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ORIGINAL ARTICLE

Water limitation mitigates high-temperature stress injuries in grapevine cultivars through changes in photosystem II efficiency and antioxidant enzyme pathways

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Abstract

Plants, via physiological and molecular processes, respond to unsuitable environmental conditions, resulting in stress tolerance. Most previous studies have focused on plant responses to a single abiotic stress, but the effects of combined water deficit and high temperature stresses are more severe and complex than those due to a single stress. Therefore, our study aimed to explore the differences in the damage caused by combined vs. single stresses. Grapevines were subjected to water deficit, high temperature, and water deficit plus high temperature treatments. The transcript levels of heat- and drought-stress genes, activities of photosystem II (PS II) and antioxidant enzymes (superoxide dismutase, catalase, and peroxidase), and changes in abscisic acid (ABA) biosynthesis were evaluated. The activities of PS II and antioxidant enzymes were lower under the water deficit plus high temperature treatment than under the heat treatment alone. The concentration of ABA and the transcript levels of ABA biosynthesis-related genes increased under both types of stress. The enhanced thermo-tolerance observed under drought stress could be attributed to increased PS II efficiency, as well as to changes in antioxidant pathways, mediated by a common regulatory system or including a substantial cross talk between heat- and drought-stress signaling.

Keywords Antioxidant pathways · Grapevine · High temperature · PS II · Water deficit

Abbreviations

ABA	Abscisic acid
CV	Conductivity value
D	Water deficit treatment
DH	Water deficit plus high temperature treatments
GOLS	Galactinol synthesis
H	High temperature treatment
HSF	Heat shock transcription factors
HSP	Heat shock protein
MBF	Multiprotein bridging factor
MDA	Malondialdehyde
OD	Optical density
PS II	Photosystem II
TBA	Thiobarbituric acid

Introduction

Recently, the study of abiotic stresses in plants has advanced considerably. However, most experiments focus on a single environmental stress, whereas several different stresses may occur simultaneously in nature, including high irradiance, extreme temperature, and low water availability, which may affect plant metabolism in a complex manner. For instance, drought and heat stresses often limit plant growth and productivity (Suzuki et al. 2014). Although drought and heat stresses have been widely studied (Queitsch et al. 2000; Wang et al. 2012), their combined impact on plants is poorly understood, and the effect of these stresses on plants is definitely accompanied by physiological and biochemical changes, as well as the transcript levels of resistance genes and proteins.

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Plants respond and adapt to biotic or abiotic stresses via biochemical and physiological processes, thereby resulting in stress tolerance mechanisms (Bruce et al. 2007). Chlorophyll *a* fluorescence was reported as a method to analyze heat-induced changes in the photosystem (PS) II of plant leaves (Camejo et al. 2005; Wen and Ding 2005). Photosynthesis, especially the activity of PS II, is highly sensitive to abiotic stress (Reddy et al. 2004), as it inhibits photosynthetic electron transfer and reduces the photochemical efficiency of leaves. This results in the photosynthetic center mechanism being destroyed, because the leaves cannot effectively absorb light energy (Camejo et al. 2005). Abiotic stresses can also disrupt the equilibrium between the production and clearance of reactive oxygen species (ROS) in plants, and produce harmful substances such as malondialdehyde (MDA). However, plants can limit the ROS-induced harm to their cells by increasing the antioxidant enzyme activities (Meloni et al. 2003; Shah and Nahakpam 2012).

Several studies have suggested that plants are simultaneously or successively subjected to multiple environmental stresses, which enhance their ability to resist environmental stress, a process known as plant cross adaptation. Zhang et al. (2016) found that, in winter wheat, drought improved the resistance to drought stress, and induced heat-tolerance through abscisic acid (ABA), which triggers responses against drought stress (Grossmann et al. 1996; Cho et al. 2012). It is well known that PS II is sensitive to abiotic stresses, and ABA can protect the PS II in leaves from photoinhibition (Ivanov et al. 1995; Kurepin et al. 2015). Furthermore, 9-*cis*-epoxycarotenoid dioxygenase gene (*NCED*) and cytochrome protein 707A1 (*CYP707A1*) are the known ABA synthesis genes. *NCED* is a key enzyme involved in the cleavage of the 11, 12 double bonds of C40 carotenoids during in ABA biosynthesis (Qin and Zeevaart 1999), while *CYP707A1* encodes for ABA 8′-hydroxylase (Wang et al. 2015).

It has been observed that, under field conditions, grapevines are able to respond effectively to different biotic or abiotic stresses in water-depleted soils. This led us to hypothesize that drought priming could ameliorate the physiological and biochemical reactions occurring due to heat stress. Therefore, we conducted an experiment under controlled laboratory conditions to investigate the effects of drought on grapevines under heat stress. Biochemical and physiological processes were analyzed to elucidate the underlying mechanisms of drought-induced heat stress responses.

Materials and methods

Plant material and growth conditions

This study was conducted in 2016 at the experimental station of Shanghai Academy of Agricultural Science (30°89 N, 121°39 E), Shanghai, China. Grapevines of the widely grown local cultivars, Shenfeng and Shenhua (*Vitis vinifera* × *V. labrusca*), were grown in plastic pots of 40 cm height × 15 cm diameter. A mixture of peat moss and perlite (*v*:*v* = 1:1) filled each pot. Grapevines (15–20 leaves) were allowed to acclimatize for approximately 2 weeks at 25 °C, 500 µmol m^{-2} s^{-1} light intensity, and 65–70% relative humidity. The plants were divided into four groups. A completely randomized experimental design with three biological replicates and three technical replicates was used. One group was kept under normal conditions (N treatment; 25 °C and 0 kPa), another group was subjected to a water deficit treatment until the soil water potential reached − 20 kPa, with the temperature maintained at 25 °C (D treatment; 25 °C and − 20 kPa), the third group was subjected to high temperature (H treatment; 45 °C and 0 kPa), and the fourth group was subjected to double stress (DH treatment; 45 °C and − 20 kPa). Chlorophyll *a* fluorescence and relative conductivity were measured in Shenfeng and Shenhua and in Shenhua alone, at 3 and 6 h, respectively, after the high temperature treatment, for all treatments. Simultaneously, functional healthy leaves of Shenfeng and Shenhua were immediately preserved in liquid nitrogen and stored at − 80 °C for further analyses.

