Adaptive Response of a Native Mediterranean Grapevine Cultivar Upon Short-Term Exposure to Drought and Heat Stress in the Context of Climate Change

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Abstract: The impact of climate change on viticulture is of major importance. Several international and indigenous grapevine cultivars have been examined for their adaptive performance to drought and heat stresses. However, the underlying physiological mechanisms are not well known. In the present study we examined the short-term effects of light and moderate drought stress (DS) as well as heat stress (HS) on physiological and biochemical attributes in two grapevine cultivars: Chardonnay (international) and Xynisteri, an indigenous adapted to the specific Cypriot microclimate. Xynisteri plants exhibited decreasing leaf stomatal conductance and photosynthetic rate as well as increasing total phenols and antioxidant capacity under DS conditions. These reactions were concomitant to a rapid accumulation of hydrogen peroxide and lipid peroxidation in leaves, associated to an increase of the antioxidant superoxide dismutase activity. However, Chardonnay plants did not exhibit the same responses as Xynisteri against DS (i.e., stomatal closure, total phenolics and antioxidants) over the first four days. Additionally, Chardonnay showed leaf damage with increased lipid peroxidation levels and activation of catalase and peroxidase. Interestingly, HS increased leaf stomatal conductance and decreased total phenolic content, flavonoids and antioxidant capacity in Chardonnay after 20 d of stress. In both cultivars, HS had milder effects compared to DS, and again Xynisteri showed better performance than Chardonnay in terms of damage index and antioxidative activities. Overall, Xynisteri adaptation to DS and HS was higher than that of Chardonnay, and both cultivars reacted more to the short-term DS than to HS. Although the Xynisteri performances under stress conditions provide an important resource for adaptation to stressful conditions, the impacts on earliness, yield and grape quality remain to be explored.

Keywords: climate change; drought stress; heat stress; photosynthesis; antioxidants; lipid peroxidation

1. Introduction

Plants will be increasingly challenged by climate change characterized by frequent and severe events of heat and drought. Long-living trees have particularly evolved a diversity of adaptive mechanisms to minimize their fitness costs under stressful conditions [1]. These adaptations operate at the physiological and molecular levels and involve regulation of stomatal opening, change of photosynthetic rate, Rubisco activity and chlorophyll binding proteins at the chloroplast, imbalance in redox homeostasis, production of reactive oxygen species (ROS) and synthesis of protective compounds and enzymes [2–6]. Plants can also cope with environmental challenges by altering leaf structure by

lowering the leaf area/volume ratio, increasing leaf rolling, having smaller and more dense stomata, decreasing epidermal cells size, increasing elasticity of the cell wall, leaf cuticle, lignification and cell inclusions, such as mucilage, starch and crystals [7–9]. In fruit trees, physiological responses mainly depend on morphological traits (leaf size, leaf length, width) and anatomical traits (leaf thickness, palisade and spongy parenchyma). Leaf anatomical structure, especially that of the palisade and spongy parenchyma, is a very important factor for leaf photosynthetic rates because it may affect photosynthesis by changing the CO_2 diffusion rate from ambient air to carboxylation sites in the chloroplast [10]. Moreover, a greater extent of palisade parenchyma is reflected with more frequent sap movement (due to higher vessel density in stem anatomy) [11] and transmission of water and minerals to the main leaves, vegetative shoots and fruits [12].

The Mediterranean basin and Europe, in general, have a significant tradition and contribution to viticulture industry. They are major key players with the largest wine production and vineyard areas in the word [13]. Grapevine (*Vitis vinifera* L.) cultivation in the Mediterranean region is often subjected to multiple abiotic/environmental stresses, including high air temperature, increased solar radiation, strong winds, long drying periods and water scarcity, while these stresses are maximized during the summer period [14,15]. In general, plants that grow in open fields are subjected to various stresses at the same time that could affect many of their physiological properties.

Grapevines cultivated in the Mediterranean area are commonly exposed to drought stress (DS) because of the clear/no cloudy sky, high evapotranspiration and low soil water availability [16], which have the potential to alter plant physiology, nutrition and metabolism [17]. Due to global warming and the increased length of the dry periods, climate change (CC) is one of the most discussed issues. Multiple studies aim to understand, explain and prevent its detrimental impacts on living organisms and the environment. To estimate the overall impacts of any single or combination of CC-related factor(s), several regional climate models have been presented [13,18,19]. Moreover, different irrigation strategies (deficit irrigation, treated waste water, partial root drying, irrigation systems, etc.) are explored in order to increase the water use efficiency for irrigation [20,21].

The rise of temperature because of climate change is reflected by heat stress (HS) conditions that affect a plant's physiology and development. Within the last 30 years, temperature has shown an increment of 1.8 °C in the Mediterranean region [22]. Despite the great number of grape cultivars (about 10,000 cultivars documented in the Vitaceae [23]), the effects of CC-related factors on grape cultivars have been well documented [24]. In general, temperature is commonly known to affect vine physiology, vegetative and reproductive cycles, as well as the quality of harvested grapes, as reviewed by de Orduna [24]. This results in the acceleration of plant metabolic activities, fruit maturation and earliness of harvesting [19], affecting also wine quality and of course marketability [24,25].

When grapevines are exposed to high temperatures, lipids replace starch in leaf chloroplasts [26]. In temperatures >30 °C, berry size and weight are decreased [27], and metabolic processes and sugar accumulation may be retarded or completely paused [28]. Aroma of white wines is favored in cool climates along with accumulation of isoprenoids and pyrazines, while sun exposure is necessary for monoterpene accumulation that may impart fruity, floral or spicy aromas [22,24]. Until now, there are no convincing data on the effects of high temperatures on aroma compounds, while accumulation of anthocyanins in berries is decreased [29,30]. Moreover, the effect of high temperature on aroma compounds is discussed in Pons et al. [31].

Both drought and heat are known to affect cell metabolism, leading to reactive oxygen species (ROS) production. This negatively affects plant metabolism through oxidative damage of lipids, proteins and nucleic acids [32,33]. Plants react to oxidative stress by increasing their capacity to scavenge or detoxify ROS through the activation of antioxidative enzymes and accumulation of nonenzymatic components [34]. The antioxidative enzymes include superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), glutathione peroxidase (GPX), glutathione *S*-transferases (GST), ascorbate peroxidase (APX) and glutathione reductase (GR), while nonenzymatic antioxidant molecules are represented by ascorbate, glutathione, α-tocopherol, proline and so forth. The high levels of both

enzymatic and nonenzymatic antioxidants are associated with low lipid peroxidation under stress conditions [35] and are also connected to stress tolerance [34].

