.* 2010). Catalase degrades H_2O_2 into water and molecular O_2, whereas peroxidase decomposes H_2O_2 by oxidation of co-substrates such as phenolic compounds and/or antioxidants (Chrysargyris *et al.* 2018). However, enzymes' activation against ROS should not be generalized, as Doupis *et al.* (2011) reported that the ROS detoxification caused by drought, UV-B radiation, and a combination of them, was reflected by the proline accumulation in water deficit treatment rather than the antioxidant enzymes' activity. The capacity to maintain higher levels of antioxidants and/or the capacity for upregulation of antioxidant enzymes is closely related to genotypic ability for adaptation to various environmental stresses including drought (Ramachandra *et al.* 2004).

It is not surprising that Maratheftiko is a well-adapted indigenous cultivar in Cyprus, under dry seasons and heat waves. However, a noticeable VWC reduction (Figure 1) was found in soil, when plants were subjected to full irrigation under HS. This might be the first output of HS impacts causing light DS, and this can be accumulated through the time of plant growth. Therefore, irrigation management needs further consideration in high temperature-stressed crops. Usually, plants grown outdoors experience more

than one stress condition simultaneously, and therefore, the combination of HS and DS could yield detrimental effects.

CONCLUSIONS

The exposure of plants to DS and HS affected significantly both physiological and biochemical parameters. Under drought and HS conditions, grapevines favored the increase in antioxidant enzyme activities to scavenge and detoxify ROS accumulation. Stomatal closure is an adaptive mechanism of plants exposed to DS and Maratheftiko followed that successfully. The cumulative negative effects of combinations of environmental stresses needs to be studied further as it is a common issue in agriculture and crop production subjected to CC challenges. Additionally, indigenous cultivars such as Maratheftiko need further study at commercial vineyards to explore CC's effects on grapevines' productivity and/or quality of the grapes/juice.

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