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Physiological changes and differential gene expression of tea plant under dehydration and rehydration conditions

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ABSTRACT

Drought is one of the major constraints for crop growth and productivity worldwide. Here, responses to soil drying and rewatering were measured at morphological, physiological and molecular levels in two adult field-grown tea plant [*Camellia sinensis* (L.) O. Kuntze] cultivars including drought-susceptible 'Zhuyeqi' (T1) and drought-tolerant 'Ningzhou 2' (T2). After eight days drought stress (DS), most of leaves in T1 were reddish brown, curled and withered, whereas only a few needle spots and yellow patches were observed in T2. Based on the morphological symptoms, T2 recovered more quickly than T1. The malondialdehyde (MDA), soluble sugars (SS) and proline (Pro) contents as well as superoxide dismutase (SOD) and catalase (CAT) activities in two cultivars increased significantly as DS progressed and then rapidly decreased following rehydration. In contrast, the abscisic acid (ABA) and salicylic acid (SA) content peaked in the early stage of DS and then decreased rapidly, while these changes were more apparent in T2 than in T1. T1 had a higher concentration of MDA, SS and Pro than T2 throughout dehydration and rehydration. T2 was characterized by lower ABA and higher SA accumulation, but the opposite results were observed in T1. Furthermore, T2 had stable SOD and higher CAT activities during the stress and recovery. Under recovery rewatering, two cultivars still maintained high CAT activities. SS level in T1 was 1.2 times higher than control value on the fourth day after rehydration, while in T2 it was nearly equal to control level. In general, T2 showed more drastic changes in the expression of five selected genes during and after DS, these changes were positively correlated with corresponding physiological indicators. Nevertheless, expression levels of Δ^1-pyrroline-5-carboxylate synthetase (P5CS) gene and phenylalanine ammonia lyase (PAL) gene in T1 were subjected to feedback inhibition. Overall, these findings were consistent with the results from the controlled indoor test and duplicate field test, providing new insights into the drought-tolerant mechanisms in tea plants.

1. Introduction

Drought is a major factor limiting plant growth and agricultural productivity worldwide. These effects may be more severe because of changes in climate, particularly global warming (Eisenstein, 2013). Therefore, understanding of morphological, physiological and molecular aspects of drought responses in plants is critical importance (Mir et al., 2012).

Plants display a series of morphological, physiological and molecular responses to drought stress (DS), making drought tolerance a complex multigenic trait (Jimenez et al., 2013; Shinozaki and Yamaguchi, 2007; Valliyodan and Nguyen, 2006). Due to frequent dry-wet climatic cycles, plants are vulnerable to DS and subsequently return to normal growth. Although there are many studies on the complex mechanisms of plant responses to DS, the research on post-drought recovery mechanisms is still limited (Xu et al., 2010). Plants experiencing DS may survive through maintaining cell turgor, reducing evaporative water loss by accumulating osmolytes, scavenging reactive oxygen species (ROS) and synthesizing new substances such as phytohormones, among other

Abbreviations: ABA, abscisic acid; CAT, catalase; DS, drought stress; GADPH, glyceraldehyde-3-phosphate dehydrogenase; Glu, glutamate; MDA, malondialdehyde; NCED, 9-*cis*-epoxycarotenoid dioxygenase; PAL, phenylalanine ammonia lyase; P5CS, Δ^1-pyrroline-5-carboxylate synthetase; Pro, proline; ROS, reactive oxygen species; SA, salicylic acid; SOD, superoxide dismutase; SS, soluble sugars; SWC, soil volumetric moisture content.

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mechanisms (Aroca, 2012; Seki et al., 2007). A myriad of genes involved in these physiological responses have been identified by genomics and molecular genetics methods, and relative expression levels of drought-related genes were quantified by quantitative real-time polymerase chain reaction (qRT-PCR) analyses.

Tea plant [*Camellia sinensis* (L.) O. Kuntze], an evergreen, thermophilic, hygrophilous and woody perennial crop, is one of the most popular beverage crops in the world. Drought is a major constraint for tea plant growth, yield and quality (Sharma and Kumar, 2005). Approximately 260,000 hectares of tea plantations in Yunnan province China were harmed by continuous drought in the spring of 2010 (Liu and Chen, 2014). It was reported that DS affected tea production by 14–33%, with nearly 6–19% plant mortality (Cheruiyot et al., 2010). As previously reported, tea plants adapt to resist DS through regulation of photosynthesis and osmosis as well as scavenging ROS (Guo et al., 2009; Upadhyaya and Panda, 2004). However, few studies have focused on changes to the phytohormone content in tea plants experiencing DS; interaction of osmolytes, antioxidases and phytohormones in tea plants are not well understood under DS and rewatering. Furthermore, a set of drought responsive genes and their pattern of expression were identified by using cDNA-amplified fragment length polymorphism (cDNA-AFLP) and suppression subtractive hybridization (SSH) (Gupta et al., 2012, 2013). Nevertheless, during and after DS, comprehensive physiological and molecular studies on the responses of susceptible and tolerant mature field-grown tea cultivars are still lacking.

The present study was conducted to investigate morphological, physiological and gene expression changes under dehydration and rehydration in drought susceptible 'Zhuyeqi' (an elite clone selected from a landrace of Hunan Province, T1) and tolerant 'Ningzhou 2' (an elite clone selected from Jiangxi Province, T2) tea cultivars. We analyzed the activity of antioxidant enzymes, changes to malondialdehyde (MDA), osmolyte and phytohormone content and expression of five drought-related genes after four and eight days of DS and after four days of rewatering. Comprehensive studies of the mechanisms of drought tolerance will provide more insight into the mechanisms of drought resistance in tea plants.

2. Materials and methods

2.1. Plant material, growth and stress conditions

Prolonged drought in July and August 2013 affected tea production in Hangzhou, the capital of east China's Zhejiang Province. In July, the average rainfall was only 8.8 mm (1.5 mm between July 5th and 6th, and 7.3 mm on July 21st). A heavy thunder shower (34.1 mm) occurred on August 1st, and conditions were cloudy and without drought on August 2nd–5th.

The experiment was conducted at the China National Germplasm Hangzhou Tea Repository from July to August 2013. Thirty-one national tea cultivars were 10 years old, grown in the same habitat, managed in the same way and slightly pruned in April. During this severe DS period, they were subjected to varying degrees of DS. Their drought-tolerant capability was preliminarily evaluated by observing the morphological symptoms according to the method of Chen et al. (2005). Consequently, two tea cultivars, drought-susceptible 'Zhuyeqi' (T1) and drought-tolerant 'Ningzhou 2' (T2) were selected for this work. When the soil volumetric moisture content (SWC) was below approximately 18% in silt–clay loam soil, the plants were subjected to the start of DS (Rab et al., 2011), and DS was achieved on July 26–31. The control tea plants were obtained on July 22nd, which was cloudy and had SWC above 18%. The rehydration of drought-stressed plants was determined to have occurred at 96 h after rewatering. Before DS as well as during DS and recovery, samples of 'two and a bud' (one young shoot with two leaves and a bud) from over 20 plants were collected, immediately frozen in liquid N_2, and stored at −80 °C. Leaf materials were collected once every four days from 17:00 to 17:30. Three independent experiments were performed, and the data were displayed as the mean ± SE.

To validate the results from the field trials, the controlled indoor test and duplicate field test were conducted. Three-year-old T1 and T2 clone cuttings were used for the indoor test. Plants were cultured in plastic pots (30-cm diameter, 35-cm height) in a substrate of 40% humus soil, 40% subsoil, 10% vermiculite and 10% pearlite grain under greenhouse conditions in May 2013. One year later, these plants were transferred to an artificial climate chamber in the months of June to July in 2014. Temperatures were between 20 °C to 28 °C and relative humidity was 60 ± 5%. The tea plants were watered periodically to soil capacity until application of stress. Afterwards, water stress was induced by withholding water in experimental plants, while the control plants were regularly watered. Furthermore, a shed for raining protect was built using a colorless and transparent plastic film over T1 and T2 plants under field conditions in the months of June to July in 2014. After acclimation for 20 days, the plants for drought test were withheld watering, while the control plants were regularly watered. After DS trials, all the plants were immediately watered to soil moisture capacity for recovery test.