Climate change fingerprints show that soil and air temperature will increase in the near future, with a consequence of high water demands for irrigation, especially for susceptible cultivars. Sustainable grapevine irrigation management either by selected techniques (i.e., regulated deficit irrigation, partial root drying and/or low-quality irrigation water by using treated waste water) or by selecting cultivars with proven better performances towards environmental stresses is attracting scientific interest [14]. The objective of the current study was to elucidate and compare a commercial (Chardonnay) and a native (Xynisteri) grapevine cultivar in terms of their physiological responses to CC-related abiotic stress, such as drought and heat stress. Several grapevine growth parameters, soil properties and physiological and biochemical stress markers have been examined in response to the above stressors. Xynisteri is the most wide-spread white grape cultivar in the Cypriot vineyard, and according to farmer observations, Xynisteri performs well in high temperatures and drought conditions. Therefore, the evaluation of drought and heat tolerance properties of this cultivar compared to the international Chardonnay cultivar may enhance local viticulture resources under climate change.

2. Materials and Methods

2.1. Grapevine Cultivars and Experimental Set Up

The present study implemented at the experimental farm of the Cyprus University of Technology, Limassol, Cyprus, during spring–summer period. Two white grapevine (*Vitis vinifera* L.) cultivars were used, one wide-spread commercial (Chardonnay) and one native Cypriot (Xynisteri). Chardonnay is a sensitive cultivar to drought stress [36], while Xynisteri is observed by farmers as a tolerant cultivar. A total of sixty, one-year-old grapevine cuttings for each cultivar were grown in 8 L pots containing soil taken from the original vineyards of both Chardonnay and Xynisteri cultivation, to avoid soil variation and maintain steady plant growth. Soil had a clay-loam texture, with 2.19% organic matter; total $CaCO_3$ 66.9%; pH 7.42; electrical conductivity (EC) 0.28 mS cm^{-1}. The soil physicochemical properties during short-term (4, 8, and 20 d) DS or HS were determined (pH, EC, organic matter, available $CaCO_3$, and nitrogen (N), potassium (K), phosphorus (P) and sodium (Na) content) as described previously [21], and selected results are presented in Table S1. The region's climate is dry with a summer (June–August) rainfall of less than 30 mm. Average day and night temperature and relative humidity during stress study for outdoor and indoor (greenhouse) set up derived by meteorological stations are shown in Figure S1.

Cuttings were collected (early January) from the commercial vineyards following common pruning agronomical practices, and then they were buried in soil until March for rooting. Rooted cuttings were planted in the pots and pruned during spring to a two-bud spur. Two shoots were allowed to grow after the bud break. During the first 3.5 months, plants were uniformly irrigated to soil capacity every day through a drip irrigation system. Irrigation needs during that period were adjusted by weighting selected pots containing cuttings with a balance, and the delivered amount of water was adjusted to reach the initial control weight (full irrigation). Before and after adjustment, the volumetric water content (VWC) was recorded on the same pots. Thereafter, each cultivar (15 plantlets in each treatment) was given different levels of irrigation, based on the measurement on the VWC. The study was divided into two subexperiments, namely, drought stress (DS) and heat stress (HS). The DS experiment had three treatments: (i) well-watered plants with full irrigation regime (FI) as a control, in which plants were irrigated every day to soil capacity; (ii) light water stress (LS), in which plants received 80% of the total water provided to well-watered plants during the week; and (iii) moderate water stress (MS), in which the plants received only 50% of the total water provided to full irrigation plants during the week. The HS experiment had two treatments: (i) well-watered plants with full irrigation regime (FI) as a control, in which plants were outdoors and were irrigated every day to soil capacity and (ii) full irrigated plants under heat stress (HS) treatment, in which the plants were grown inside a greenhouse

(with increased temperature of +8–9 °C) and irrigated every day to soil capacity. Different parameters were measured at 4, 8 and 20 d. Volumetric water content in soil was measured every 2–3 d by a field-scout TDR300 with 20 cm rods (Spectrum Technologies Inc, Aurora, IL, USA), as presented in Figure 1.
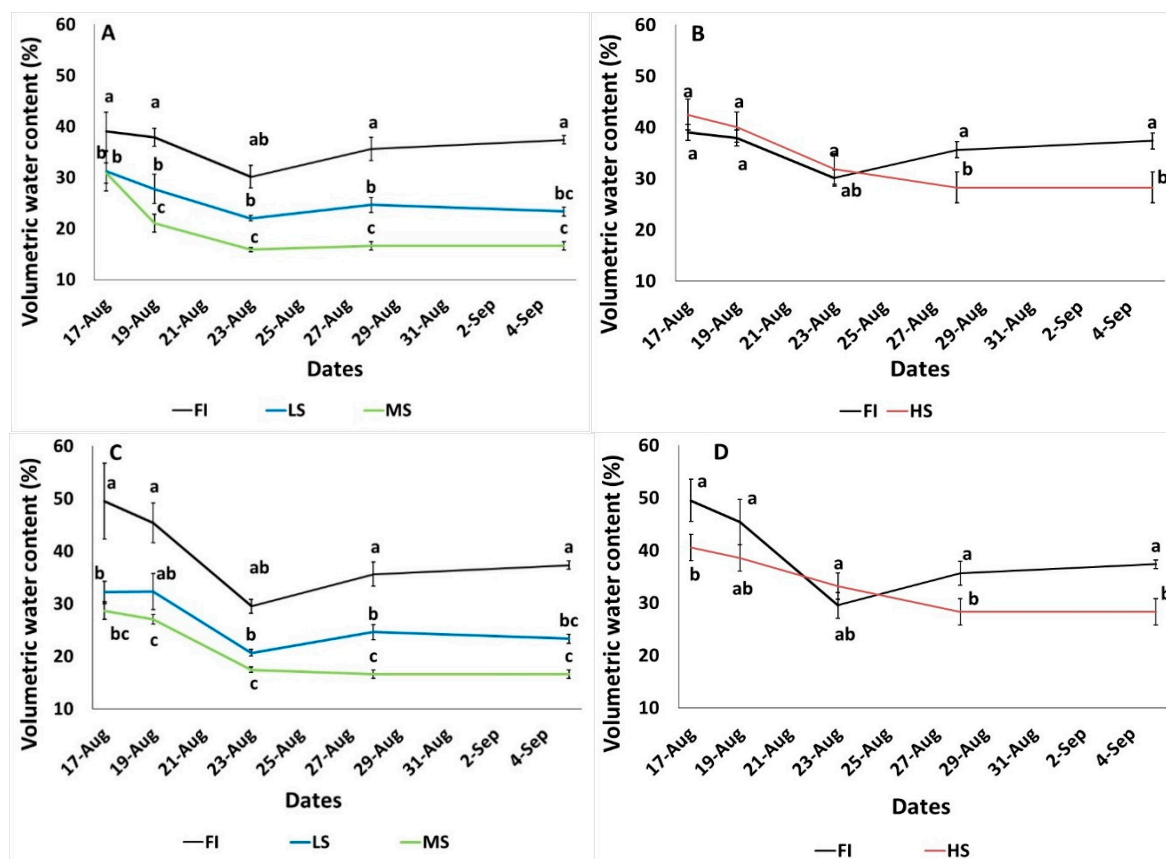
Figure 1. Impact of short-term drought and heat stress on soil volumetric water content in two grapevine cultivars, Chardonnay (**A**,**B**) and Xynisteri (**C**,**D**). Vegetative cuttings from both cultivars grown in pots were submitted to full irrigation (FI); light water stress (LS) with 80% of the FI, moderate water stress (MS) with 50% of the FI, and heat stress (HS). Data are means ± SE (*n* = 4), and significant differences (*P* < 0.05) among treatments for the different dates are indicated by different letters (a, b, c, ab, bc).