Leaf chlorophyll *a* fluorescence

The polyphasic chlorophyll *a* fluorescence transient was measured using a Plant Efficiency Analyzer (Hansatech Instruments Ltd., King's Lynn, Norfolk, UK; Strasser et al. 2000). The formulae presented in Table S1 illustrate the biophysical parameters.

Measurement of relative conductivity

Leaf discs (10-mm diameter) were punched from five fresh, whole leaves, added to 10 mL ddH_2O, and shaken continuously for 3 h. The initial conductivity value (CV_1) and the value measured after boiling the sample for 10 min (CV_2) were determined using a conductivity meter (DDS-6110; Chendu Ruizi Analysis and Control Instrument Co., LTD, Chendu, China). The conductivity of ddH_2O blank samples (CV_0) was determined simultaneously. The relative conductivity (*L*) for each sample was determined as:

$$L(\%) = (CV_1 - CV_0) \times 100/(CV_2 - CV_0).$$

Measurement of ABA concentrations

The samples were extracted with 100 mM phosphate buffer (pH 7.4), and the ABA concentration in the supernatant was then measured using the Phytodetek ABA enzyme immunoassay test kit (Agdia, Elkhart, IN, USA; Zhang et al. 2014).

Quantitative real-time (RT)-PCR

Total RNA was extracted using the E.Z.N.A. Plant RNA Kit (Omega, Bio-Tek, Doraville, GA, USA). First-strand cDNA was obtained using PrimeScript™ RT reagent Kit with gDNA Erase (Perfect Real Time) (Takara, Tokyo, Japan). Gene transcript levels were quantified using the cDNA samples and SYBR Green (Takara), in a LightCycler 480 System (Roche Diagnostics GmbH, Mannheim, Germany). The results were normalized using glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and elongation factor 1r (*EF1r*) following Guillaumie et al. (2013). The stability values (*M*) of the reference genes were 0.261 (< 1.5) and 0.587 (< 1.5) in Shenfeng and Shenhua, respectively, which indicated that the reference genes were suitable for our experiment. The normalized transcript levels of *GLOS1* (Pillet et al. 2012), *HSFA2* (Pillet et al. 2012), *HSP70* (Hren et al. 2009), *MBF1* (Yan et al. 2014), *NCED1* (Sun et al. 2010), *CYP707A1* (Kondo et al. 2014), and *MnSOD* (Carvalho et al. 2006) were calculated using the geNorm software (https://genorm.cmgg.be/; Vandesompele et al. 2002). The qRT-PCR primer sequences are listed in Table S2. Three technical replicates were performed for each qRT-PCR assay.

Measurement of MDA content

The samples were extracted with 100 mM phosphate buffer (pH 7.0) and the supernatant was isolated. The MDA content in each sample homogenate was determined using a commercially available kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) based on thiobarbituric acid (TBA) reactivity, following the manufacturer's protocol. Briefly, after mixing trichloroacetic acid with the homogenate and centrifuging, TBA was added to the resulting supernatant. The red color developed after the reaction was measured in a DU-800 UV/Vis spectrophotometer (Beckman Coulter, Fullerton, CA, USA) at 532 nm. The MDA content was determined as follows:

$$\mathrm{MDA\,(nmol/mL)} = (\mathrm{OD_s} - \mathrm{OD_{ck}})/(\mathrm{OD_{ss}} - \mathrm{OD_b}) \times C_{ss}/C_s,$$

where $\mathrm{OD_s}$ is the OD value for the sample, $\mathrm{OD_{ck}}$ is the value for the control, $\mathrm{OD_{ss}}$ is the value for the standard, $\mathrm{OD_b}$ is the value for the blank, C_{ss} is the concentration of the standard substance, and C_s is the concentration of the sample.

Extraction of antioxidant enzymes and measurement of their activities

The samples were extracted with 100 mM phosphate buffer (pH 7.0) and the supernatant was isolated (Li et al. 2013). The activities of catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), and total antioxidant capacity (T-AOC) were determined using commercial assay kits (Nanjing Jiancheng Bioengineering Institute) and a DU-800 UV/Vis spectrophotometer (Beckman Coulter).

One unit of SOD activity was defined as the quantity of SOD required to produce 50% inhibition of nitrite reduction in a 1 mL reaction solution, as measured by the change in absorbance at 550 nm. The activity of SOD was calculated as:

$$\mathrm{SOD\,(U/mg)} = ((\mathrm{OD_{ck}} - \mathrm{OD_s})/\mathrm{OD_{ck}})/50\% \times V_m/V_s,$$

where $\mathrm{OD_s}$ is the OD value for the sample, $\mathrm{OD_{ck}}$ is the value for the control, V_m is the total reaction volume, and V_s is the sampling amount.

The activity of the CAT enzyme was calculated as the decrease in absorbance at 405 nm due to the degradation of H_2O_2. The CAT activity was calculated as:

$$\mathrm{CAT\,(U/mg)} = (\mathrm{OD_{ck}} - \mathrm{OD_s}) \times 271 \times 1/(60 \times V_s),$$

where $\mathrm{OD_s}$ is the OD value for the sample, $\mathrm{OD_{ck}}$ is the value for the control, V_s is the sampling amount, and 271 is the reciprocal of the slope.

