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ORIGINAL ARTICLE

Differential expression of gibberellin- and abscisic acid-related genes implies their roles in the bud activity-dormancy transition of tea plants

Chuan Yue[1,2] · Hongli Cao[1,2] · Xinyuan Hao[2] · Jianming Zeng[2] · Wenjun Qian[2] · Yuqiong Guo[1] · Naixing Ye[1] · Yajun Yang[2] · Xinchao Wang[2]

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Abstract

Key message **Thirty genes involved in GA and ABA metabolism and signalling were identified, and the expression profiles indicated that they play crucial roles in the bud activity-dormancy transition in tea plants.**

Abstract Gibberellin (GA) and abscisic acid (ABA) are fundamental phytohormones that extensively regulate plant growth and development, especially bud dormancy and sprouting transition in perennial plants. However, there is little information on GA- and ABA-related genes and their expression profiles during the activity-dormancy transition in tea plants. In the present study, 30 genes involved in the metabolism and signalling pathways of GA and ABA were first identified, and their expression patterns in different tissues were assessed. Further evaluation of the expression patterns of selected genes in response to GA_3 and ABA application showed that *CsGA3ox, CsGA20ox, CsGA2ox, CsZEP* and *CsNCED* transcripts were differentially expressed after exogenous treatment. The expression profiles of the studied genes during winter dormancy and spring sprouting were investigated, and somewhat diverse expression patterns were found for GA- and ABA-related genes. This diversity was associated with the bud activity-dormancy cycle of tea plants. These results indicate that the genes involved in the metabolism and signalling of GA and ABA are important for regulating the bud activity-dormancy transition in tea plants.

Keywords Abscisic acid (ABA) · Bud dormancy · Gene expression · Gibberellins (GA) · Tea plant

Introduction

To withstand unfavourable environmental factors such temperature stress, changes in day length and nutrient deficiency, perennial evergreen plants enter dormancy and later regrow under favourable conditions (Cooke et al. 2012; Horvath et al. 2003; Olsen 2010; Rohde and Bhalerao 2007; Tanino 2004; Tanino et al. 2010). Because this activity-dormancy cycle is important for plant survival and reproduction, the regulation of the mechanism underlying this cycle has gained much attention. Phytohormones, especially gibberellin (GA) and abscisic acid (ABA), have been demonstrated to play important roles in the activity-dormancy cycle (Cooke et al. 2012; Druart et al. 2007; Olsen 2010; Ruttink et al. 2007), but the expression profiles of GA- and ABA-related genes during the natural bud activity-dormancy cycle have not been elucidated. In general, GA and ABA play antagonistic roles in the regulation of plant growth and development. Increased ABA levels induce the onset

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✉ Yajun Yang
yjyang@tricaas.com

✉ Xinchao Wang
wangxinchao@caas.cn

[1] College of Horticulture, Key Laboratory of Tea Science in Universities of Fujian Province, Fujian Agriculture and Forestry University, Fuzhou 350002, China

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

of bud and seed dormancy, whereas increases in GA levels release buds and seeds from dormancy (Cooke et al. 2012; Druart et al. 2007; Zheng et al. 2015). The metabolism and signalling of GA and ABA are well characterized and have been broadly identified in many plant species (Du et al. 2015; Pearce et al. 2015; Xue et al. 2008; Wang et al. 2015). In addition, the response to GA and ABA is known to be controlled by several genes involved in GA and ABA metabolism and signal transduction; however, the expression profiles of these genes in perennial evergreen plants during winter dormancy are largely unknown.