2.2. Physiological Parameters

Leaf tissue (four replications per treatment; each replicate consisted 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 (total Chl) contents were calculated as described by Chrysargyris et al. [37]. Maximum F_v/F_m photochemical quantum yields of photosystem II (PSII) were measured with an OptiSci OS-30p Chlorophyll Fluorometer (Opti-Sciences). To this end, leaves were incubated in the dark for 20 min prior to F_v/F_m measurements. Leaf photosynthetic rate (*pn*), stomatal conductance (*g*s) and internal leaf concentration of CO_2 (Ci) were measured using a portable infra-red gas analyzer (Li-6400, Li-Cor, Biosciences, Lincoln, USA). Measurements of *pn*, *g*s and Ci were carried out between 9:00–11:20AM, when the leaf temperature within the chamber was 28 ± 2 °C, with photon flux density of 1200 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.

2.3. Polyphenol Content and Antioxidant Activity

Total phenolics were determined with the Folin–Ciocalteu method at 755 nm according to Chondraki et al. [38], and results were expressed as equivalents of gallic acid per gram of fresh weight (mg of GAE g^{-1} fresh weight). The antioxidant capacity was measured using the ferric reducing antioxidant power (FRAP) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) methods, and total flavonoids content was analyzed as previously described [39,40]. The results for antioxidant activities were expressed as trolox equivalents (mg of trolox g^{-1} fresh weight) and for the content of total flavonoids as rutin equivalents (mg rutin g^{-1} fresh weight).

2.4. Hydrogen Peroxide and Lipid Peroxidation

Hydrogen peroxide (H_2O_2) content in leaves was determined as described by Chrysargyris et al. [39]. 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 and measured in terms of the malondialdehyde content (MDA) [39]. 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.

2.5. Antioxidative Enzyme Activities

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 [39].

Catalase activity (CAT) (EC 1.11.1.6) and superoxide dismutase activity (SOD) (EC 1.15.1.1) were assayed following the methods described previously [39]. 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 was used. The reaction began by exposing the mixture to a light source of two 15 W fluorescent lamps for 15 min, 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. Peroxidase activity (POD) (EC 1.11.1.6) was determined as described previously [41] following the increase in absorbance at 430 nm. Calculations were performed using the coefficient of extinction of 2.47 mM cm^{-1}. One POD unit was defined as the amount of enzyme to decompose 1 µmol of H_2O_2 per minute. Results were expressed as units of peroxidase per milligram of protein.

2.6. Statistical Methods

Statistical analysis was performed using IBM SPSS version 22 comparing data means (±SE) with one way-ANOVA, and Duncan's multiple range tests were calculated for the significant data at $P < 0.05$. Measurements were done in four biological replications/treatment (each replication consisted of a pool of two to three individual measures/sample). Pairwise metabolite effect correlations were calculated by Pearson's correlation test using the R program.

3. Results

3.1. Microclimate, Soil Water Content and Soil Physicochemical Properties

Grapevines plants were exposed to DS and HS conditions up to 20 d, and during that period, daytime temperature averaged 30.85 ± 0.37 °C and 39.31 ± 0.49 °C for outdoors and indoors (inside the greenhouse), respectively (Figure S1). During night, temperature averaged 22.16 ± 0.48 °C and 23.88 ± 0.58 °C for outdoors and indoors, respectively. During HS study, indoor plants were subjected up to 42.73 °C, being the average maximum temperature recorded over the period of HS, while the maximum temperature outdoors was 33.50 °C. In general, the increased temperatures during the daytime reflected relative humidity decreases (from 50.78% to 24.17%), while the opposite occurred during night time.

The volumetric water content (VWC) in soil was in line with the levels requested with the deficit irrigation practice, i.e., 80% (LS) and 50% (MS) of the full irrigation, as presented in Figure 1A,C. Grapevine plants grown under HS had less available water after 12 d of stress, as the VWC decreased up to 24.3% and 20.6% for Chardonnay and Xynisteri, respectively (Figure 1B,D).

Both cultivars were grown under the same soil from the commercial grown viticulture area of Limassol prefecture. Soil properties varied under the examined abiotic stresses, mainly the pH, EC and N levels (Table S1 and Figure 2). For example, in Chardonnay, soil pH increased under DS and HS during the stress period of 20 d (Figure 2A–C). Nitrogen content in soil increased during the 20 d of LS but decreased at 4 and 20 d of MS (Figure 2A,C). Heat stress resulted in a decrease of soil organic matter, N, K and Na levels (Figure 2A–C).