The activity of POD was measured as the change in absorbance at 420 nm. The formula used to calculate POD activity was:

$$\mathrm{POD\,(U/mg)} = ((\mathrm{OD_s} - \mathrm{OD_{ck}})/12 \times V_m/V_s)/t \times 1000,$$

where $\mathrm{OD_s}$ is the OD value for the sample, $\mathrm{OD_{ck}}$ is the value for the control, V_m is the total reaction volume, V_s is the sampling amount, and t is the reaction duration.

The T-AOC was measured as the change in absorbance at 593 nm. The ability to reduce Fe^{3+}-tripyridine triazine (Fe^{3+}-TPTZ) to produce blue Fe^{2+}-TPTZ in an acidic environment reflects the T-AOC. The standard curve was $y = 0.3154x + 0.065$; $R^2 = 0.9989$, and the T-AOC was calculated as:

$$\mathrm{T\text{-}AOC\,(U/g)} = ((\mathrm{OD_s} - \mathrm{OD_{ck}}) - 0.065)/0.3154 \times V_m/(V_s/V_t \times W),$$

where $\mathrm{OD_s}$ is the OD value for the sample, $\mathrm{OD_{ck}}$ is the value for the control, V_m is the total volume of the reaction, V_s is the sampling amount, V_t is the total sampling amount, and W is the fresh weight.

Statistical analysis

Data are presented as mean ± standard deviation. Each experiment was carried out in tri-replicates, and the differences among treatments were assessed using one-way analysis of variance (ANOVA) in SPSS 22.0 software (SPSS Inc., Chicago, IL, USA).

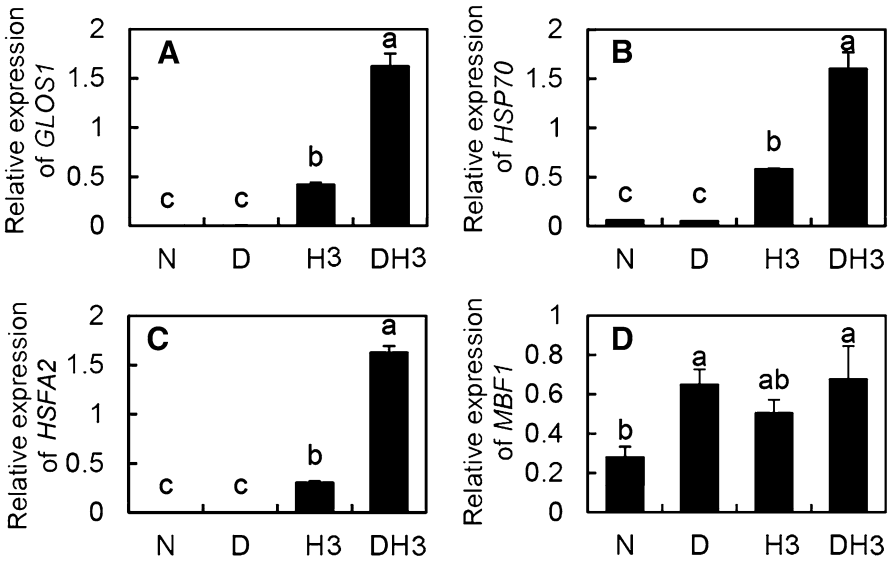

Fig. 1 The transcript level of stress-related gene in Shenfeng under different treatments. N: 0 kPa and 25 °C; D: − 20 kPa and 25 °C; H3: 0 kPa and 45 °C at 3 h; DH3: − 20 kPa and 45 °C at 3 h. Significant differences ($P \leq 0.05$) were performed by different lower case letters

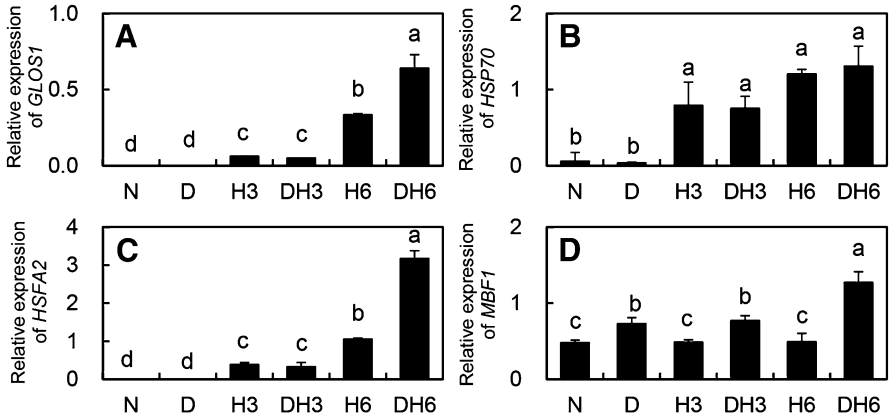

Fig. 2 The transcript level of stress-related gene in Shenhua under different treatments. N: 0 kPa and 25 °C; D: − 20 kPa and 25 °C; H3: 0 kPa and 45 °C at 3 h; DH3: − 20 kPa and 45 °C at 3 h; H6: 0 kPa and 45 °C at 6 h; DH6: − 20 kPa and 45 °C at 6 h. Significant differences ($P \leq 0.05$) were performed by different lower case letters

Results

Shenfeng and Shenhua, which are commercially important table-grape cultivars in China, were selected to study the responses to drought and heat stresses. Although drought and high temperature damaged both cultivars, they showed different degrees of damage under the stress treatment at 45 °C. Shenfeng showed heat injury symptoms after 3 h at high temperature and died after 6 h under this treatment, whereas Shenhua showed heat injury symptoms after 6 h at high temperature and eventually died by the end of the treatment duration. Therefore, leaves of Shenfeng and Shenhua after 3 h, and leaves of Shenhua after 6 h at high temperature, were used for the subsequent experiments.