2.2. Physiological measurements

The soil volumetric moisture content (SWC) was measured in $m^3\ m^{-3}$ with a time-domain reflectometer (TDR) (TDR100; Campbell Scientific Inc., Logan, UT, USA) at the 0–40 cm soil layer. The particle-size composition was determined by the pipette method (LY/T 1225-1999). The catalase (CAT) activity as well as MDA, soluble sugars (SS) and free proline (Pro) content were determined as described by Wang (2006) with some modifications. Briefly, one CAT activity unit (U) was defined as the conversion of 1.0 μmol of hydrogen peroxide per minute, and the activity was expressed as AU g^{-1} FW. Superoxide dismutase (SOD) activity was determined by measuring the rate of enzymatic inhibitioning of $O_2^{-\bullet}$ produced by xanthine morpholine and xanthine oxidase using a SOD detection kit provided by Nanjing Jiancheng Bioengineering Institute (Nanjing, China). One SOD activity unit (U) was defined as the quantity of SOD required to inhibit 50% of the nitrite reduction, and the activity is expressed as AU g^{-1} FW.

Abscisic acid (ABA) and salicylic acid (SA) were extracted and purified according to the method of Liu et al. (2013) and Pan et al. (2010) with some modifications. Briefly, a high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) system consisting of a triple-stage quadrupole mass spectrometer with linear ion trap capability (Waters, Milford, MA, USA) was used to conduct the analysis. SA and ABA matrix-matched calibration curves were used to quantify the analyte content. A Symmetry C_{18} column (Waters, Milford, MA, USA) (3.9 mm × 150 mm, 5.0 μm) was used, and the injection volume was 10.0 μL. Two eluents, A and B, were used: 0.1% formic acid and methanol, respectively. The eluent flow rate was 0.3 mL/min, and the column oven temperature was thermostated to 35 °C. Electrospray-ionization tandem mass spectrometry (ESIMS/MS) analysis was performed in the negative ionization mode, and the capillary voltages, ion source temperature, desolvation temperature, desolvation gas flow rate and cone gas flow rate were 3.0 kV, 80 °C, 350 °C, 550 L/h and 50 L/h, respectively. Qualitative analysis was performed using multiple-reaction monitoring (MRM).

2.3. RNA extraction and quantitative RT-PCR

9-*cis*-Epoxycarotenoid dioxygenase (NCED) is the key enzyme involved in the biosynthesis of ABA (Seo and Koshiba, 2002). Phenylalanine ammonia lyase (PAL) is involved in the biosynthesis of SA and plays an important role in the response to abiotic stresses (Andre et al., 2009; Kim and Hwang, 2014). Δ^1-pyrroline-5-carboxylate synthetase (P5CS) is a key enzyme in plants Pro synthesis (Dobra et al., 2011; Deng et al., 2013). Additionally, evidence has suggested that DS enhances antioxidative enzyme gene expression, e.g., expression of *SOD* and *CAT* (Bian and Jiang, 2009; Marok et al., 2013). Therefore, to examine how changes in gene expression could lead to drought tolerance and recovery and investigate the correlation between the physiological and gene expression responses in the studied cultivars, transcript levels of five drought-related genes (*NCED1*, *PAL*, *P5CS*, *SOD* and *CAT*) under dehydration and rehydration were quantitated by qRT-PCR.

The total RNA was extracted using the RNeasy Plant Mini Kit according to the manufacturer's instructions (Qiagen, Germany) and treated with RNase-free DNase II (Takara, Dalian, China). The RNA quality and concentration were assessed using a NanoDrop ND-2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and 1% formaldehyde–agarose gel electrophoresis. Complementary DNA (cDNA) synthesis was performed using 500 ng DNase-treated total RNA and the PrimeScript™ RT reagent kit (Takara, Dalian, China) by incubation at 37 °C for 15 min followed by incubation at 85 °C for 5 s. Primers (Table 1) for qRT–PCR were designed using the software Primer Premier 5 (Premier Biosoft International, Palo Alto, California, USA) and synthesized by Shanghai HuaGene Biotech Co., Ltd (Shanghai, China). Glyceraldehyde-3-phosphate dehydrogenase gene (GADPH) was used as an internal control for gene expression normalization. The qRT-PCR analysis was performed using an ABI 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA), 100 ng reverse transcriptase template and SYBR Premix Ex Taq™ II (Perfect Real Time, Takara, Dalian, China). The PCR conditions were as follows: 30 s at 95 °C followed by 40 thermal cycles of 5 s at 95 °C and 34 s at 60 °C. The reactions were followed by melting curve detection to check for the generation of non-specific products and primer-dimer using the following conditions: 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s. The relative gene expression levels were calculated using the comparative delta-delta CT method and expressed as the fold change relative to expression in the null controls (expression = 1).

2.4. Statistical analysis

A one-way analysis of variance (ANOVA) followed by a post-hoc Fisher's LSD test was used to examine the significant differences between measurements at different stages. For the two cultivars, bivariate correlations between traits and the corresponding gene expression levels were calculated using Spearman's rank correlation, and the differences were considered to be significant at a probability level of $P < 0.05$. In the figures, differences among the stages of each cultivar at each sampling point are indicated by letters. These analyses were conducted using SPSS18.0 (Chicago, IL, USA).

3. Results

3.1. Soil water and leaf morphological status indicate the degree of drought stress

The soil texture was determined to be a silt–clay loam consisting of 54.6% clay, 31.4% silt, and 14.0% sand. The field capacity SWC was 33.2% ± 1.6%. DS was induced gradually by withholding water for four days until the SWC decreased to 74.0% of that for the control plants (normal moisture level) (Fig. 1A). After four days of drought, a number of curly yellow leaves with brown spots and some reddish brown leaves were observed in T1, whereas no significant changes were observed for the T2 leaves (Fig. 2A). After eight days of DS, most of the T1 leaves were reddish brown (mainly 1st to 4th leaf), curled and withered, while for T2, only needle spots and yellow patches were observed. During the recovery period, the morphological symptoms of DS including reddish brown and curled leaves were still observed in T1, while T2 cultivar sprouted more new buds and its own damaged organs were more rapidly eliminated in comparison to T1, indicating T2 recovered more quickly than T1 (Fig. 2B). According to soil water and leaf morphological status, T2 had higher DS resistant capacity than T1.

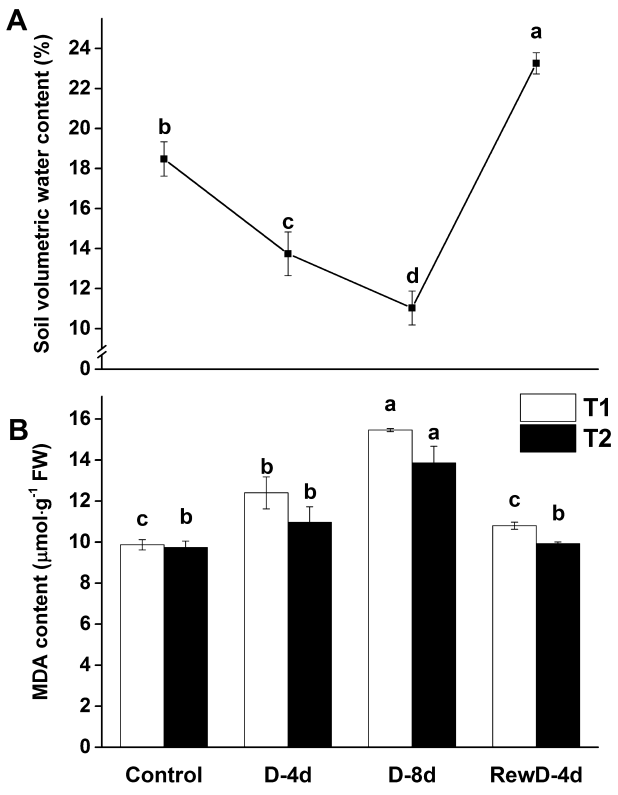

Fig. 1. Physiological parameters of the tea cultivars at four (*D*—4 d) and eight (*D*—8 d) days of drought stress and after four days rewatering (*D*—8 d followed by rewatering, Rew*D*—4 d). (A) SWC content, (B) MDA content. Data are displayed as the mean of three replicates with standard error. Statistically significant differences among stages of each cultivar at each sampling point are indicated with different letters ($P < 0.05$).

3.2. Lipid peroxidation

MDA is a major cytotoxic product of lipid peroxidation, and changes to its abundance represent the degree of lipid-membrane oxidation by oxidants such as free radicals (Ozkur et al., 2009). MDA contents were higher in T1 than in T2 throughout the DS and recovery periods (Fig. 1B). At eight days DS, MDA concentrations in T1 and T2 were increased by 56.7% and 41.3%, respectively, relative to the control (Fig. 1B), with T1 accumulating 1.2-fold more MDA than T2. Changes in MDA content of two cultivars during dehydration further indicated that T1 was more vulnerable to DS in comparison to T2. After rewatering, these values decreased significantly in the two cultivars compared with the value at eight days DS and were close to the control levels.