In Xynisteri, soil pH increased during the 8 d of exposure to LS, while P content increased following LS and MS, especially after day 8 (Figure 2D,E). Heat stress decreased soil pH and increased organic matter content as well as Na levels at 4 d, while the opposite trend was found after 8 and 20 d of stress (Figure 2D–F). Nitrogen levels increased at 4 and 8 d of HS but decreased thereafter at 20 d (Figure 2D–F). Potassium levels in soil were following exposure to HS.

3.2. Physiological Parameters

Chlorophyll content was mainly affected after 8 d of drought stress for Chardonnay, but such changes were produced later, at 20 d, for the Xynisteri cultivar (Figure 3). Interestingly, chlorophyll a, chlorophyll b and total chlorophyll increased at 8 d for Chardonnay, but decreased following longer drought stress period (i.e., 20 d). In the case of Xynisteri, and in comparison with Chardonnay, chlorophyll content increased after 20 d. Heat stress seemed to have no major effect on the chlorophyll content, with the exception for the chlorophyll b content, which decreased in HS-exposed Chardonnay plants compared to the control (FI-outdoor plants) after 20 d of stress. However, in Xynisteri, HS significantly ($P < 0.05$) decreased Chl a, Chl b and total Chl content after 20 d of stress (Figure 3).

Leaf photosynthetic rate, stomatal conductance, internal CO_2 concentration and chlorophyll fluorescence were differently affected among the Chardonnay and Xynisteri cultivars when subjected to DS and HS (Figure 4). Chardonnay exhibited lower leaf photosynthetic rates and stomatal conductance, while in the case of Xynisteri, photosynthetic performance and stomatal conductance were maintained at high levels under controlled, fully irrigated conditions.

In Chardonnay, when subjected to DS, leaf photosynthetic rates decreased (up to 68.6%) in drought (LS and MS) conditions compared to FI treatment (control) after 8 d (Figure 4). Similarly, internal CO_2 concentration was decreased under MS conditions. Leaf stomatal conductance did not follow a clear trend. In the case of Xynisteri, leaf photosynthetic rates, stomatal conductance and internal CO_2 concentration decreased up to 79.9%, 91.7% and 37.9%, respectively, in MS treatment compared to the control. Chlorophyll fluorescence did not change in Chardonnay among stress and time of exposure, but it decreased in Xynisteri after 8 d of DS.

Xynisteri plants subjected to HS showed decreased photosynthetic rate, stomatal conductance and internal CO_2 concentration, mainly after 8 d (Figure 4). Similarly, leaf photosynthetic rate decreased in

Chardonnay. However, Chardonnay stomatal conductance increased in full-irrigated plants when subjected to HS even at early exposure (i.e., 4 d). No differences were found in chlorophyll fluorescence in plants subjected to HS (greenhouse) or control (outdoors) conditions.

A comparison of the photosynthetic rates among the two cultivars as affected by different levels of stress conditions through time is presented in Figure S4. Leaf photosynthetic rates were higher in Xynisteri compared to Chardonnay in full irrigation and in LS treatments (after the 8th day). Interestingly, in MS, both cultivars had similar photosynthetic rates. The levels of photosynthesis decreased in Xynisteri compared to Chardonnay after 8 d of HS (Figure S4).
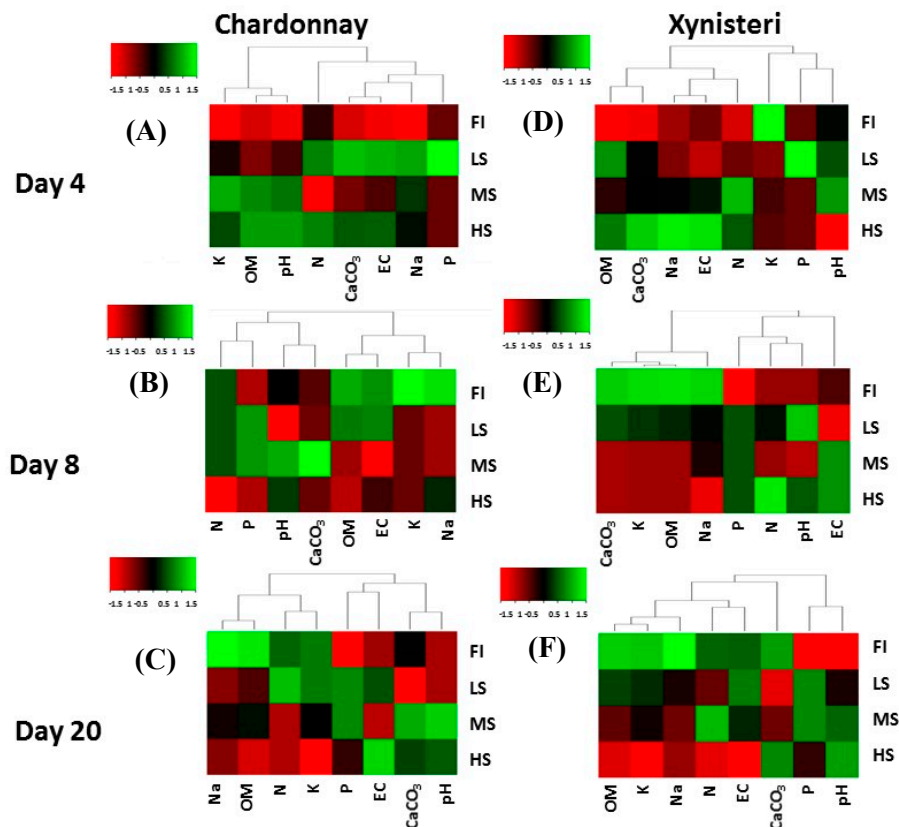