Abiotic stresses might induce the transcript levels of several stress-related genes, including the heat-related genes *GLOS1*, *HSFA2*, and *HSP70*, and drought-related gene *MBF1*. In Shenfeng (Fig. 1), *GLOS1*, *HSFA2*, and *HSP70* showed similar expression patterns under all treatments, with a significant induction under treatments H3 and DH3, while the transcript levels of *MBF1* were significantly induced under treatments D and DH3. Therefore, in Shenfeng, the transcript level of heat-related genes was specifically enhanced by high temperature, whereas that of *MBF1* was specifically induced by water deficit. In Shenhua, the transcript levels of the *GLOS1*, *HSFA2*, and *HSP70* were induced under treatments H3, DH3, H6, and DH6 (Fig. 2), whereas the drought-related gene *MBF1* was enhanced under treatments D, DH3, and DH6 (Fig. 2). These results indicate that the gene responses characteristic of heat and water deficit stresses were typically found in both cultivars.

Chlorophyll *a* fluorescence is an important index that allows the most effective identification of plants under stress. In Shenfeng (Fig. 3, Table 1), the initial fluorescence value of OJIP (F_O) increased under different stresses, and the F_O of H3 was the highest among all treatments. This trend was also observed in F_J and F_I. However, the highest F_P value was found in N, and F_P was the lowest in H3. One of the

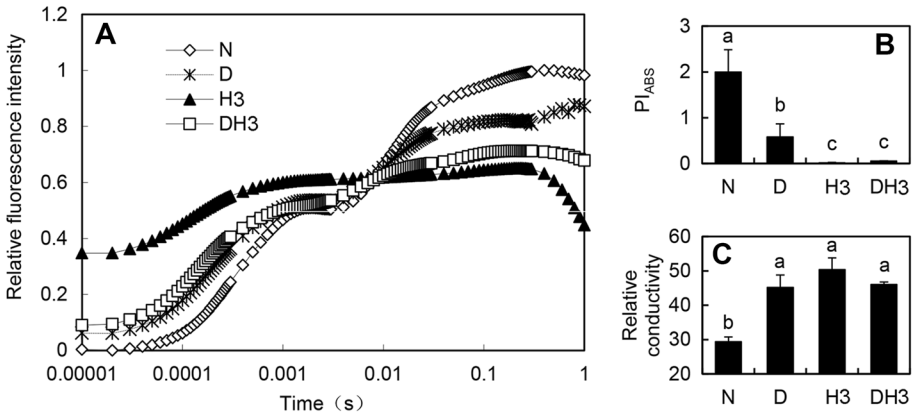

Fig. 3 The OJIP curve, PI_{ABS}, and relative conductivity in Shenfeng under different treatments. N: 0 kPa and 25 °C; D: − 20 kPa and 25 °C; H3: 0 kPa and 45 °C at 3 h; DH3: − 20 kPa and 45 °C at 3 h. Significant differences ($P \leq 0.05$) were performed by different lower case letters

Table 1 The inflection point of chlorophyll fluorescence OJIP curve in Shenfeng

Treatment	F_O	F_J	F_I	F_P
N	536 ± 54.0b	854.33 ± 79.6b	1550.67 ± 54.6a	2555.33 ± 62.7a
D	654.75 ± 57.3b	1126.75 ± 123.4a	1615.5 ± 137.6a	2307.25 ± 229.9ab
H3	1234 ± 140.9a	1582.2 ± 261.5a	1766.4 ± 383.4a	1848.6 ± 394.4b
DH3	712.4 ± 161.0b	1232 ± 70.6a	1615.4 ± 62.9a	1973.8 ± 383.5b

Different lower case letters indicate significant differences ($P \leq 0.05$)

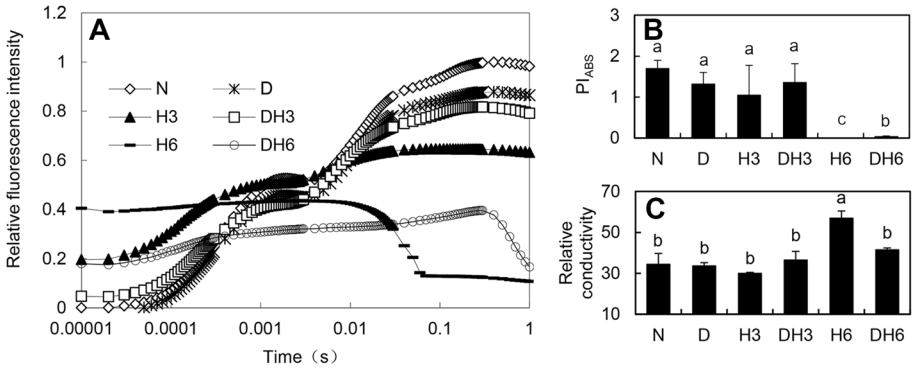

Fig. 4 The OJIP curve, PI_{ABS}, and relative conductivity in Shenhua under different treatments. N: 0 kPa and 25 °C; D: − 20 kPa and 25 °C; H3: 0 kPa and 45 °C at 3 h; DH3: − 20 kPa and 45 °C at 3 h; H6: 0 kPa and 45 °C at 6 h; DH6: − 20 kPa and 45 °C at 6 h. Significant differences ($P \leq 0.05$) were performed by different lower case letters

most sensitive indicators of chlorophyll *a* fluorescence, PI_{ABS}, decreased under all stress treatments relative to the N treatment and, in Shenfeng, its expression under H3 and DH3 treatments differed significantly from those under N and D treatments (Fig. 3). The relative electrical conductivity of Shenfeng leaves increased under the different stress treatments; it was highest in H3, followed by DH3, and D (Fig. 3). In Shenhua (Fig. 4, Table 2), the initial fluorescence value of OJIP under treatments D, H3, DH3, H6, and DH6 was 491, 935, 626, 1365, and 900, respectively. Thus, the F_O value increased under heat stress compared with the N treatment, but was lower under the water deficit plus high temperature treatment. This trend was also observed in F_J and F_I values, but F_P was lower under high temperature (H3 and H6) than under the remaining treatments (N, D, DH3, and DH6). The PI_{ABS} values in D, H3, and DH3 were not different from that in the N treatment, and the PI_{ABS} values in H6 and DH6 were significantly lower than those in other treatments. In fact, the PI_{ABS} of H6 was the lowest (Fig. 4). The relative electrical conductivity of Shenhua leaves increased significantly under H6 and DH6, showing the highest value under H6. The relative electrical conductivity under D, H3, and DH3 treatments did not differ from that under the N treatment (Fig. 4).