Table 1
Forward and reverse primers used for qRT-PCR reactions.

Gene name	Accession number	Primer sequence	Product length (bp)
GADPH	–	F: TTG GCA TCG TTG AGG GTC T R: CAG TGG GAA CAC GGA AAG C	213
NCED1	KC816734	F: GGC CAC TCC GGC ATC GCT CGC CTC R: CGT CTT CCG ACA TGG CTA AGA GGC	140
PAL	D26596	F: GCT CTT CGG ACT TCA CCT CAA TGG R: GCC TTG TTC CTC GAA ACA TCG ATC A	128
P5CS	KJ143742	F: TGT TGG TGA AAG GCT CAT TGG A R: CCA TCA GCA TGA CCC AGA ACA G	162
SOD	AY694187	F: CAT TTC AAT CCT GCT GGC AAA GA R: GCA TGG ACA ACA ACG GCC CTA CC	176
CAT	AY641732	F: TGC AGA GAA TGA GCA GCT TG R: GTG CCT CTG GGT ATC AGC GTA G	115

3.3. Phytohormone response

In the absence of DS, two tea cultivars accumulated low ABA (Fig. 3A); T1 showed the higher ABA content than T2. DS caused a rapid increase of ABA levels in the two cultivars. T2 showed a more rapid increase in ABA content, rising 121.9% after four days DS compared to the control, whereas T1 showed an increase of 82.3%. However, prolonged DS induced a rapid reduction in ABA levels for T1 and T2. T2 showed more rapid reduction in ABA content, dropping 55.9%, while for T1 (33.6%) at eight days DS compared to at four days DS. During DS, the ABA content was higher in T1 than in T2. After four days of recovery, a significant decrease in the ABA content was observed in T1, whereas the T2 ABA content increased significantly compared with the control values.

At four days of DS, the SA levels increased markedly in two cultivars (Fig. 3B). T2 increased 2.6-fold compared with the control, whereas a 1.2-fold increase for T1. Prolonged DS also induced a rapid reduction of SA levels in two cultivars. At eight days DS, two cultivars showed a significant decrease in SA content compared with the levels at four days (27.4% for T1 and 21.5% for T2). At eight days DS, the T2 SA content was higher than the corresponding value for the control, while the T1 SA content was lower than the control. During the DS periods, the T2 SA content was higher than T1. After four days of recovery, a significant decrease in the SA content was found in T1 and T2 compared with the levels at eight days stress.

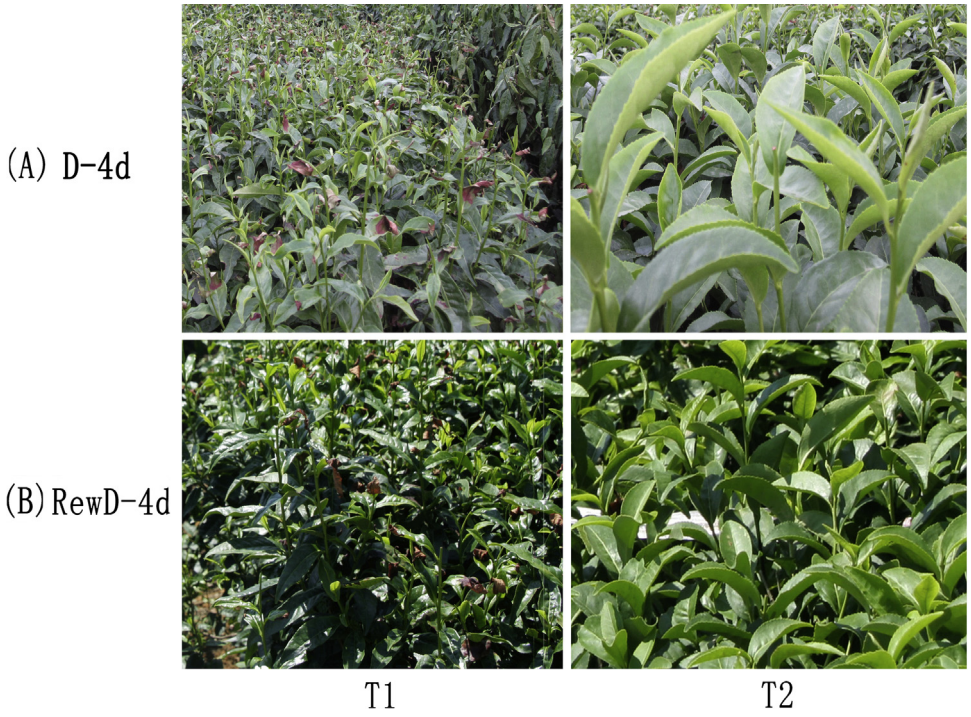

Fig. 2. Leaf morphology of the tea cultivars under drought stress and recovery irrigation. (A) *D*—4 d, (B) Rew*D*—4 d.

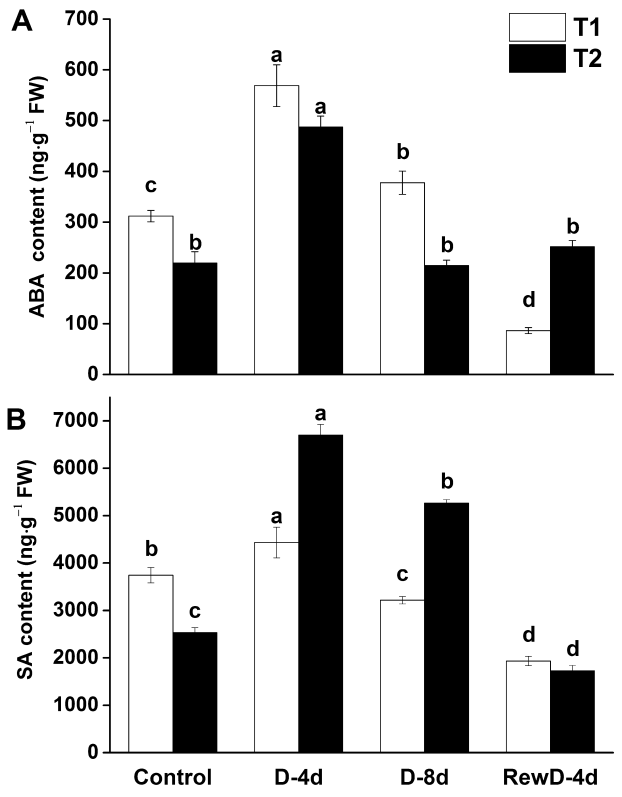

Fig. 3. ABA and SA content after four and eight days of drought stress and recovery irrigation. (A) ABA content, (B) SA content. Data are displayed as the mean of three replicates and standard error. Statistically significant differences among stages of each cultivar at each sampling point are indicated with different letters ($P < 0.05$).

3.4. Changes in soluble sugars and proline content

As shown in Fig. 4A, the SS increased in a drought-dependent manner and peaked at eight days of DS. After four days DS, the cultivars showed a slight increase in the SS content (9.3% for T1 and 2.5% for T2) compared with before DS. After eight days DS, the SS content was increased by 25.1% and 18.1% for T1 and T2, respectively, compared to the control. Following four days of rehydration, SS level in T1 was 1.2 times higher than control value, while in T2 it was nearly equal to the control level. As expected, T1 showed more SS accumulation at each stage.

At eight days DS, the Pro levels were increased 1.4-fold for T1 and approximately 1.5-fold for T2. T1 showed more accumulation of Pro during DS and recovery. After four days of recovery, the Pro levels in two cultivars fell significantly to levels equal to the control (Fig. 4B).

3.5. Changes to antioxidant activity

After four days of DS, the SOD activities were significantly increased in T1 (85.2%) compared to the control, while T2 only showed a slight increase of 6.1% (Fig. 4C). The SOD activities increased noticeably and peaked at eight days DS compared with the control (2.0-fold for T1 and 1.2-fold for T2). After four days of recovery, the SOD activities decreased markedly by 34.4% and 25.9% compared with those at eight days DS for T1 and T2, respectively. In the absence of stress, T2 maintained higher SOD activity compared with T1.