Figure 2. Relative changes in soil physicochemical properties after exposure of grapevine cultivars, Chardonnay (**A–C**) and Xynisteri (**D–F**) to water and heat stress. Heat map representing relative composition level of soil physicochemical properties after 4, 8 and 20 d of light water stress (LS) with 80% of the FI; moderate water stress (MS) with 50% of the FI, and heat stress (HS) as compared to control (full irrigation, FI) plants. Red shades indicate the lower level (less than −1.5 fold), deep red corresponds to −1 fold, black signifies that the level is not different from the control, deep green corresponds to 1 fold, clear green indicates that the level is more than 1.5-fold higher than the control. Na, N, K and P are expressed in $g\,kg^{-1}$, $CaCO_3$, OM in % and EC ($\mu S\,cm^{-1}$).
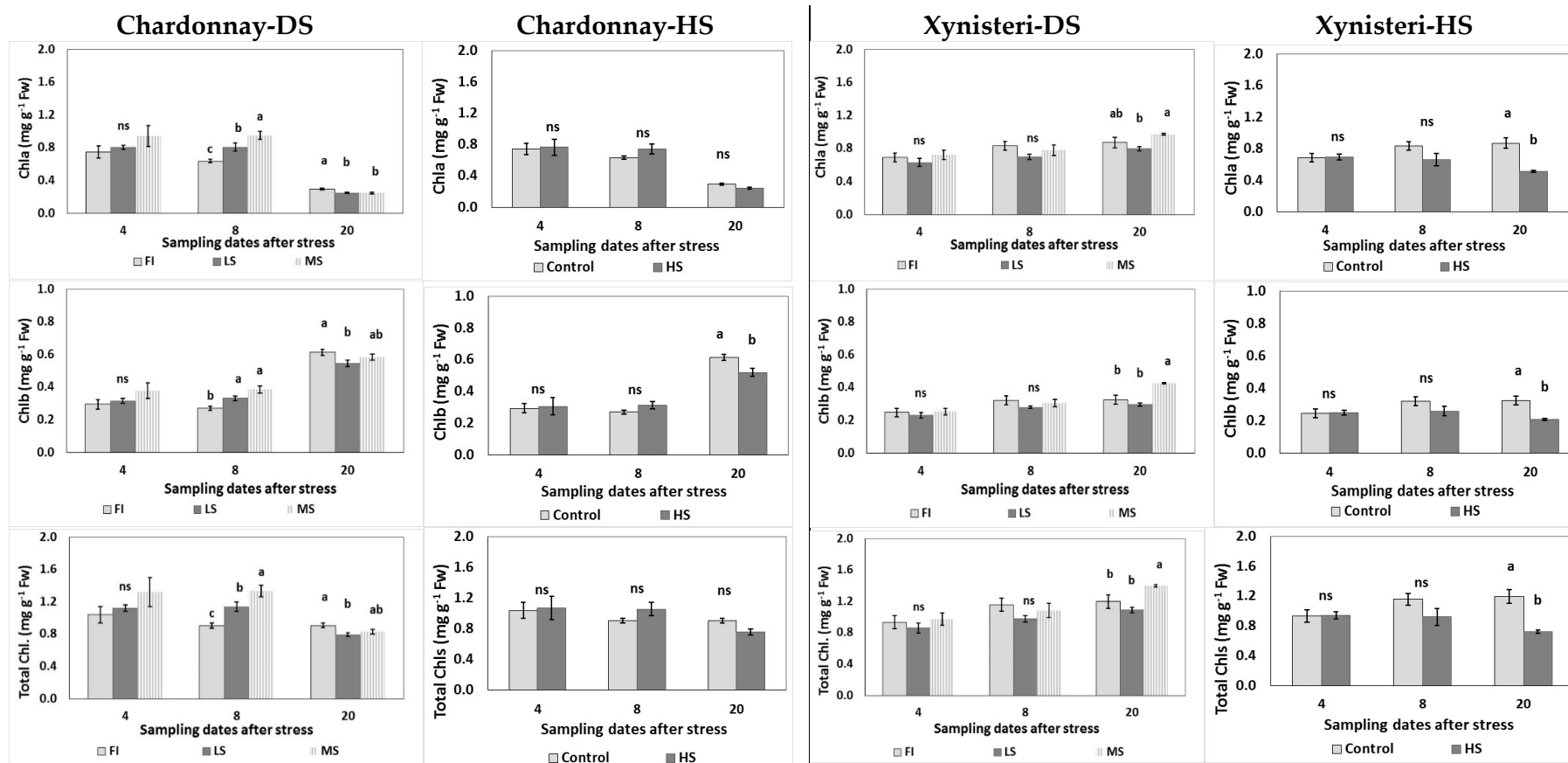
Figure 3. Impact of short-term drought and heat stress on chlorophyll content in Chardonnay and Xynisteri cultivars. Plants were full irrigated (FI) or exposed to light water stress (LS) with 80% of the FI, moderate water stress (MS) with 50% of the FI, and heat stress (HS). Chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll (total Chl) were determined after 4, 8, and 20 d of stress. Data are means ± SE (*n* = 4), and significant differences (*P* < 0.05) among treatments are indicated by different letters (a, b, c, ab). ns: not significant.