Stress induces a rapid rise in the OJIP parameters. Therefore, the amplitude of phase *K* (W_k) can be used as an indicator of the damage caused to the PS II donor site. In Shenfeng, W_k significantly increased under H3 and DH3 treatments (Fig. 5), but no difference was found between treatments D and N. In Shenhua, W_k increased significantly under H3, H6, and DH6 treatments, but no difference was observed between D and DH3 treatments compared with the N treatment (Fig. 6). In Shenfeng, the values of φ_{Po}, φ_{Eo}, and Ψ_{Eo} displayed no significant changes between N

Table 2 The inflection point of chlorophyll fluorescence OJIP curve in Shenhua

Treatments	F_O	F_J	F_I	F_P
N	530 ± 55.9b	897.67 ± 154.4c	1599.67 ± 242.8a	2592 ± 186.6a
D	491 ± 25.0b	833.5 ± 57.1c	1458 ± 92.6a	2341 ± 134.1a
H3	935 ± 158.7a	1358 ± 307.0ab	1605 ± 198.4a	1864 ± 120.2b
DH3	626 ± 100.1b	985 ± 111.5bc	1438 ± 197.5a	2216 ± 209.9a
H6	1365 ± 225.4a	1389.5 ± 170.8a	1428.5 ± 174.5a	1428.5 ± 209.1c
DH6	900 ± 128.3a	1081 ± 55.4bc	1192 ± 93.9b	1347 ± 56.3c

and D treatments, but decreased under H3 and DH3 treatments (Fig. 5). In Shenhua, the values of φ_{Po}, φ_{Eo}, and Ψ_{Eo} significantly decreased under H6 and DH6 treatments and were lower in H6 than in DH6. However, no differences in φ_{Po}, φ_{Eo}, and Ψ_{Eo} were observed in the D, H3, and DH3 treatments compared with the N treatment. Changes in the approximate initial slope of the fluorescence transient (M_o) were induced under H3 and DH3 treatments in Shenfeng (Fig. 5) and under H6 and DH6 treatments in Shenhua (Fig. 6). The redox state of photosystem I (δ_{Ro}) did not display significant changes under any of the treatments in Shenfeng (Fig. 5), but increased under the DH6 treatment in Shenhua (Fig. 6).

Water deficit and heat stress treatments significantly increased leaf ABA compared with the N treatment. In Shenfeng, the ABA concentrations were significantly higher in D and DH3 than in N and H3 treatments (Fig. 7). In Shenhua, both stresses enhanced the ABA concentration (Fig. 8). In Shenfeng, the transcript level of *NCED1* was induced under D and DH3 treatments and the transcript level of *CYP707A1* increased under the H3 treatment (Fig. 7). The transcript levels of *NCED1* and *CYP707A1* increased under the different stress treatments and showed the highest value under the H6 treatment in Shenhua (Fig. 8).

In Shenfeng, the activity of antioxidant enzymes varied under the different treatments (Fig. 9). The activities of SOD, POD, CAT, T-AOC, and MDA content, and the transcript level of did not differ between the D and N treatments, but the activities of SOD, CAT, and MDA content, and the *MnSOD* transcript level increased under the H3 treatment.

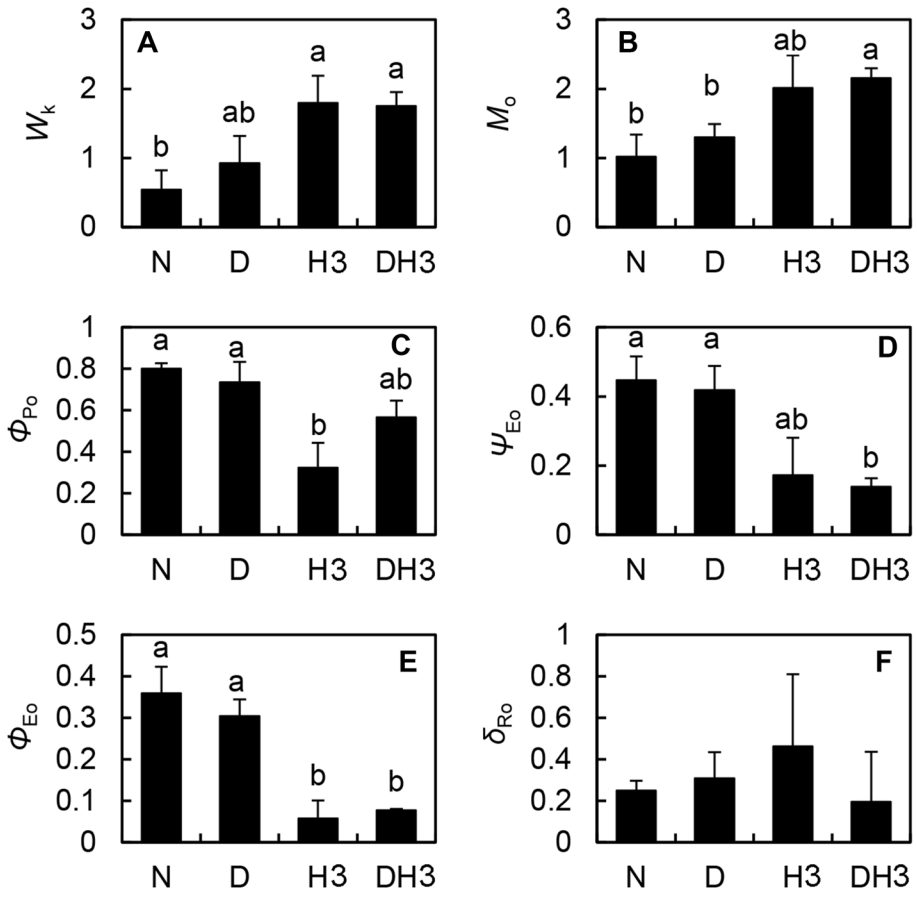

Fig. 5 The chlorophyll fluorescence parameters in Shenfeng under different treatments. N: 0 kPa and 25 °C; D: −20 kPa and 25 °C; H3: 0 kPa and 45 °C at 3 h; DH3: −20 kPa and 45 °C at 3 h. Significant differences ($P \leq 0.05$) were performed by different lower case letters

