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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).