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Comprehensive transcriptome analysis reveals common and specific genes and pathways involved in cold acclimation and cold stress in tea plant leaves

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ABSTRACT

Freezing temperatures during the winter and unusual temperature fluctuations during the winter and early spring are the most harmful ambient factors threatening tea plant winter survival and may cause marked economic losses in tea production during the spring. In this study, we simulate natural climate change, to established cold acclimation (CA) and rapid cold stress conditions to investigate the transcriptome changes involved in CA and cold stress. Results revealed transcriptional changes occurring during the initial period of CA and the cell wall changes that occur throughout the entire CA process; these changes play crucial roles in increasing freezing tolerance during this process. Comparing cold-acclimated plants without further treatment against cold-acclimated plants under cold-stress, different cold response mechanisms were rapidly activated under cold stress; however, the subsequent freezing-induced accumulation of reactive oxygen species could be the major signal and harmful factor stimulating stress-associated gene expression and impairing tea leaf physiology. Moreover, we investigated 60 differentially expressed genes shared by both processes and highlighted the importance of β-ketoacyl CoA synthases, HXXXD-type acyl-transferase family proteins, NAC domain containing protein 80, sugar SWEET transporters and enolases in the responses to various cold conditions. The results provide useful information for understanding the regulation mechanism in tea plant responding to complex low temperature conditions.

1. Introduction

Frequently plants must adapt to cope with cold climates throughout their life cycle (Korner, 2016). This adaptation varies according to species, geographical distribution, and even the development stage of the plant. Moreover, the differences between plant adaption in response to extremely low temperature and their response to gradually decreasing temperatures are distinct at the physiological, molecular and metabolic levels (Hincha and Zuther, 2014). Recently, global climate change has become a more intensive major factor impacting plant survival during the winter (Rapacz et al., 2017). Tea plants are thermophilic perennial evergreen woody plants with a wide geographical distribution. Cold acclimation (CA) during the late fall or early winter is a crucial strategy for tea plants to survive the winter cold (Wang et al., 2013). However recently, the increasing frequency of unusually warm temperatures during the late winter and early spring, have resulted in unexpected deacclimation that threatens the winter survival of perennial plants, particularly in tea plants (Pagter and Arora, 2013). Unfortunately, the current understanding of CA in tea plants is limited. In addition, though the major molecular mechanism and cold inducible genes have already been reported in other species (Byun et al., 2014; Chinnusamy et al., 2007, 2010; Hincha and Zuther, 2014), the regulatory mechanisms involved in response to sudden freezing cold after deacclimation from CA is mostly unknown in tea plants.

The phenomenon of CA has been studied for decades. The physiological, biochemical and transcriptional changes that occur in plants during CA have been well reviewed (Miura and Furumoto, 2013; Shi et al., 2015; Theocharis et al., 2012). Moreover, alterations in the accumulation of proteins in response to low-temperature stress in major cereal crops have been comprehensively described by Janmohammadi

Abbreviations: bZIP, basic-leucine zipper; CA, cold acclimation; CAMTA, CALMODULIN BINDING TRANSCRIPTION ACTIVATOR; CBF, C-repeat binding factor; CDPK, calcium-dependent protein kinase; CIPK, calcineurin B-like protein-interacting protein kinase; COR, cold-regulated gene; DEG, differentially expressed gene; ENO, cytosolic enolase gene; ERF, ethylene responsive element binding factor; FLS, flavonol synthase; GO, gene ontology; HAI, highly ABA-induced PP2C gene; ICE, inducer of CBF expression; KCS, 3-ketoacyl-CoA synthase; KEGG, Kyoto encyclopedia of genes and genomes; KIN, cold-induced; LOS, lipooligosaccharide; MAP, mitogen-activated protein; MAPK, mitogen-activated protein kinase; NAC, NAC domain containing protein; PCA, principal component analysis; PCA, principal component analysis; PEL/DCR, HXXXD-type acyl-transferase family protein; PTB, polypyrimidine tract-binding protein; ROS, reactive oxygen species; STEM, short time-series expression miner; SWEET, sugars will eventually be exported transporter

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et al. (2015). In addition to the well-identified key cold-response genes, including the *C-repeat binding factor* (*CBF*) gene and its associated genes, such as the *cold-regulated genes* (*CORs*), *cold-induced 1 and 2* (*KINs*), and *inducer of CBF expression 1* (*ICE1*), the important roles of small RNAs and hormonal signaling in the response to cold tress have recently become better understood (Eremina et al., 2016; Liu et al., 2017; Medina et al., 2011). In tea plants, *CsICE1* and *CsCBF1* were the first two cold-response-related genes cloned based on achievements in other species, and the direction of the regulatory relationship between these genes was validated by Wang et al. (2012). Subsequently, the potential roles of the tea plant *H1 histone* genes in cold resistance were explored by overexpression studies in tobacco (Wang et al., 2014b). Multiple *CsbZIP* (basic-leucine zipper) genes also showed expression alterations in response to cold stimulation, and *CsbZIP6* was identified as a negative regulatory gene in freezing tolerance in tea plants (Cao et al., 2015; Wang et al., 2017). Recently, an increasing number of transcription factors or functional genes involved in cold tolerance have been identified in tea plants, including *NAC*, *aquaporin*, *spermine synthase* and *galactinol synthase* (Li et al., 2016a; Wang et al., 2016b; Yue et al., 2014; Zhou et al., 2017; Zhu et al., 2015). The application of global transcriptomic analyses has greatly improved our understanding of cold responses in tea plants during natural CA (Wang et al., 2013). Many cold-related differentially expressed genes (DEGs) were screened during CA and deacclimation, and carbohydrate metabolism and calcium signaling were considered two crucial pathways in the response to cold stress in tea plants. The roles of sugar metabolism in CA were further confirmed by detecting the contents of multiple specific sugars and performing expression analyses of sugar metabolism-related genes during the entire winter season (Yue et al., 2015). Previous studies have provided important clues regarding the major regulation mechanism involved in CA. However, the entire CA process in nature typically requires a long time and is easily affected by climate fluctuations. Therefore, performing transcriptome analyses under controlled conditions with intensive sampling time points is essential to comprehensively uncover the key genes or pathways involved in CA in tea plants.