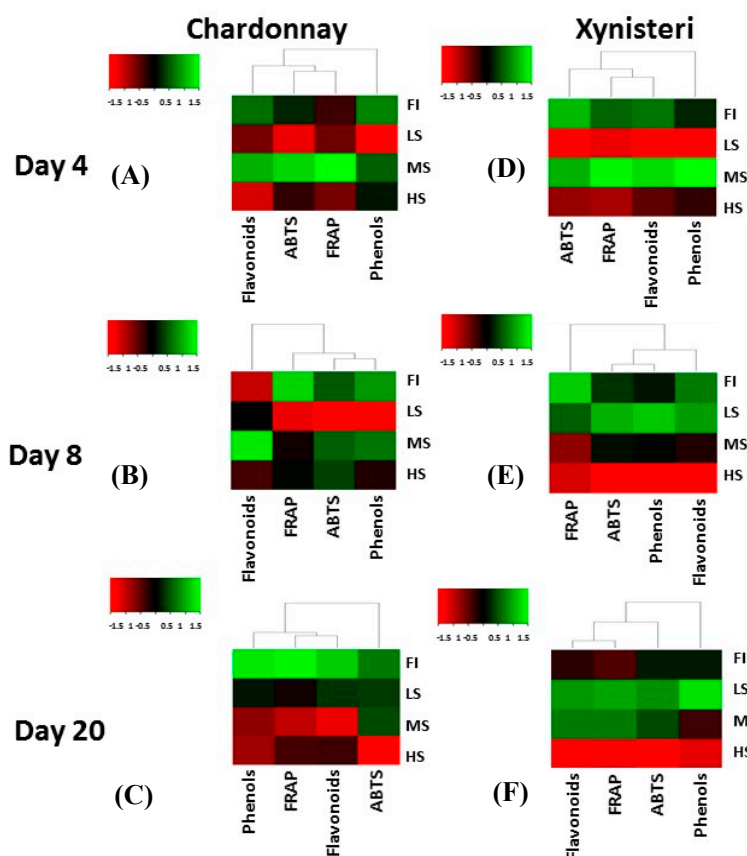**Chardonnay-DS** | **Chardonnay-HS** | **Xynisteri-DS** | **Xynisteri-HS**
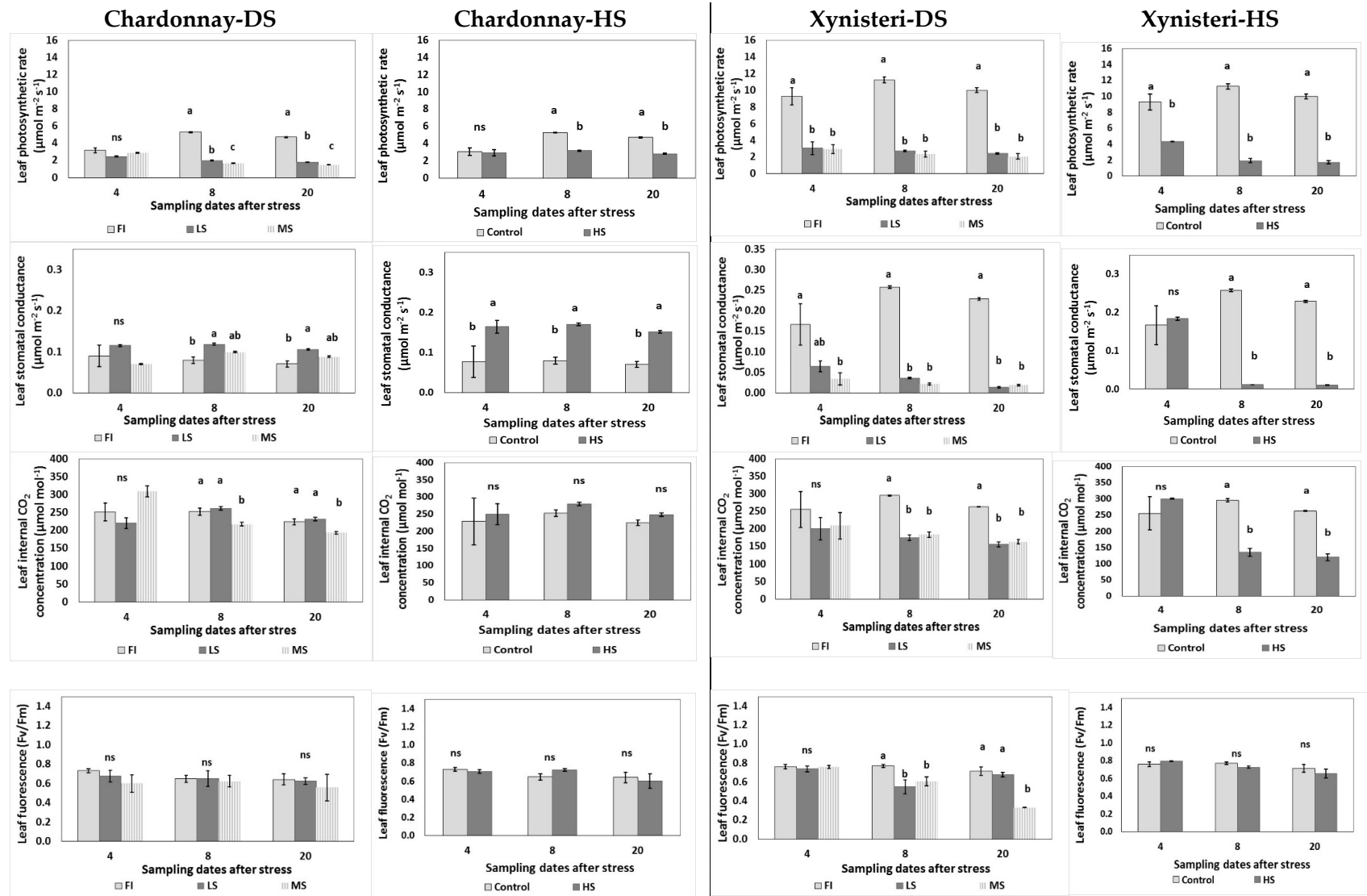
Figure 4. Impact of short-term drought and heat stress on photosynthetic rate, stomatal conductance, internal CO_2 concentration and maximum PSII quantum efficiency in two grapevine cultivars, Chardonnay and Xynisteri. Plants were full irrigated (FI) or exposed to light water stress (LS) with 80% of the FI, moderate water stress (MS) with 50% of the FI, and heat stress (HS). Leaf photosynthetic rate (*Pn*), stomatal conductance (*gs*), internal CO_2 concentration (*Ci*), and maximum PSII quantum efficiency (F_v/F_m) were measured after 4, 8, and 20 d of stress. Data are means ± SE (*n* = 4), and significant differences (*P* < 0.05) among treatments are indicated by different letters. ns: not significant.

### 3.3. Polyphenols, Flavonoids and Antioxidant Activity

The effects of DS and HS on the polyphenols, flavonoids and antioxidant capacity of Chardonnay and Xynisteri are presented in Figure 5 and Figure S2. Following 20 d of DS, total phenolics and antioxidant activity (assayed by FRAP) in leaves were decreased in Chardonnay but were increased in Xynisteri compared to their respective controls (FI). The content of flavonoids was decreased in Chardonnay under MS conditions but was stimulated in Xynisteri after 20 d of LS (Figure 5 and Figure S2). Total phenolics, flavonoids and antioxidant activity (FRAP, ABTS) were decreased after 20 d of HS for both examined cultivars in comparison with control (outdoors). Flavonoids were also decreased in Chardonnay at 4 d of HS exposure.