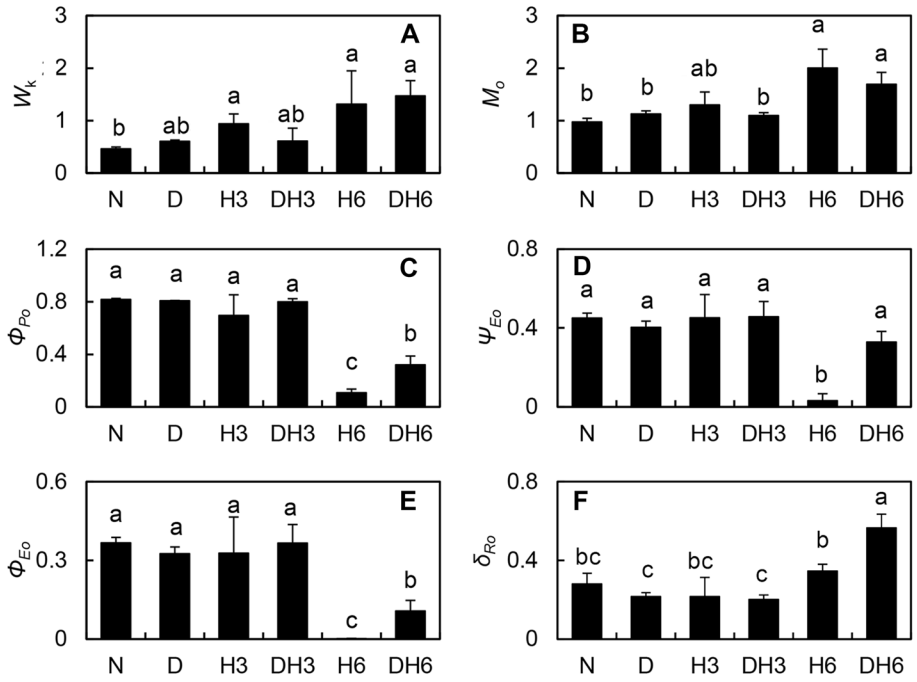

Fig. 6 The chlorophyll fluorescence parameters in Shenhua under different treatments. N: 0 kPa and 25 °C; D: − 20 kPa and 25 °C; H3: 0 kPa and 45 °C at 3 h; DH3: − 20 kPa and 45 °C at 3 h; H6: 0 kPa and 45 °C at 6 h; DH6: − 20 kPa and 45 °C at 6 h. Significant differences ($P \leq 0.05$) were performed by different lower case letters

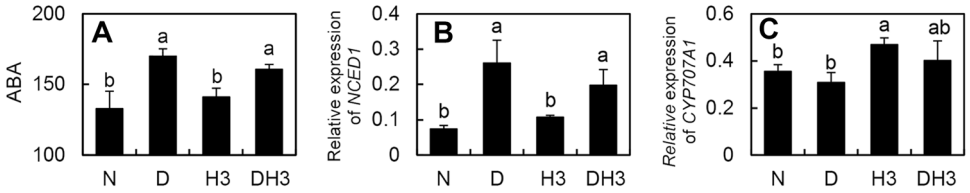

Fig. 7 The ABA concentrations and the transcript level of related genes in Shenfeng under different treatments. N: 0 kPa and 25 °C; D: − 20 kPa and 25 °C; H3: 0 kPa and 45 °C at 3 h; DH3: − 20 kPa and 45 °C at 3 h. Significant differences ($P \leq 0.05$) were performed by different lower case letters

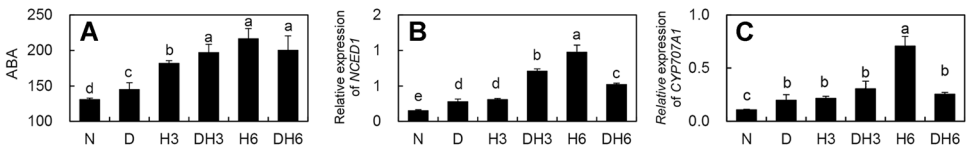

Fig. 8 The ABA concentrations and the transcript level of related genes in Shenhua under different treatments. N: 0 kPa and 25 °C; D: − 20 kPa and 25 °C; H3: 0 kPa and 45 °C at 3 h; DH3: − 20 kPa and 45 °C at 3 h; H6: 0 kPa and 45 °C at 6 h; DH6: − 20 kPa and 45 °C at 6 h. Significant differences ($P \leq 0.05$) were performed by different lower case letters

The DH3 treatment had no effect on the activities of SOD, POD, and CAT, or the MDA content. In Shenhua, SOD activity showed no significant difference across treatments, whereas POD activity increased under all stress treatments. The CAT activity increased under H3 and DH6 treatments and the MDA content increased under D, H3, H6, and DH6 treatments. The T-AOC increased under H6 and DH6 treatments, while the *MnSOD* expression increased under H3, H6, and DH6 treatments (Fig. 10).