In plants, cold tolerance, particularly freezing tolerance (typically below 0 °C), can be significantly enhanced after the completion of CA (Hincha and Zuther, 2014). Certain woody species can survive prolonged exposure to temperatures below −40 °C and minimum temperatures below −60 °C after CA (Strimbeck et al., 2015). However, plants can also suffer sudden temperature declines prior to CA or after deacclimation (Kovi et al., 2016). In particular, cold spells during the late spring severely damage tea plant leaves (particularly young shoots) and cause enormous economic losses. Unlike apple, citrus, grape, etc., tea plants are woody plants giving leaves and/or shoots as agricultural main product. Therefore, cold spells have become the most serious ambient factors affecting tea production in China and other temperate regions. Although alterations in the expression patterns of many genes, proteins and metabolites in response to cold stress have been reported in other species, knowledge regarding the cold stress responses occurring in tea plant after deacclimation remains limited (Kovi et al., 2016; Wang et al., 2013; Zheng et al., 2016; Zhu et al., 2015).

In this study, we combined CA and cold stress conditions in a CA/deacclimation/cold stress environment in a climate chamber for tea plants by simulating natural climate change and investigated the intensive transcriptomic changes in the tea plant leaves under the designed conditions. By performing a comprehensive comparative analysis, we identified the major signaling pathways and key genes involved in or shared by CA and cold stress. These results improved our understanding of the molecular mechanism in tea plants responding to low temperature stress and provide meaningful information for further function study of key genes involved in.

2. Materials and methods

2.1. Plant material and treatments

The 8-year-old potted tea cultivar "*Camellia sinensis* (L.) O. Kuntze cv. *Longjing 43* (LJ43)" was used as the plant material, and regular pest management and fertilization were applied. Based on our investigation over the years, LJ43 is a widely planted cultivar with relative high cold/frost resistance. The tea plants were moved to a climate chamber for treatment after the new leaves fully unfolded after sprouting in the spring. The treatment was divided into the following four stages: pre-treatment stage (control), which included one week of suitable growth conditions simulating the outside conditions to which the plants were exposed prior to relocation; gradual cooling stage (CA), which included five weeks of CA simulating the conditions in the fall; resuming stage under warmth (deacclimation), which included one week of deacclimation simulating the conditions in the early spring and even occasionally the winter; and freezing stage (cold stress), which included one day of frost simulating the conditions in late spring during tea plant sprouting. 10,000-lux light and 65% humidity was applied. The other detailed conditions of each treatment stage, including the day length, temperature and sampling time points, are listed in Fig. 1A. We sampled the third and fourth fully unfolded leaves without the shoots tips.

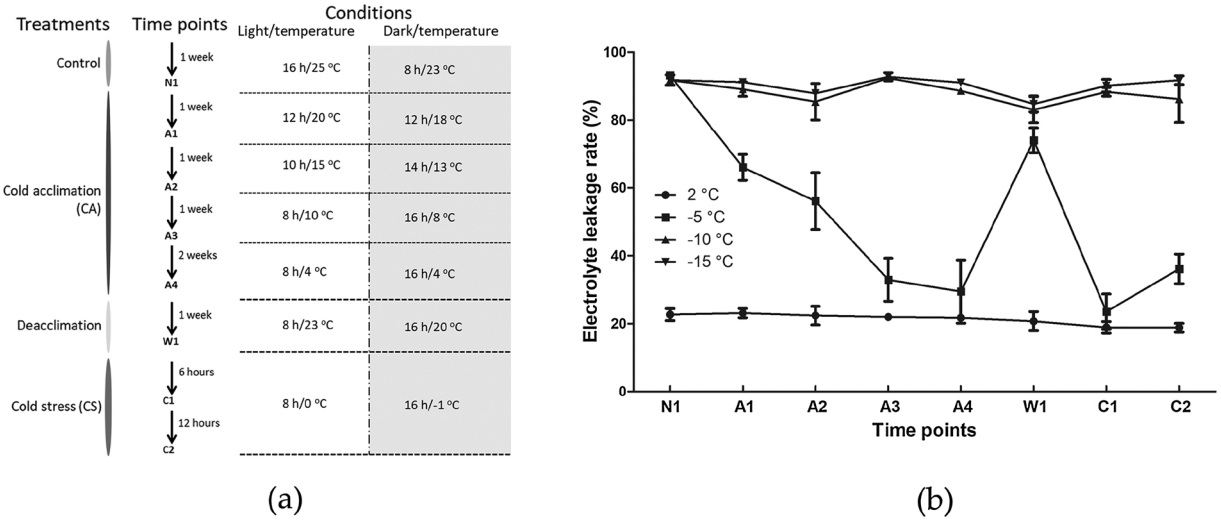

Fig. 1. Experimental design (a) and detection of electrical conductivity (b). The time point labels given in (a) correspond to the labels at plot in (b). Briefly, at each time point, the samples leaves were treated with different low temperature and then their electrolyte leakage rate was measured. Values represent the means of three replicates ± standard deviation in (b).