Figure 5. Relative changes in total phenols, flavonoids and antioxidant activity (FRAP, ABTS) after exposure of grapevine cultivars, Chardonnay (**A–C**) and Xynisteri (**D–F**), to water and heat stress. Heat map representing relative composition level of molecule traits after 4, 8 and 20 d of light water stress (LS) with 80% of the FI, moderate water stress (MS) with 50% of the FI, and heat stress (HS) as compared to control (full irrigation, FI) plants. Red shades indicate the lower level (less than −1.5 fold), deep red corresponds to −1 fold, black signifies that the level is not different from the control, deep green corresponds to 1 fold, clear green indicates that the level is more than 1.5-fold higher than the control. Total phenols are expressed as GAE g^{-1}, flavonoids in mg rutin g^{-1} and FRAP and ABTS in mg trolox g^{-1}.

3.4. Hydrogen Peroxide, Lipid Peroxidation and Antioxidative Enzyme Activity

The impacts of short-term DS and HS on the damage index (H_2O_2 production and lipid peroxidation (MDA)) and antioxidative enzyme (SOD, CAT, POD) activity were examined in both cultivars (Figure 6 and Figure S3). In Chardonnay, H_2O_2 production increased after 20 d of DS, while MDA content increased after 8 d of DS. These changes were followed by a decrease of SOD and increase of CAT and POD activities, mainly in response to moderate stress (Figure 6). Xynisteri subjected to DS had increased MDA and H_2O_2 production, accompanied by the increased SOD activity and decreased CAT and POD, in general, with more pronounced effects at 20 d of DS (Figure 6).
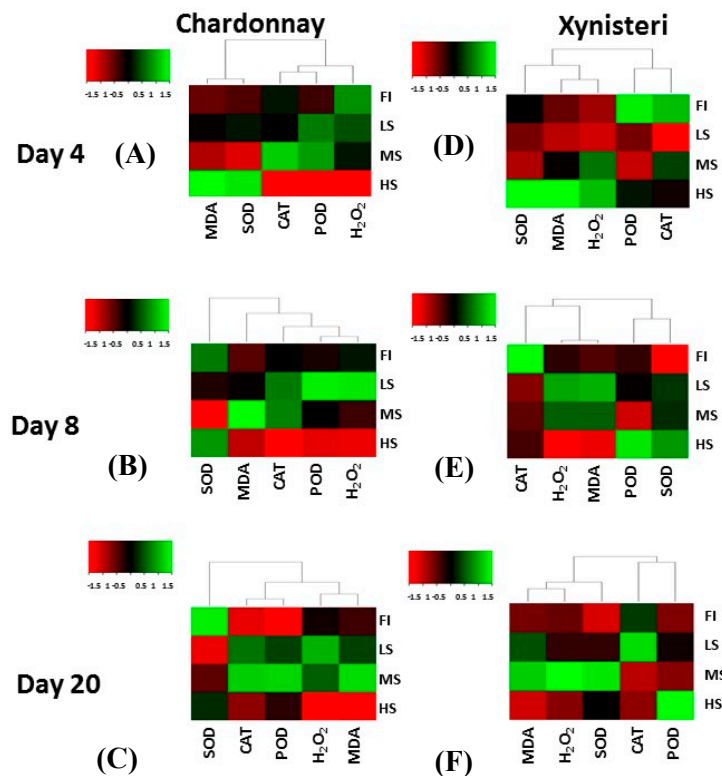
Figure 6. Relative changes in damage index (malondialdehyde (MDA), H_2O_2) and antioxidative enzymes (superoxide dismutase (SOD), catalase (CAT), peroxidase (POD)) after exposure of grapevine cultivars, Chardonnay (**A**–**C**) and Xynisteri (**D**–**F**), to water and heat stress. Heat map representing relative composition level of molecules traits after 4, 8 and 20 d of light water stress (LS) with 80% of the FI, moderate water stress (MS) with 50% of the FI, and heat stress (HS) as compared to control (full irrigation, FI) plants. Red shades indicate the lower level (less than −1.5 fold), deep red corresponds to −1 fold, black signifies that the level is not different from the control, deep green corresponds to 1 fold, clear green indicates that the level is more than 1.5-fold higher than the control. MDA and H_2O_2 are expressed in μmol/g; SOD, CAT and POD in units/mg protein.

Chardonnay plants subjected to HS showed a decrease of MDA and H_2O_2 production, associated to a decreased SOD and increased POD activity at 20 d. Interestingly, SOD activity increased only at the early stage (i.e., 4 d) of short-term HS, and decreased thereafter (Figure 6). In Xynisteri, MDA and H_2O_2 production increased only at the early stage (i.e., 4 d) of short-term HS. An activation of SOD (at > 4 d) and POD (at > 8 d) activities was observed. Both MDA and H_2O_2 decreased following 8 and 20 d of HS.

4. Discussion

The results of the present study demonstrated that Xynisteri, a Cypriot indigenous cultivar, expressed and maintained some adaptive traits to environmental challenges of climate change

compared to Chardonnay, an international cultivar. Indeed, both cultivars were affected more by the short-term drought stress than the heat stress conditions. Under stress conditions, plants develop adaptive mechanisms that reflect their sensitivity or tolerance to the challenged stress [42]. Therefore, leaf stomata closure decreased the photosynthesis and stomatal conductance as a result of drought stress [43]. Stomata closure to limit water loss by evapotranspiration during drought periods is an early physiological response that decreases the risk of hydraulic failure [44]. These adaptive processes are, in most cases, accompanied by hormonal changes including synthesis of abscisic acid in roots and its transport to the shoot and leaves. They act as a main signaling pathway in plants subjected to drought stress and control stomatal closure [44–46]. Recent studies indicated that stomatal conductance is controlled by hydraulic signals, abscisic acid and/or their interaction in response to drought stress [47]. Moreover, differences in leaf anatomical structure may affect the photosynthetic rates by changing the CO_2 diffusion rate from ambient air to carboxylation sites in the chloroplast, as documented in apple leaves [11]. As such, leaf structure of the examined grapevine cultivars should be considered in future studies.