With prolonged periods of DS, the CAT activities were significantly increased for two cultivars (Fig. 4D). After four days DS, the CAT activities increased by 32.7% and 19.8% compared with the control for T1 and T2, respectively. At eight days DS, two cultivars showed a peak and significant increase in CAT activities. After four days of rehydration, a slight reduction of CAT activities was observed for T1 and T2, compared with the activities observed for eight days DS. At multiple stages, T2 appeared to maintain higher CAT activity compared with T1.

3.6. Relationships among eight physiological indicators during and after drought stress

There were negative correlations between SWC with seven physiological indicators in two cultivars after four days DS, while positive correlations among these seven physiological indicators were observed (Fig. 5). In contrast, no significant correlations between SS with ABA and SA were observed for T2 at four days DS. In addition, Pro in T2 was positively but not significantly correlated with SS.

After eight days DS, SWC in T1 had positive correlations with SA, whereas that in T2 was negatively and significantly with SA (Fig. 5). Additionally, there were no significant correlations between the ABA and SA with Pro, SS, SOD and CAT in T1 at eight days DS, only SA in T2 was positively and significantly correlated with Pro and CAT. During DS, a significant positive correlation was observed between the SS and Pro with the SOD and CAT.

On the fourth day after rewatering, ABA in T1 was positively and significantly correlated with MDA, SS, Pro and SOD, while that in T2 was negatively correlated with these four indicators. ABA in T1 had significant positive correlation with SA, whereas the opposite result was observed in T2. Moreover, low-level correlations between CAT with SWC, MDA, ABA, SA, SS, Pro and SOD were observed for T1, while CAT in T2 was strongly correlated with these indicators except ABA.

3.7. Expression of five drought-related genes

The expression levels of five genes were dramatically increased under DS in two cultivars (Fig. 6). After four days of recovery, the expression levels of the five genes were dramatically reduced for T2, while the expression levels of *PAL* and *P5CS* in T1 were continuously up-regulated. *NCED1* expression increased to a peak at eight days DS in T1; however, expression in T2 peaked at four days DS and then dropped at eight days DS, which was consistent with its synthesis of ABA, indicating that T2 responded more rapidly to drought signals than T1. For T1 and T2, the expression levels of *SOD* and *CAT* peaked at eight days DS, whereas T2 showed higher up-regulation of *SOD* expression levels.

3.8. The relationship between gene expression and corresponding physiological indicators

During dehydration and rehydration, a significant positive correlation was observed between the *NCED1* expression levels and ABA content in T1 and T2 (Table 2). The *PAL* expression levels were significantly and negatively correlated with SA content in T1, but no significant correlations were observed in T2. The *P5CS* expression levels were significantly and positively correlated with Pro content in T2, while no significant correlation was observed in T1. The *SOD* and *CAT* expression levels were significantly and positively correlated with SOD and CAT activity in two cultivars.

3.9. The indoor and annual seasonal field test validates the field results

To validate these field trial results, we conducted the controlled indoor test and duplicate field test. In both cultivars, MDA contents increased markedly with the drought degree aggravated and then

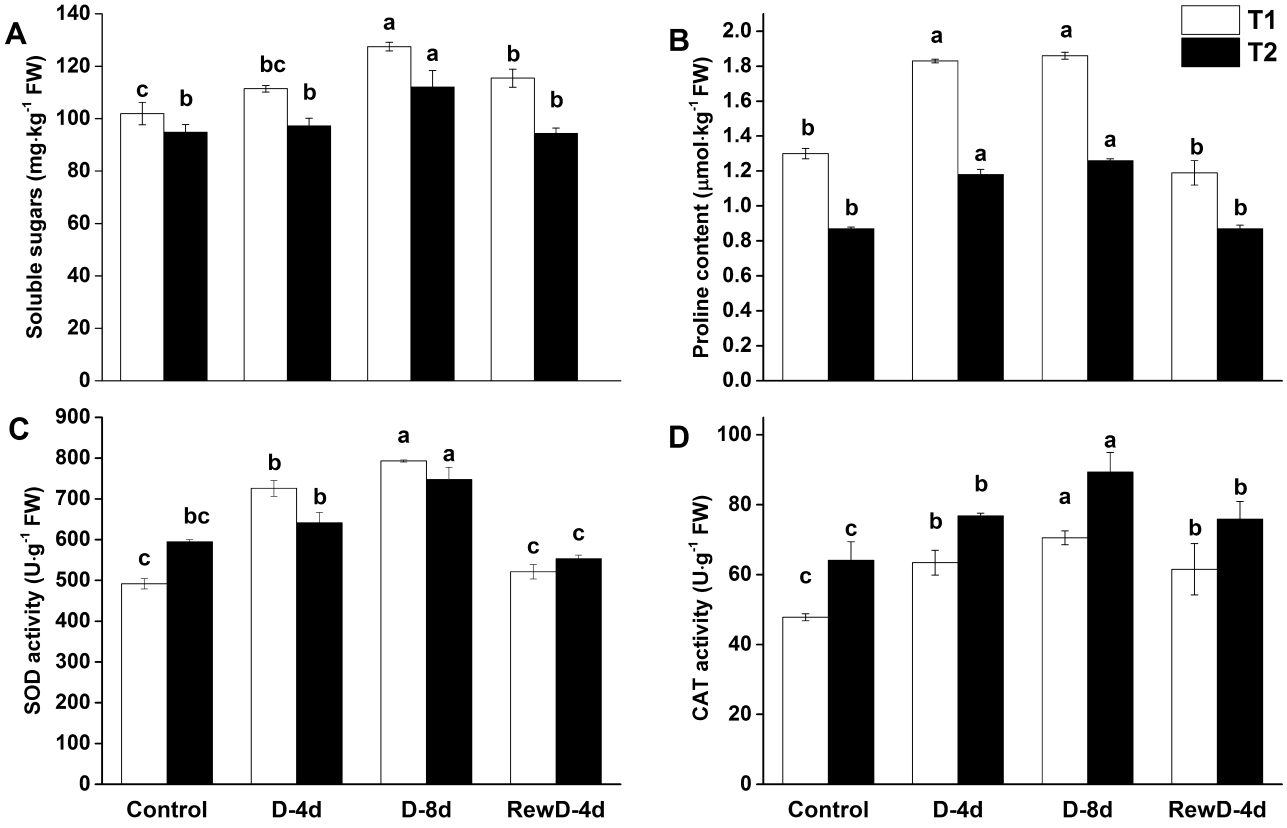

Fig. 4. SS and Pro content and SOD as well as CAT activities following four and eight days of drought stress and recovery irrigation. (A) SS content, (B) Pro content, (C) SOD activity, and (D) CAT activity. Mean values of the three replicates with standard error are displayed. Statistically significant differences among stages of each cultivar at each sampling point are indicated with different letters ($P < 0.05$).

rapidly decreased following rehydration under two different treatments (Figs. 7B and 8B). Changes in ABA and SA contents were also consistent with the results from 2013 field trial (Figs. 7C and D and 8C and D). However, compared with the control and the field trial in 2013, a small change in the amplitude of SA content in T1 and T2 was observed in 2014 field test (Fig. 8D). This may be mainly attributed to no significant difference in degree of drought between at five days DS and at 10 days DS.