Discussion

Water deficit and heat stress damage plants

Plants undergo various biochemical, physiological, and molecular damages due to heat or drought stresses. The transcript levels of heat stress-related genes *HSFA2*, *HSP70*, and *GLOS*, and that of drought stress-related gene *MBF1*, significantly increased under heat or water deficit stress,

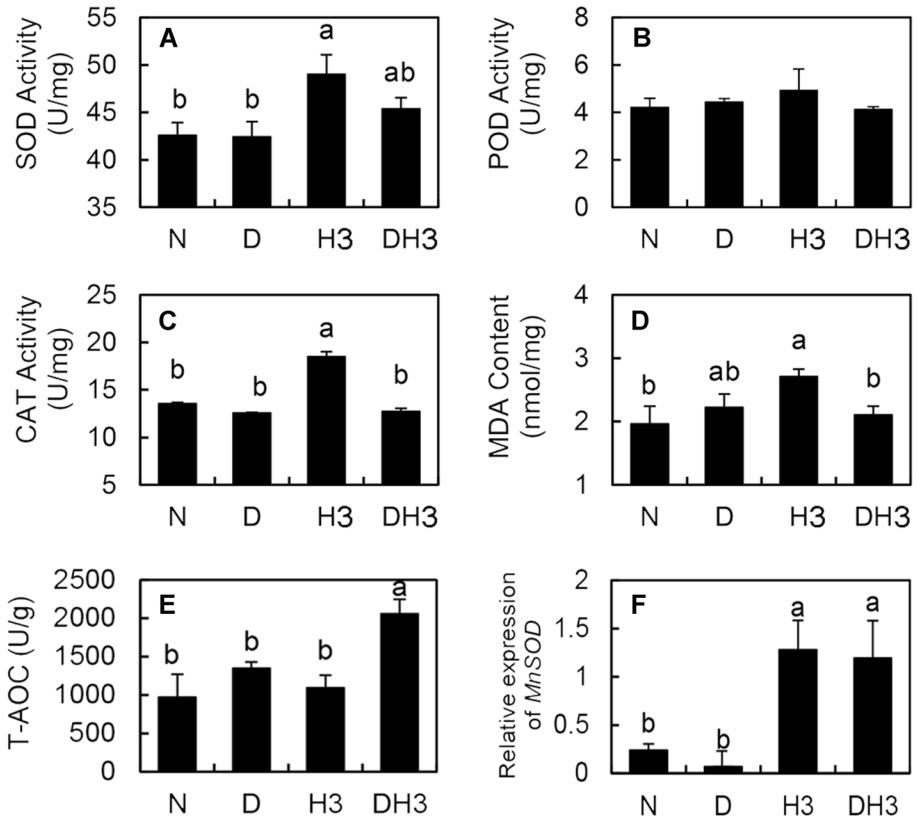

Fig. 9 The activities of SOD, POD, CAT, and MDA content in Shenfeng under different treatments. N: 0 kPa and 25 °C; D: − 20 kPa and 25 °C; H3: 0 kPa and 45 °C at 3 h; DH3: − 20 kPa and 45 °C at 3 h. Significant differences ($P \leq 0.05$) were performed by different lower case letters

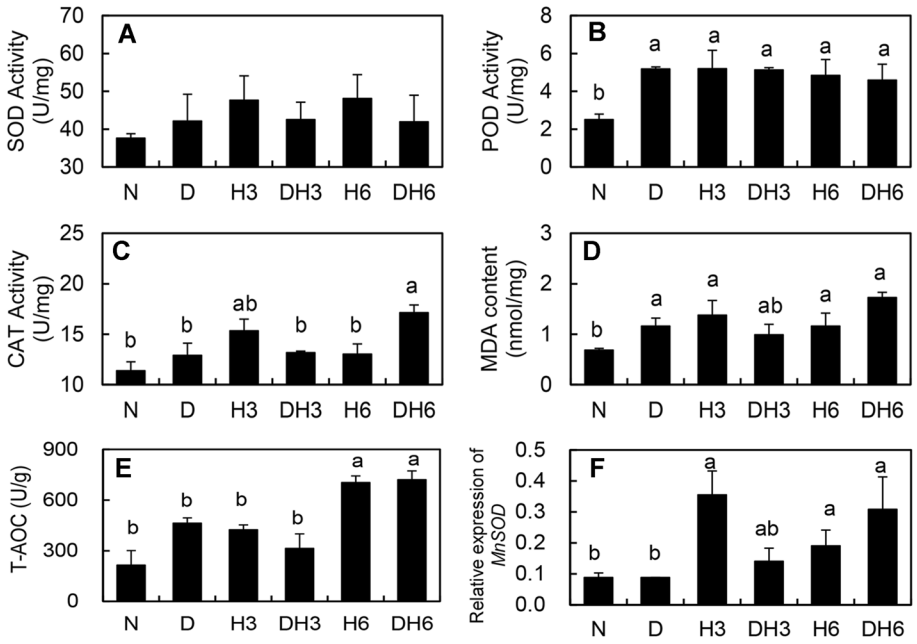

Fig. 10 The activities of SOD, POD, CAT, and MDA content in Shenhua under different treatments. N: 0 kPa and 25 °C; D: − 20 kPa and 25 °C; H3: 0 kPa and 45 °C at 3 h; DH3: − 20 kPa and 45 °C at 3 h; H6: 0 kPa and 45 °C at 6 h; DH6: − 20 kPa and 45 °C at 6 h. Significant differences ($P \leq 0.05$) were performed by different lower case letters

respectively. Previous studies conducted using Shenfeng and Shenhua had also revealed that *HSFA2*, *HSP70*, and *GLOS1* expression increased under high- temperature treatments (Zha et al. 2016a, b) and that *MBF1* expression was induced by water deficit stress (Yan et al. 2014; Zandalinas et al. 2016). This indicated that specific molecular damage is caused by each of these stresses. Relative electrolyte conductivity was enhanced under water deficit, heat, and water deficit plus heat stress in Shenfeng and Shenhua, but stress injuries and their degrees differed among the different treatments. The relative electrolyte conductivity is greatly influenced by both of these stresses (Bajji et al. 2002; Yin et al.