Chlorophyll is also sensitive to environmental challenges, thereby impacting photosynthetic performance and plant growth, as a positive correlation between chlorophyll content and photosynthetic rates has been reported [10]. Chlorophyll content is expected to be decreased in plants subjected to drought stress, through the suppression of key enzymes involved in its synthesis or the activation of its degradation [11,48]. Chlorophyll content was transiently increased at 8 d in Chardonnay, then decreased at 20 d, while in Xynisteri chlorophyll content increased after 20 d of drought stress. This fluctuation of chlorophyll content might be related to the susceptibility to the short-term exposure to DS (i.e., 4 or 8 d). A decrease of leaf chlorophyll content was also observed in another endemic red cultivar in Cyprus (namely Maratheftiko; Chrysargyris et al. [33]) or even in grapevines that were exposed to drought stress for a longer period of 45 d [49]. However, in mature grapevines that were 14 years old, chlorophyll content was unchanged in nonirrigated compared to irrigated plants [50], and this could be dependent on cultivar tolerance, plant age and the intensity of environmental stress. However, short-term exposure to HS did not affect chlorophyll content. This could be related to some thermotolerance capacity of grapevine cultivars.

Photosynthesis is one of the main processes affected by drought, and because of stomatal closure, both water loss and carbon flux to the carboxylation sites decrease [51]. Xynisteri is considered a drought-tolerant cultivar, while it responds impressively under a full irrigation regime by increasing the rates of photosynthesis and stomatal conductance. However, under DS conditions, photosynthesis and stomatal conductance decrease, slowing down the metabolic activity and adapting to the new DS conditions. This is consistent with other observations in a red cultivar resistant to DS as Xynisteri [33]. Such adaptation was not evidenced for the Chardonnay cultivar, as different cultivars exhibit different drought signaling mechanisms and abilities to withstand water stress [14]. Differences in stomatal response were also observed under DS among the examined cultivars, even when they were grown under the same environment and soil. Although additional proof is needed, this result could be attributed to changes in either hormonal status or accumulation of protective molecules (osmolytes, antioxidants), as suggested for other cultivars [20]. Short-term DS alters the grapevine metabolism and decreases leaf growth rate, in order to maintain an optimal leaf water state, by avoiding excessive water loss through transpiration [52]. Xynisteri also reacted to HS by lowering its metabolic activity through reduction of photosynthesis, stomatal conductance and internal CO_2 concentration, mainly after 8 d of stress. In the case of Chardonnay, the leaf stomata were not closed in response to HS, but surprisingly, stomatal conductance was increased, with probably greater water losses from evapotranspiration and decreased photosynthetic rates.

Under drought stress, polyphenols, antioxidant activity (as assayed by FRAP) and flavonoid content were increased in Xynisteri but decreased in Chardonnay after 20 d of stress conditions. Similar results to Xynisteri were found in the Cypriot red indigenous cultivar Maratheftiko [33]. It has been shown that the increased flavonoid content is related to the decreased proline levels under stress

conditions. This is consistent with the altered flux of nitrogen and carbon, which are used for flavonoids or proline synthesis [53,54]. Further proof is needed.

Stressed plants trigger enzymatic and nonenzymatic mechanisms of protection to scavenge and detoxify ROS [34,55]. The first target of ROS is to damage cell membranes. This increases lipid peroxidation and is highly correlated with the increase of MDA concentration. Beis and Patakas [14] reported that Mavrodafni grapevines had a higher MDA content compared to Sabatiano plants following DS. Here, we showed that the activity of antioxidative enzymes was significantly increased in water-stressed plants, following the increments of MDA and H_2O_2. These reactions were more pronounced in the Xynisteri cultivar than in Chardonnay. The enzyme activities were more precocious in Xynisteri in response to both DS and HD. The activation of antioxidative mechanisms is closely related to the genotypic ability to adapt to various environmental stresses including drought [56]. Initially, plants activate SOD to dismutate the superoxide ion and produce H_2O_2; thereafter, CAT, APX and POD are activated to detoxify the H_2O_2 into nontoxic components [33]. Nonetheless, the capacity of the plant to rapidly regulate the antioxidant system has been found to be closely associated with reduced oxidative damage and, therefore, drought tolerance in different species [14,57,58]. Nevertheless, activation of enzymes against ROS should not be generalized, as Doupis et al. [54] stated that the detoxification of ROS induced by drought, UV-B radiation and their combination was associated with the accumulation of proline in response to water deficit rather than the action of antioxidants.

It is not surprising that under semidry seasons and heat waves in Cyprus, Xynisteri is a well-adapted native cultivar. Drought-resistant cultivars can be cultivated without requiring irrigation, which is an important asset for sustainability, in a context where water resources become increasingly scarce [59]. Other strategies on water scarcity include the modification of training systems (e.g., goblet bush vines, or trellised vineyards at wider row spacing) or selecting soils with greater water holding capacity [59]. Noticeably, a substantial decrease in the water content in soil (Figure 1) under heat stress condition was observed despite the fact that the crop was fully irrigated. This could be the first response of HS with the reduction of soil water content, which causes light drought stress to the crop, and can be accumulated during plant growth period. Therefore, in cases of high temperatures that might cause HS, the current irrigation management practices (full irrigation for Chardonnay and dry-farmed Xynisteri) need to be reconsidered, as the effects of HS might cause water shortages to crops. Plantations of Xynisteri outside Cyprus would probably survive without irrigation in most regions, which is a big leap forward in terms of sustainability. Before doing so, the potential quality of Xynisteri outside Cyprus should, of course, also be taken into account. Normally, plants grown outdoors undergo more than one stress condition at the same time; thus, the combination of HS and DS could have adverse effects.

5. Conclusions

Drought and heat stress significantly affected physiological and biochemical parameters of the examined grapevine cultivars. Under drought and heat stress conditions, grapevines favored the increase in antioxidant enzyme activities to scavenge and detoxify ROS accumulation, with more pronounced effects in the indigenous cultivar, Xynisteri. Plant adaptation to DS included closure of stomata and the reduction of plant photosynthetic rates, and Xynisteri responded successfully to that. According to this study, we can conclude that the Chardonnay cultivar showed comparatively poorer physiological and biochemical properties than that of Xynisteri. The combined negative effects of environmental stresses need to be further explored, as it is a common issue facing climate change challenges in agriculture and crop production. Further studies on indigenous cultivars versus the international ones will add knowledge on the mechanisms adapted by grapevines through the decades to challenge climate change and specific edaphoclimatic conditions of an area. Moreover, exploitation of climate change effects on grapevines should take place at commercial vineyards, examining the impacts of climate change on grapevine productivity and/or quality of the grapes/juice.

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