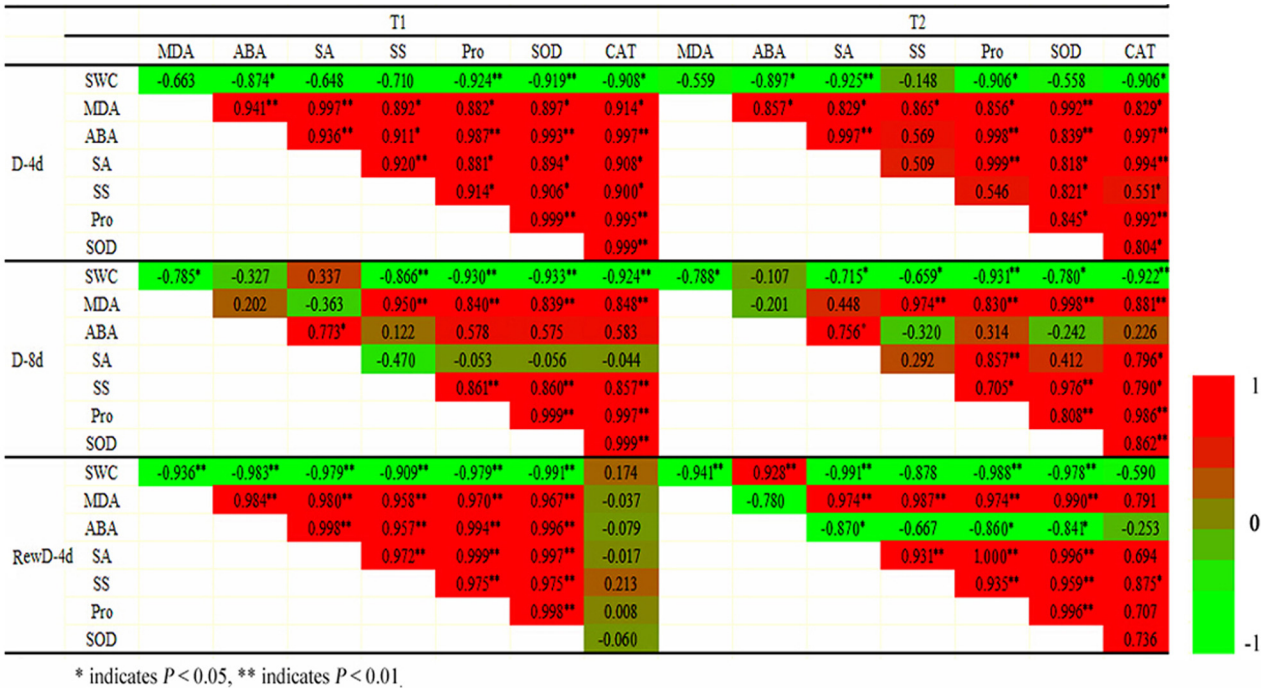

Fig. 5. Relationships among eight physiological indicators of control, drought stress (D—4 d, D—8 d) and rewatering (Rew$D$—4 d) in two tea cultivars.

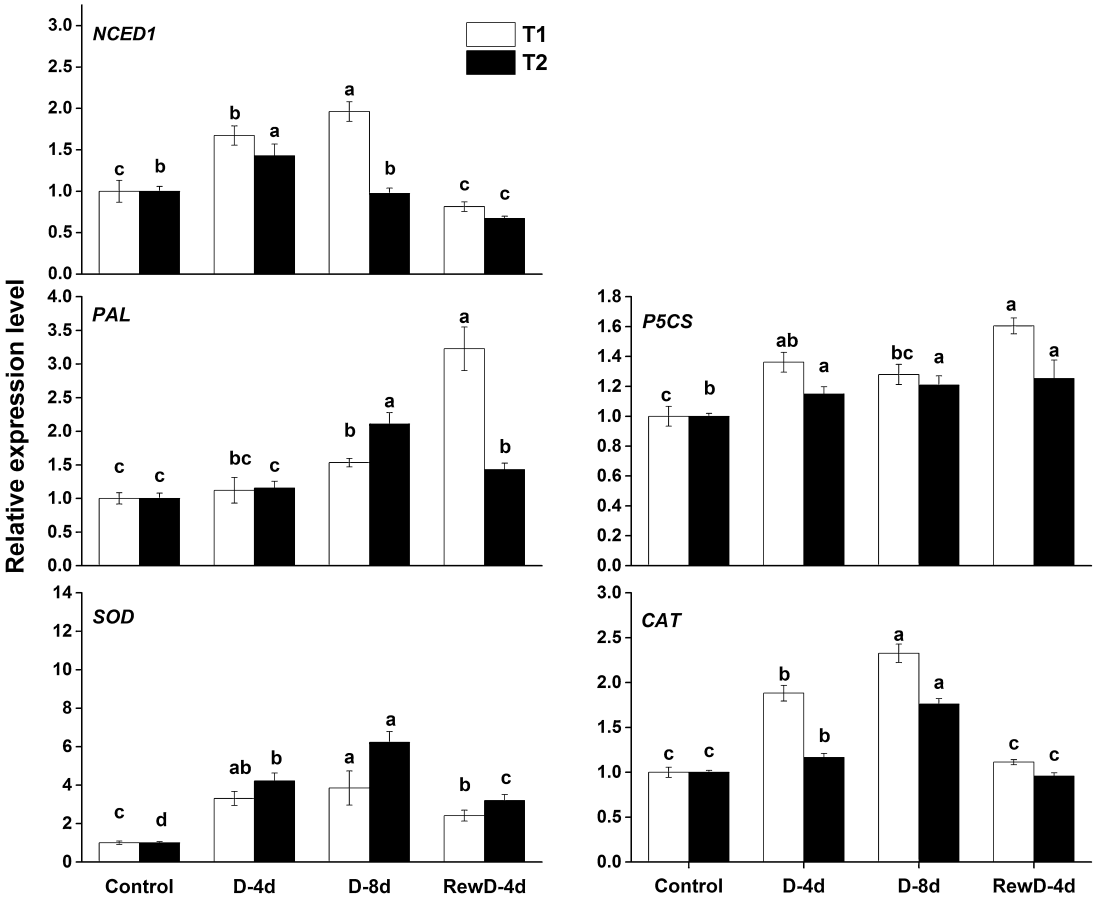

Fig. 6. Expression of studied genes in tea plants exposed to drought stress and recovery irrigation detected by qRT-PCR.
GAPDH was used as a control. Data are displayed as mean values of the three replicates with standard error. Statistically significant differences among stages for each cultivar at each sampling point are indicated with different letters ($P < 0.05$).

The indoor and duplicate field test further confirmed that the expression of *CAT*, *SOD* and *P5CS* was clearly induced by drought in both cultivars (Figs. 9 and 10). Peak expression of *NCED1* was observed earlier than that of the other four genes (Figs. 9 and 10). The expression pattern of *PAL* and *P5CS* were almost the same with the results in 2013 (Figs. 9 and 10). Moreover, in accordance with the results in 2013, the expression of *PAL* was increased with SA content decreasing.

4. Discussion

Changes in leaf morphological and physiological traits could more accurately indicate the degree of stress (Corina et al., 2009; Sperdouli and Moustakas, 2012). Monitoring the SWC, leaf morphology and MDA content throughout the dehydration and rehydration ensured that the stress levels were adequate and equivalent for two cultivars. Our data found that, with the DS prolonged, the content of MDA, Pro and SS increased significantly, and the activities of SOD and CAT also enhanced. After rehydration treatment, the content of MDA, Pro and SS as well as the activities of SOD and CAT were all decreased. In contrast, the ABA and SA content increased markedly at four days DS and then decreased rapidly at eight days DS and recovery irrigation. Similar changes in ABA and SA content under DS were also observed in bluegrass (*Poa annua* L.), poplar (*Populus* × *canadensis* Moench) and *Arabidopsis thaliana* (Jing et al., 2014; He et al., 2014; Yu and Ma, 2014), while research on these hormones in response to rewatering is still limited. Our results were consistent with previous studies (Upadhyaya and Panda, 2004; Liu et al., 2010; Netto et al., 2010), which indicated that the response of tea plants to DS was severely affected by DS intensity and duration. By comparing the susceptible and tolerant cultivars in response to DS, our research found that their morphological, physiological and molecular responses to drought were characterized at the different stages.

Table 2
Correlation coefficients for gene expression levels and the corresponding physiological indicators.

	T1					T2				
	ABA	SA	Pro	SOD	CAT	ABA	SA	Pro	SOD	CAT
NCED1	0.80*					0.81*				
PAL		−0.72*					0.28			
P5CS			−0.06					0.53*		
SOD				0.97**					0.81**	
CAT					0.64*					0.97**

* Indicates $P < 0.05$.
** Indicates $P < 0.01$.

