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Research article

Molecular cloning and expression analysis of tea plant aquaporin (*AQP*) gene family

Chuan Yue [a, c, 1], Hongli Cao [a, c, 1], Lu Wang [a, b, c], Yanhua Zhou [a, c], Xinyuan Hao [a, c], Jianming Zeng [a, b, c], Xinchao Wang [a, b, c, *], Yajun Yang [a, b, c, **]

[a] Tea Research Institute of the Chinese Academy of Agricultural Sciences, Hangzhou 310008, China
[b] National Center for Tea Improvement, Hangzhou 310008, China
[c] Key Laboratory of Tea Biology and Resources Utilization, Ministry of Agriculture, Hangzhou 310008, China

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ABSTRACT

The role of aquaporin proteins (AQPs) has been extensively studied in plants. However, the information of AQPs in the tea plant (*Camellia sinensis*) is unclear. In this manuscript, we isolated 20 full-length *AQP* cDNAs from the tea plant, and these sequences were classified into five subfamilies. The genes in these subfamilies displayed differential expression profiles in the studied tissues. The *CsAQP* expression patterns correlated with flower development and opening (FDO) and bud endodormancy (BED). To better understand the short-term expression patterns of *CsAQPs* in response to abiotic stress, tea plants were treated with abscisic acid (ABA), cold, salt or drought. ABA treatment down-regulated the expression of various *CsAQPs*. Salt up-regulated the transcription of most *CsAQP* genes. Cold treatment resulted in a complicated transcriptional regulation pattern for various *CsAQPs*. The expression of *CsAQPs*, especially plasma membrane intrinsic proteins (*CsPIPs*) and tonoplast intrinsic proteins (*CsTIPs*), was induced by drought and remained relatively high after rehydration in leaves, whereas almost all the *CsAQPs* were repressed in roots. Our results highlighted the diversity of *CsAQPs* in the tea plant and demonstrated that the *CsPIP* and *CsTIP* genes play a vital role in the stress response as well as in FDO and BED. Furthermore, certain *CsSIPs* (small basic intrinsic proteins), *CsNIPs* (NOD26-like intrinsic proteins) and *CsXIPs* (X intrinsic proteins) may regulate BED and FDO.

1. Introduction

Tea is an important economic crop that is cultivated in more than 20 countries and is consumed as a beverage throughout the world. Tea plant (*Camellia sinensis*) originates from tropical or subtropical areas, and its distribution and productivity are mainly limited by temperature and other environmental factors. These limitations are closely related to water, suggesting that the water status in plants, especially the ratio of free water to bound water, not only reflects the plant growth conditions but also mediates the stress responses. Studies on tea plant resistance to abiotic stress have focused on enzymatic antioxidative systems (Vyas et al., 2007; Vyas and Kumar, 2005), transcript factors (Wang et al., 2012; Li et al., 2010) and transcriptome (Wang et al., 2013), but have rarely evaluated water regulation. However, water is the main regulator of plant development and growth, particularly during flowering and bud dormancy. In the tea plant, the developmental regulation of processes such as bud dormancy (Krishnaraj et al., 2011) has been studied, but the regulatory mechanisms involved in bud endodormancy (BED) and in flower development and opening (FDO) during annual growth remain unknown. Hence, we focused on the water status changes under various conditions and on the tea plant responses to external stimuli or yearly growth, as these parameters provide new insight into tea plant physiology.

Aquaporin protein (AQP), also called major intrinsic proteins (MIPs), control water transport and are ubiquitously distributed in

Abbreviations: AQP, aquaporin protein; CsAQP, *Camellia sinensis* aquaporin protein; FDO, flower development and opening; BED, bud endodormancy; PIP, plasma membrane intrinsic protein; TIP, tonoplast intrinsic protein; SIP, small basic intrinsic proteins; NIP, NOD26-like MIPs or NOD26-like intrinsic protein; XIP, X intrinsic proteins; MIPs, major intrinsic proteins; TM, transmembrane; NPA, Asn–Pro–Ala; Ar/R, aromatic/arginine; ABA, abscisic acid; PEG, polyethylene glycol.

* Corresponding author. Tea Research Institute of the Chinese Academy of Agricultural Sciences, Hangzhou 310008, China. Tel.: +86 571 86653162.
** Corresponding author. Tea Research Institute of the Chinese Academy of Agricultural Sciences, Hangzhou 310008, China. Tel.: +86 571 86650226.
E-mail addresses: yccyyx@163.com (C. Yue), lili9885@126.com (H. Cao), wanglu317@tricaas.com (L. Wang), zhouyh@tricaas.com (Y. Zhou), amery.hao@yahoo.com (X. Hao), zengjm@tricaas.com (J. Zeng), wangxinchao@caas.cn (X. Wang), yjyang@tricaas.com (Y. Yang).
[1] These authors contributed equally to this work.

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higher plants; the AQP families consist of numerous homologous genes, e.g., 35 in *Arabidopsis* (Johanson et al., 2001) and 34 in rice (Nguyen et al., 2013). There are five subfamilies in plants, which are categorized according to their subcellular localization and sequence similarity: plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), NOD26-like MIPs or NOD26-like intrinsic proteins (NIPs), small basic intrinsic proteins (SIPs), and the newly identified X (or uncategorized) intrinsic proteins (XIPs) (Danielson and Johanson, 2008). These subfamilies are divided into various groups, such as the PIP1 and PIP2 groups within the PIP subfamily. AQPs have an average molecular weight between 28 and 34 kDa, and they form tetrameric quaternary structures. Each AQP contains 6 transmembrane (TM) helices (H1 to H6) connected by five loops (A-E), and two half-helices located at loops B and E form the seventh TM helix, which contains two highly conserved NPA (Asn−-Pro−Ala) motifs; this region forms the narrow portion of the pore and the two N-and C-terminal tails are cytosolic. An additional structural characteristic is the aromatic/arginine (Ar/R) selectivity filter, which is formed by the interaction of four amino acids within the pore and participates in substrate selectivity .