2008), owing to their effects on the permeability of plant cell membranes. The OJIP curve, some PS II parameters, and ABA concentrations changed under high temperature and water deficit treatments, revealing that PS II and its signaling factors respond positively to stresses, in agreement with previously reported results (Yoshida et al. 2014; Wang et al. 2010).

The difference in the response to high temperature (45 °C) between the two grape cultivars, Shenfeng and Shenhua, was most likely due to their genetic differences. Our results showed that some indicators (PI_{ABS}, relative conductivity, W_k, M_o, δ_{Ro}, φ_{Po}, φ_{Eo}, and Ψ_{Eo}) under H and DH treatments did not show significant changes in Shenhua after 3 h, but were obviously altered in Shenfeng; after a 6-h period under these treatments, Shenhua also showed differences in these parameters. In addition, unlike the Shenhua, Shenfeng was not able to resist the high temperature stress.

High temperature stress specifically damages PS II and the activity of antioxidant enzymes

In Shenfeng and Shenhua, changes between F_O and F_I under the DH and D treatments were similar, but significantly lower than those under the H treatment. The greatest increase in F_O was recorded under heat stress, indicating that heat stress caused the maximal damage to the leaf photosynthetic system. The initial chlorophyll *a* fluorescence level at stage *P* of OJIP corresponds to the state where all Q_A molecules are in the reduced state. As F_P was found to be significantly lower in H and DH treatments than in the N treatment, fast and slow reducing *PQ* centers might be present in Shenfeng and Shenhua under these stresses. Changes in the OJIP curve under the DH treatment showed a smoother trend than under the H treatment, suggesting that heat stress sharply alters OJIP curves.

Changes in chlorophyll *a* fluorescence might be used to measure changes in PS II activity, caused directly or indirectly by stress (Su et al. 2014; Sun et al. 2016). Regarding the chlorophyll *a* energy flow, the photosynthetic mechanism includes the capture of light energy, energy distribution, and electron transport, with a competitive relationship among them (Cendrero Mateo et al. 2012). In our study, the PS II parameters were changed under heat stress, but not under water deficit stress, indicating that the tested water deficit level was not fatal to the plants. In previous studies, moderate and severe drought stress often inhibited the activity of enzymes related to PS II (Dias and Brüggemann 2007; Wang et al. 2011; Zhou et al. 2017), but photosynthetic capacity showed little or no change under low drought stress (Yordanov et al. 2000).

Abiotic stresses-related decreases in photosynthesis are usually consistent with a burst of ROS production under abiotic stresses (Kurepin et al. 2015). Over-accumulated electrons with O_2 block the photosynthetic electron transport in the reaction centers of PS I and PS II resulting in the production of ROS (Wang et al. 2011; Oukarroum et al. 2015). The antioxidant pathways of plant prevent ROS overproduction via non-enzymatic and enzymatic antioxidants among others mechanisms (Noctor and Foyer 1998). Here, the activities of SOD, POD, and CAT, and the level of MDA significantly increased under the H treatment in Shenfeng and Shenhua, in agreement with the strong stimulation of oxidative capacity by heat stress, as reported in previous studies (Gomes Silva et al. 2010). Thus, water deficit effectively enhanced the antioxidant capacity.

Water deficit triggers cross tolerance to heat stress

Water deficit priming in plants could induce thermo-tolerance. In our study, PS II and the activities of antioxidant enzymes were severely affected by high temperature treatments, but only slightly affected by the combined water deficit plus high temperature treatments. Zhang et al. (2016) reported that drought priming conferred heat tolerance in wheat, as exemplified by increased grain yield, photosynthesis, and anti-oxidation and proteomic activities in leaves subject to both drought and heat stress, relative to those subjected to heat stress only. At the cellular level, the response to adversity is oxidative stress, which can stimulate or induce the improvement of ROS scavenging ability in plants (Bowler et al. 1992). Thus, cross resistance to drought and heat stresses seems to be closely related to the ability of plants to remove ROS.

The cross talk between water deficit and heat stresses involves ABA

In addition to playing an important role in plants' signal regulation in response to stress (Finkelstein et al. 2002), ABA has been correlated with drought and heat resistance (Reddy et al. 2004; Zhang et al. 2008). Drought and heat stresses induced ABA biosynthesis in Shenfeng and Shenhua. Tolerance to heat stress could be achieved either via protection of PS II activity from photoinhibition (Ivanov et al. 1995), or through the induction of the antioxidant defense system related to ABA accumulation under water deficit stress (Liu et al. 2003). Thus, ABA is likely to play an important role in the cross talk between water deficit and heat stress.

Genes related to ABA biosynthesis are known to be induced by environmental stresses. The transcript level of *NCED* in *Arabidopsis thaliana*, cowpea, and avocado was stimulated by drought stress (Chernys and Zeevaart 2000; Iuchi et al. 2000; 2001). Here, water deficit also induced the expression of *NCED1*, whereas *CYP707A*1 expression was induced by high temperature. Heat stress has also been shown to increase the ABA concentrations in a previous

study (Larkindale et al. 2005). Therefore, there is likely a cross talk between heat- and drought-stress responses, including changes in the expressions of *NCED1* and *CYP707A1*. In addition, ROS are known to mediate ABA signaling in plants through MAP kinases (Jammes et al. 2009) and several ROS-related enzyme activities and products were found to be altered by the abiotic stresses examined in our study. However, how the ROS interact with ABA remains unclear and requires further research.

Author contribution statement QZ and ALJ conceived and designed the experiment. QZ and YNH procured the materials and methods for the experiment. QZ and XXJ worked carried out data curation and formal analysis. QZ, ALJ, and XPF prepared the manuscript.

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