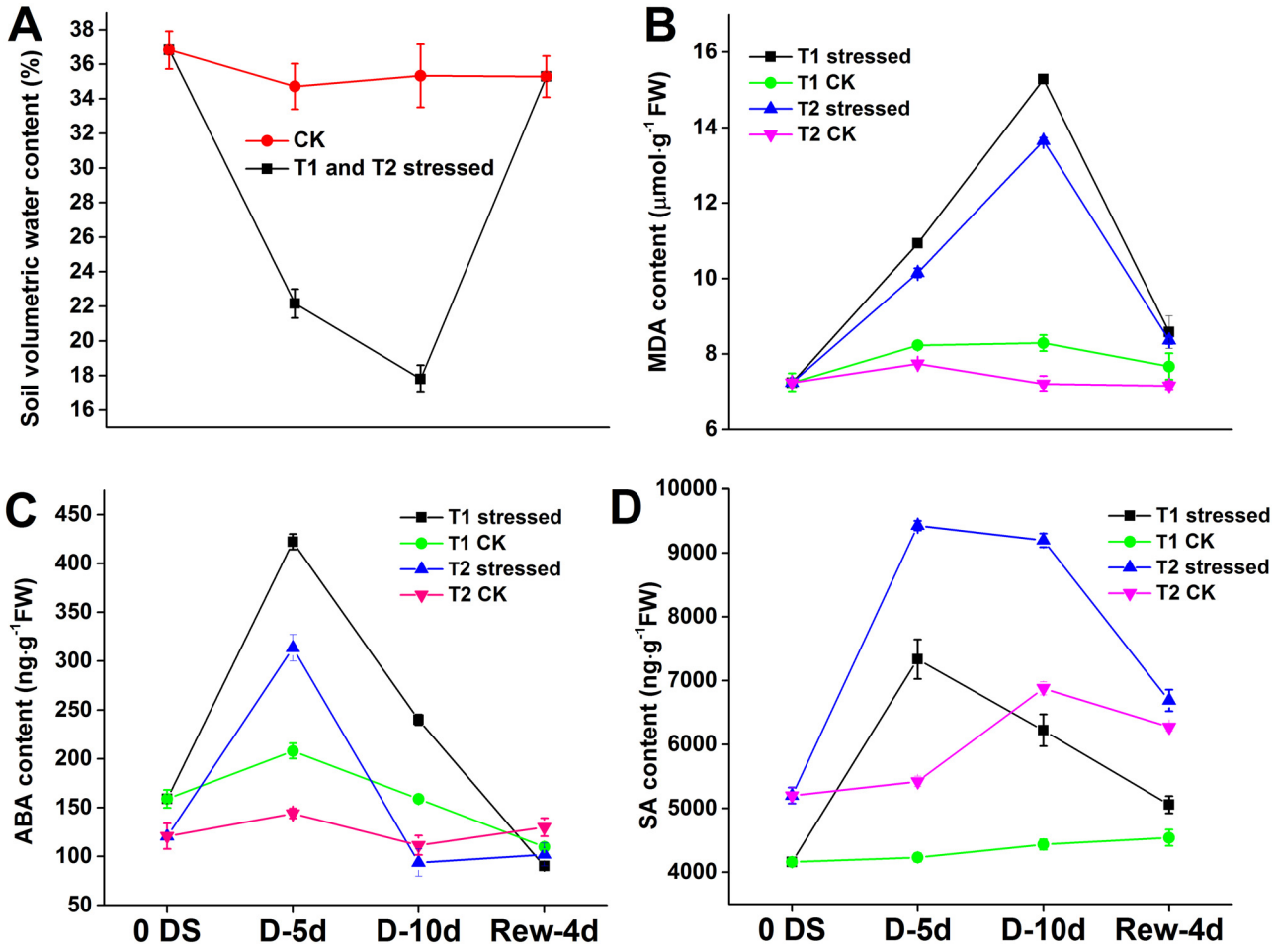

Fig. 7. Under drought stress and recovery rewatering, physiological parameters of tea plants grown in an artificial climate chamber. (A) SWC content, (B) MDA content, (C) ABA content, and (D) SA content. Mean values of the three replicates with standard error are displayed.

4.1. Different physiological responses among tea cultivars under drought stress and recovery

Based on changes in the leaf morphology and MDA content during DS, T2 showed higher drought-tolerant capacity compared with T1. Our results were in accordance with Netto et al. (2010), who reported that drought-tolerant tea cultivars had lower MDA content following 30 days of non-irrigation compared with susceptible cultivars.

Phytohormones, being important signaling molecules, are rapidly synthesized in response to a wide range of abiotic stresses such as drought, heat, cold and salinity (Miyazono et al., 2009; Sheard and Zheng, 2009; Okuma et al., 2014). Recently, several lines of evidence have suggested that ABA- or SA-pretreated plants such as maize (*Zea mays*) showed much higher osmolytes accumulation and antioxidant enzyme activity than the control (Jiang and Zhang, 2002; Costa et al., 2011). In our experiments, compared with accumulation SS and Pro as well as SOD and CAT activity, the ABA and SA levels strongly and rapidly peaked in two cultivars during the early drought stage. Additionally, three different treatment experiments revealed that the peak expression of *NCED1* was more significantly earlier than that of *CAT*, *SOD* and *P5CS* under DS. These results indicated that ABA and SA were synthesized and preferentially accumulated in comparison to the SS, Pro, CAT and SOD, thereby inducing a defense response against DS. In the present study, the ABA contents were lower in T2 than T1 during DS, suggesting that the drought-tolerant cultivar with lower accumulation of ABA induced ABA sensing, signaling and transport drought signals to cope with DS. Our data are in accordance with previous reports; for example, Stikic and Davies (2000) reported that drought-susceptible maize accumulated more ABA than tolerant maize, and Wang and Huang (2003) reported that drought-tolerant cultivars of Kentucky bluegrass were characterized by lower ABA accumulation. It was also reported that high ABA levels in the tomato were observed in severe DS phenotypes (Tung et al., 2008). However, the SA levels of T2 were obviously greater than those of T1 during DS, suggesting that T2 is characterized by higher SA accumulation. Leaves of T2 cultivar accumulated more SA and less ABA, while the opposite result existed in T1, implying that a balance of biosynthesis and catabolism controls the ABA and SA levels in response to DS. It was reported that application of exogenous ABA prevented SA accumulation in *Arabidopsis* (Mohr and Cahill, 2007). In our research, T2 showed a more rapid increase in ABA and SA content at four days DS as well as a more rapid reduction in ABA levels at eight days DS, indicating that T2 can more rapidly respond to DS than T1. Furthermore, these data suggest that ABA and SA homeostasis are important for coordination of tea plant growth under conditions of prolonged drought.

Numerous studies have shown that Pro and SS play a vital role in osmotic adjustment and signal transduction at the same time, and Pro signaling could interact with SS signaling pathway (Moustakas et al., 2011; Sperdouli and Moustakas, 2012; Mohammadkhani and Heidari, 2008). Sperdouli and Moustakas (2012) have proposed that Pro and SS protection against oxidative stress may be partly due to activation of specific ROS scavenging systems. In the present study, significant positive correlations among SS, Pro, SOD and CAT