In plant cells, the AQP channel proteins are located in the plasma membrane and cytosolic regions, and they facilitate the transport of water, small neutral solutes (such as urea, boric acid, and silicic acid), and gases (including ammonia and carbon dioxide) (Chaumont and Tyerman, 2014). Thus, AQPs are involved in various physiological processes, and their functional regulation in plants has been extensively studied in the past decade. The expression of *AQPs* is associated with plant growth, which is accompanied by cell division and differentiation and high water consumption, such as the bud active-dormancy cycle (Yooyongwech et al., 2008, 2009) and flower opening (Xue et al., 2009; Ma et al., 2008; Azad et al., 2004; Chen et al., 2013). Overexpression of *AQP* genes, such as *PIP1b* (Aharon et al., 2003) in *Arabidopsis*, substantially alter the plant vegetative and reproductive growth cycles. These transgenic plants exhibit increased growth, transpiration, stomatal density, and photosynthetic efficiency. In addition to their potential role in plant growth regulation, *AQPs* are involved in plant responses to multiple external stimuli, including drought, temperature and salinity stress (Wang et al., 2011; Matsumoto et al., 2009; Peng et al., 2007; Sreedharan et al., 2013). Because *AQPs* participate in plant resistance to adverse environments, they could be used in bioengineering to improve crop resistance and yield. Recently, *AQP* genes were identified in the genomes of various plants, including *Vitis vinifera* (Jaillon and et al., 2007), cotton (Park et al., 2010) and tomato (Reuscher et al., 2013). Their multiple functions are recognized in many species. *AQP* genes may also affect tea plant development and stress responses, but their sequences and expression profiles are unavailable.

In this study, 20 full-length *AQP* genes in the tea plant (*C. sinensis, Cs*) were identified, and certain characteristics, such as subcellular localization and TM regions, were predicted. Furthermore, the tissue specificity and developmental expression profiles of the *AQP* genes during BED and FDO, respectively, were investigated using quantitative real-time PCR (qRT-PCR). To gain further insights into their role in different stress responses, a detailed transcriptional profile of the 20 genes was generated after short-term treatments with abiotic stresses, including abscisic acid (ABA), cold, salinity and drought.

2. Materials and methods

2.1. Plant materials and growth conditions

The tea plant cultivar '*C. sinensis* cv. Longjing 43' ('LJ43') was used in this study. For the examination of dormancy-active growth and flower development, the plants were grown in the field at the Tea Research Institute, Chinese Academy of Agricultural Sciences (TRI, CAAS, N 30°10′, E 120°5′), Hangzhou, China. For tissue and abiotic stress treatments, two-year-old cutting seedlings were planted in a pot and grown with a natural photoperiod in greenhouse conditions (Fig. 1A).

2.2. ABA, salt, drought and cold stress treatments

For the ABA treatment, a freshly prepared working solution of 100 μM ABA was sprayed on the leaves (Li et al., 2010). The third leaves from the apical bud were used for gene expression analysis.

To initiate short-term salt stress, two-year-old plants were irrigated with 250 mM NaCl for 24 h. The third mature leaves from two shoots of each plant were collected, frozen in liquid nitrogen, and stored at −80 °C.

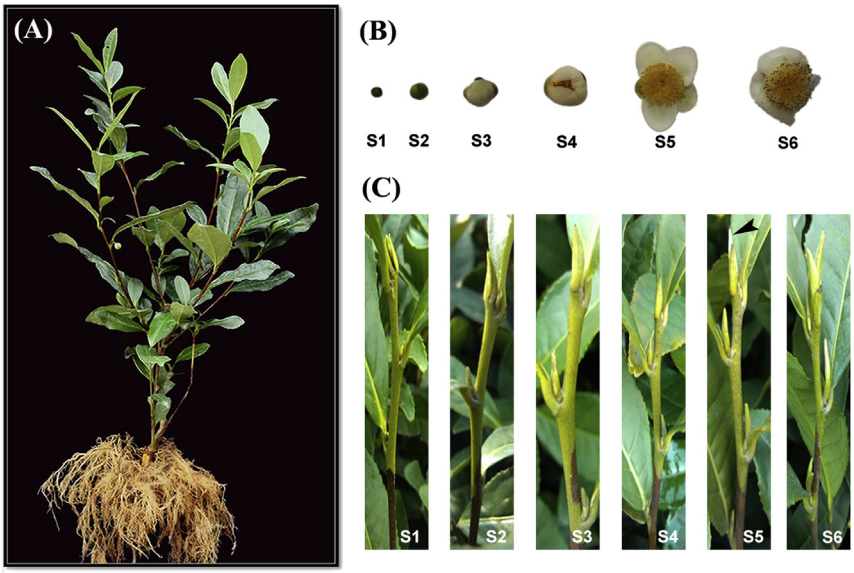

Fig. 1. Different stages of tea plant flower development and leaf bud endodormancy. Flowers and leaf buds were collected from a 20-year-old tea plant grown in a natural tea yard. Two-year-old tea plants were used for the stress treatments. A: Two-year-old tea plant grown in a greenhouse with natural light. B: Six stages sampled from flower bud set (S1) to senescence (S6). C: Six sampling time points, from leaf bud initiation (S1) through the active-dormancy cycle (S2–S6). The arrow in S5 indicates sprouting.