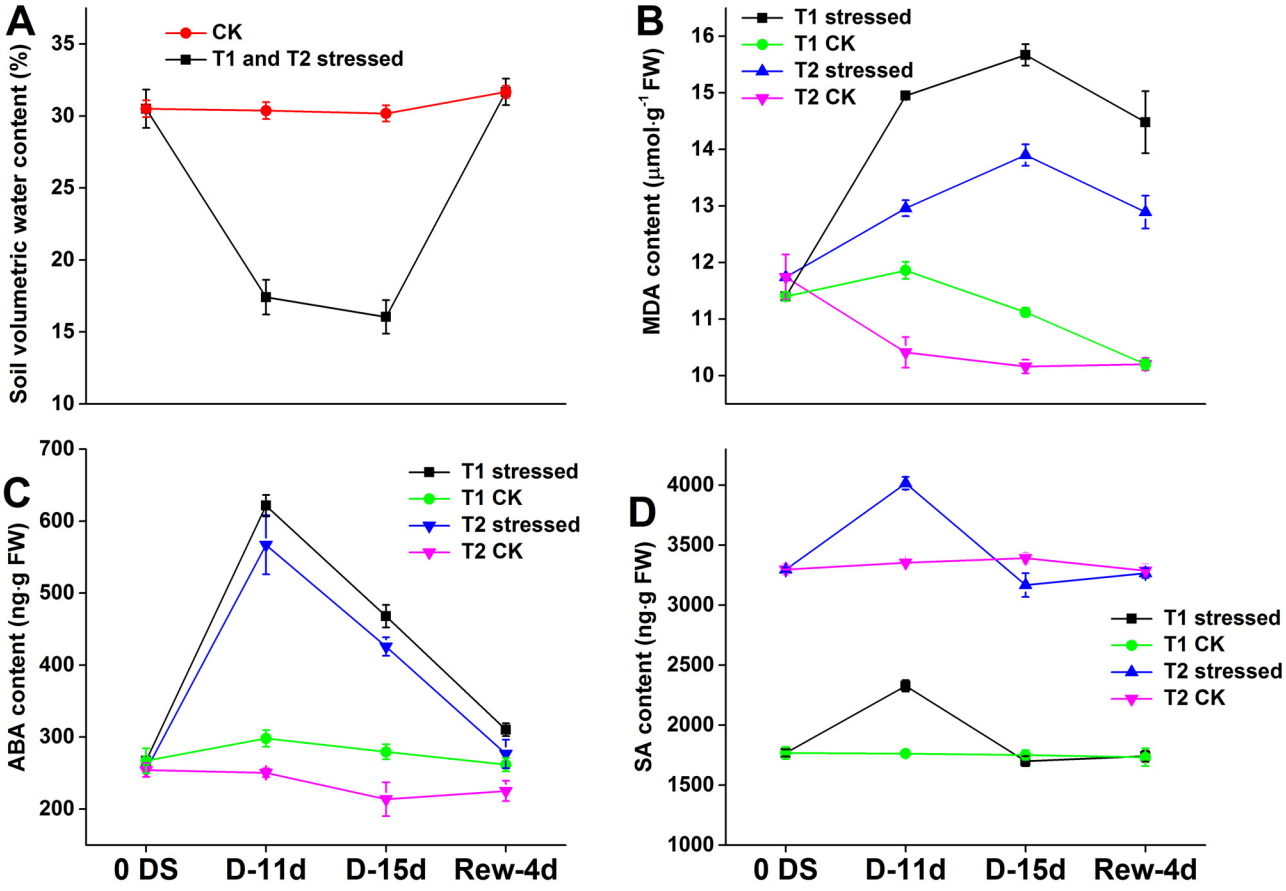

Fig. 8. Under drought stress and recovery rewatering, physiological parameters of tea plants grown in a field plastic shed. (A) SWC content, (B) MDA content, (C) ABA content, and (D) SA content. Mean values of the three replicates with standard error are displayed.

were observed in two cultivars under DS. Additionally, our results suggested that the accumulation of Pro and SS in tea plants leaves under DS was dependent on the cultivar as well as the stress intensity and duration. Among tea cultivars, glutamate (Glu) content varies significantly (Yue et al., 2010). Verslues and Sharma (2010) suggested that Pro was synthesized mainly from Glu in plants under DS. In our experiments, T1 had higher Pro levels compared to T2, possibly due to T1 with higher Glu content. After rehydration, the Pro content in two cultivars remained at low levels, while the Pro content was higher in T1 than in T2. Under DS, especially in the case of prolonged or severe DS, plants optimally used SS to maintain growth (Ruan et al., 2010). In the present study, T1 had higher Pro and SS content than T2, primarily because T1 was subjected to more significant DS.

An excessive production of ROS arising from DS can damage proteins, DNA and lipids (Apel and Hirt, 2004). The antioxidative system protects plant cells from oxidative damage through scavenging ROS during DS. The SOD acts as the first line of defense against ROS by dismutating superoxide to H_2O_2, and CAT subsequently detoxifies H_2O_2 (Apel and Hirt, 2004). Changes in the antioxidant enzyme activities during DS depend on the plant species and stress intensity (Gill and Tuteja, 2010). In the absence of stress, T2 had higher SOD activities than T1. After eight days of DS, the T2 SOD activity levels were nearly equal to that of T1. In contrast, during the DS periods, T2 had markedly higher CAT activities than T1, indicating that T2 can efficiently scavenge ROS as a result of a more effective SOD-CAT antioxidative systems. Our results were in accordance with Bai et al. (2013), who reported that CAT and SOD activities were greater in drought-tolerant maize than in drought-susceptible genotypes. Furthermore, correlation analysis revealed that SA accumulation was significantly and positively correlated with CAT in T2 during DS, while an indistinctively negative correlation was observed in T1 at eight days DS, indicating SA induces massive H_2O_2 production and activate CAT. The previous researches have demonstrated that ABA prevents the excessive accumulation of H_2O_2 (Ye et al., 2011). In addition, it was reported drought-induced increase in H_2O_2 contents in guard cells involved in the signaling cascade for the induction of stomatal closure in maize leaves (Yao et al., 2013). These results and ours further elucidated T1 with lower CAT activities and DS resistance capacity in comparison to T2.

Upon recovery irrigation, two cultivars had nearly equal MDA content, and no significant differences were observed for SA content. The Pro content and SOD activity in T1 and T2 were less than or nearly equal to that of the control; however, the T1 leave symptoms were obviously more serious in comparison to those of T2, suggesting that the tolerant cultivar recovered more rapidly than the susceptible cultivar. The T2 ABA content rapidly returned to normal levels, but the levels for T1 were lower than that of the control, indicating that the drought-tolerant cultivar T2 has a rapid recovery mechanism following drought that is absent from the more susceptible cultivar. The T1 SS content were higher than that of both the control and T2, whereas the T2 SS content was nearly equal to that of the control, indicating that T2 had a more rapid recovery of normal growth upon recovery irrigation. For two cultivars, the CAT activities were significantly higher than that of the control, indicating that they did not fully return to normal growth because they were still subject to H_2O_2 stress. MDA, Pro, ABA and SA levels as well as SOD activity nearly rapidly restored to the levels equal to

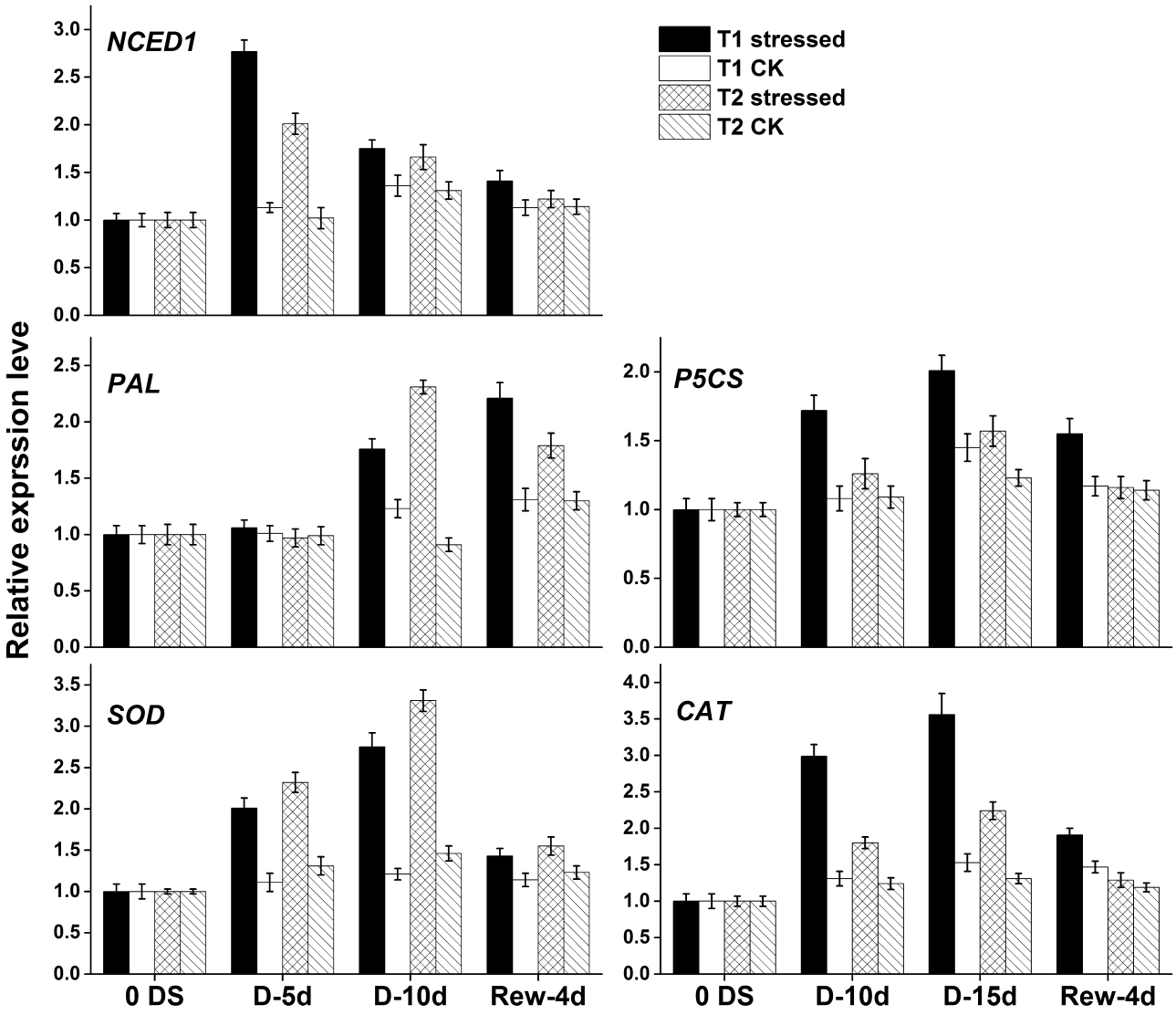

Fig. 9. Under artificial climate chamber condition, expression of studied genes in tea plants exposed to drought stress and recovery irrigation detected by qRT-PCR. *GAPDH* was used as a control. Data are displayed as mean values of the three replicates with standard error.

the control in comparison to SS content and CAT activities, indicating the index of CAT activity and SS content could be an easy screening method for high recovery capability in tea plants.

4.2. Gene expression differences among the tea cultivars under drought stress and recovery

DS signal perception and transduction by plants cells induced the expression of regulatory and functional sets of genes (Gupta et al., 2012, 2013). By comparing the expression of five drought-related genes in cultivars during the different phases of DS, we found that the expression levels of five genes were differentially up-regulated under DS.

Several studies have found that over-expression of *NCED1* in plants such as tobacco (*Nicotiana plumbaginifolia*) increased the cellular ABA levels (Nitsch et al., 2009; Tung et al., 2008; Zhang et al., 2008). Consistent with these studies, we found that the ABA content was significantly and positively correlated with *NCED1* expression levels in T1 and T2. *NCED1* expression levels in T1 were continuously increased with prolonged DS, whereas a significant decrease in *NCED1* expression levels was observed at eight days of DS for T2, further connecting the drought-tolerant cultivar T2 with lower ABA accumulation.

A negative correlation between *PAL* expression levels and SA content in T1 was observed. Moreover, the field and indoor test found that expression of *PAL* increased with SA content decreasing, indicating *PAL* expression was regulated by feedback of SA accumulation. Our results are consistent with those of Xiong et al. (2013), who reported that expression of *PAL* was reduced but PAL activity was increased in *C. sinensis* cv. *Anji Baicha*. Higher plants have two distinct enzymatic pathways for SA biosynthesis: one is the isochorismate synthase (ICS)-mediated isochorismate pathway, and the other is the PAL-mediated phenylalanine pathway (An and Mou, 2011). The bulk of SA was synthesized in tobacco by activation of the ICS pathway under both biotic and abiotic stress (Catinot et al., 2008). Therefore, it is possible that the majority of SA is synthesized from the ICS pathway in tea plants; however, the roles of these two SA biosynthetic pathways and their regulation in different physiological processes, especially tea plants, are still unclear (An and Mou, 2011).

It has been reported that higher plants have two Pro biosynthetic pathways: up-regulation of genes encoding P5CS, and a putative ornithine aminotransferase (OAT) (Szabados and Savoure, 2010; Xu

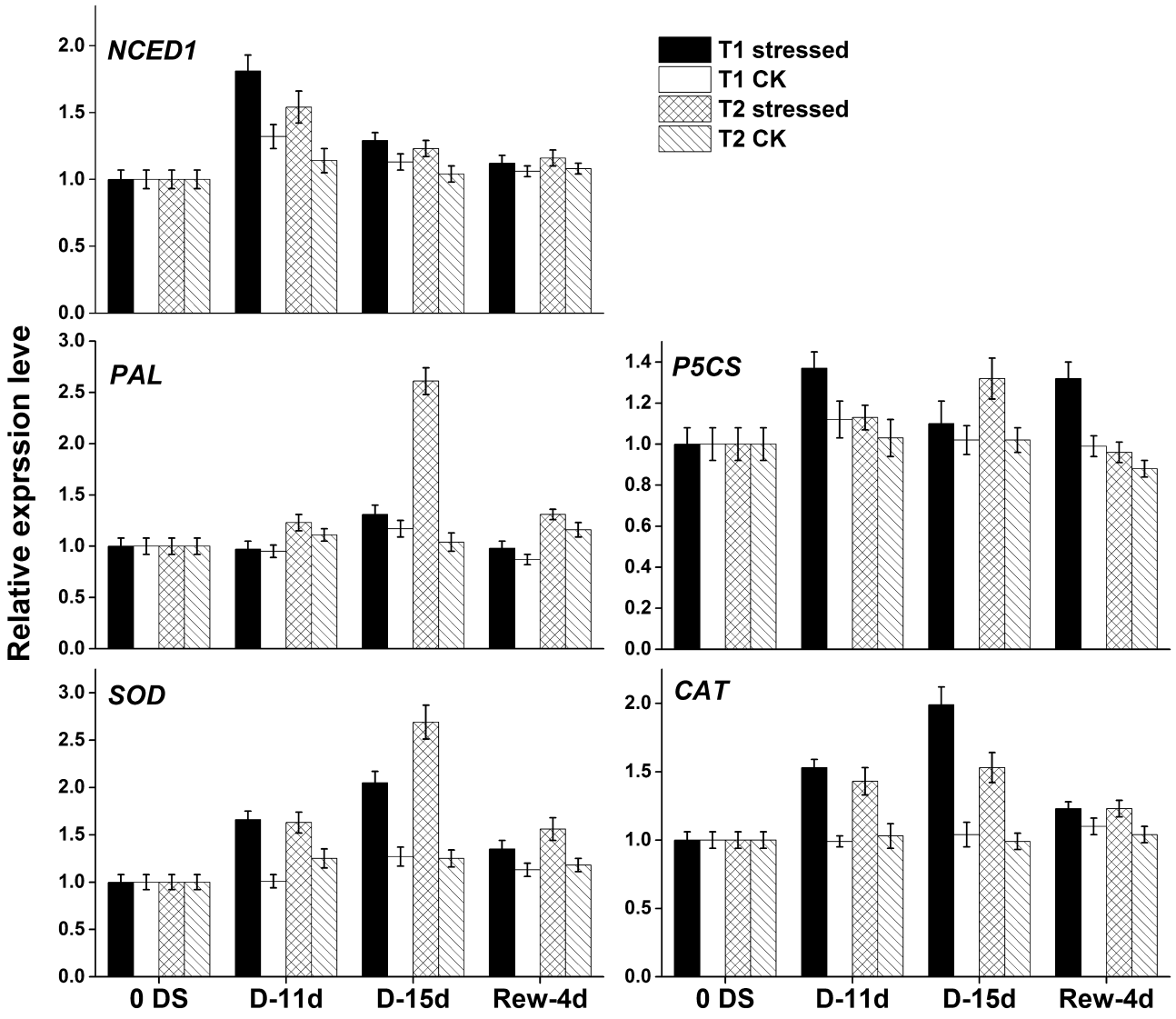

Fig. 10. Under field shed condition, expression of studied genes in tea plants exposed to drought stress and recovery irrigation detected by qRT-PCR. *GAPDH* was used as a control. Data are displayed as mean values of the three replicates with standard error.

et al., 2013). In our experiment, the expression patterns of *P5CS* in T2 were significantly and positively correlated with Pro content, whereas no correlation was observed in T1. At eight days of DS, the *P5CS* expression in T1 was slightly down-regulated and levels of Pro were continuously increased. In contrast, the Pro content and *P5CS* expression levels were increased in T2 at four and eight days of DS. Similar results from the duplicate field test also found, suggesting preferential use of one Pro biosynthetic pathway in the drought-tolerant tea cultivar and that *P5CS* expression levels were subjected to feedback inhibition by Pro in T1. Similar to maize, both Pro biosynthetic pathways are active in tea plants, and only one of them is activated during DS depending on the genotype (Corina et al., 2009). Following four days of rehydration, there was a strong correlation between the Pro content and *P5CS* expression levels in drought-tolerant cultivar T2; Pro content was decreased and *P5CS* expression levels was down-regulated. In contrast, in the drought-susceptible cultivar T1, there was a higher Pro content and significant increase in *P5CS* expression. These results suggested that T2 returned to normal growth more rapidly than T1 and the OAT pathway was involved in Pro accumulation under DS and recovery irrigation in T1.

During DS and recovery irrigation, the expression patterns of *SOD* and *CAT* were consistent with the activities of SOD and CAT in two cultivars, indicating the crucial role of these two genes in mediating SOD and CAT activity, respectively.

In summary, the results of this study indicate that the more drought-tolerant tea cultivar had a highly-integrated system, with strong drought-resistant leaf morphology, effective antioxidative defense systems, rapid signaling of phytohormones and up-regulation of drought-related genes that allowed reduction of drought damages under DS. This work not only aids in our understanding of the regulatory mechanisms of drought tolerance in tea plants in the aspects of leaf morphological status, phytohormone and osmolyte content, the activity of antioxidant enzymes and the expression of drought-related genes, but also forms a good practice guidance for breeding the drought-resistant cultivars and planting tea plant in drought-prone areas.

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