The tea plant (*Camellia sinensis* (L.) O. Kuntze) is a perennial evergreen woody plant whose tender leaves and buds are processed as tea for drinking. The bud activity-dormancy conversion influences tea plant growth under stress, including high temperature and/or drought stress in the summer and cold and/or drought stress in the winter. Importantly, by controlling the tea bud flush time, the conversion also affects the economic output of tea products, especially during early spring tea production, as earlier-harvested tea usually costs relatively more than does late-harvested tea (Hao et al. 2017; Wang et al. 2014). The regulation of bud dormancy in tea plants is, therefore, a key aspect of tea plant research. To elucidate the mechanism underlying bud dormancy, dynamic changes in phytohormone levels (i.e., auxin, GA and ABA) and in the levels of phenols, polyamines, and reactive oxygen species have been investigated; the results suggest that all of these components are correlated with tea plant bud dormancy (Kakkar and Nagar 1997; Nagar and Kumar 2000; Nagar and Sood 2006; Vyas et al. 2007). Furthermore, a number of genes that are differentially expressed in dormant and active buds were also identified using subtractive hybridization methods and transcriptome analysis. The results showed that the expression patterns of certain genes, including *CsGA20ox*, are associated with the activity-dormancy transition (Krishnaraj et al. 2011; Paul et al. 2014; Paul and Kumar 2011; Thirugnanasambantham et al. 2013; Wang et al. 2014). Recently, we performed RNA-Seq analysis of buds at different dormancy stages and identified several regulatory pathways (Hao et al. 2017); the results of this analysis suggested that GA- and/or ABA-responsive pathways play critical roles in tea plant dormancy. GA and ABA were previously shown to influence bud dormancy in tea plants (Barua 1969). However, how GA- and ABA-related gene expression is regulated during the bud activity-dormancy cycle in the winter-spring season is unclear.

In the current study, 30 genes encoding key enzymes involved in the metabolism and signalling pathways of GA and ABA were identified. The evaluated genes exhibited tissue-specific expression patterns in different tea plant organs. In addition, they showed differential accumulation profiles in response to treatment of the plants with exogenous GA and ABA. Moreover, we systematically investigated the expression patterns of these genes during the winter–spring season from 2013 to 2015. The results provide a better understanding of the contributions of GA and ABA to the activity-dormancy cycle of tea plants.

Materials and methods

Plant materials and bud collection

In this study, 15-year-old 'Longjing43' tea plants cultivated in the natural tea garden of the Tea Research Institute, Chinese Academy of Agricultural Sciences (TRI, CAAS, N 30°18′, E120°10′), Hangzhou, China were used. The lateral buds were periodically sampled and imaged for analysis from November to March of 2013–14 and 2014–15. The collected buds were separated in triplicate as biological replicates and immediately frozen in liquid nitrogen, after which they were stored at − 80 °C until needed for RNA isolation. The daily temperature from October to March of 2013–14 and 2014–15 was recorded and analysed. For tissue-specific analyses, roots, mature leaves, stems, flowers and buds were collected from 3-year-old tea plants. The materials were frozen in liquid nitrogen and stored at − 80 °C until needed for RNA extraction.

GA_3 and ABA treatment

To investigate the effects of GA_3 and ABA on the expression of GA- and ABA-related genes, tea plants were treated with various concentrations of GA_3 and ABA for varying amounts of time. 4-year-old tea plants growing in the greenhouse under natural day length and a constant temperature of 25 ± 1 °C were selected for phytohormone treatment analysis. To examine the time course of the response to phytohormones, 100 μM GA_3 and 100 μM ABA were sprayed onto the leaves of the tea plants at 9:30 a.m., and the 3rd and 4th leaves from the apical buds were collected after 0, 3, 6, 12 and 24 h. To initiate the concentration-dependent response analysis, 0, 50, 100, and 150 μM GA_3 or ABA were sprayed onto the tea plant leaves, and the leaves were sampled 3 h later. The collected leaves were frozen in liquid nitrogen and stored at − 80 °C until needed for gene expression analysis.

Identification of GA- and ABA-related genes in tea plant

In the present study, the genes annotated as ent-kaurene synthase *(KS), ent*-kaurene oxidase (*KO*), *ent*-kaurene acid oxidase (*KAO*), gibberellin 20-oxidase (*GA20ox*), gibberellin 3-oxidase (*GA3ox*), gibberellin 2-oxidase (*GA2ox*), GA receptor (*GID1*) and GA repressor (*DELLA*) were identified from our previous transcriptomes and from data on

the tea plant cultivar 'Longjing43' in the NCBI database. Moreover, the annotated unigenes that encode key enzymes involved in ABA metabolism (zeaxanthin epoxidase (*ZEP*), 9-cis-epoxycarotenoid dioxygenase (*NCED*), short-chain dehydrogenase/reductase (*SDR*), abscisic aldehyde oxidase (*AAO*) and *CYP707A*) and in signal transduction involving *PP2C* and *PYL* were also selected from the transcriptome of the 'Longjing43' cultivar. To verify the annotations of the sequences and query their putative full-length open reading frames (ORFs), the selected sequences were subjected to Basic Local Alignment Search Tool (BLAST)X searches in the NCBI database. For sequences not containing a complete ORF, rapid amplification of cDNA ends (RACE)-PCR was used to amplify the full-length sequences. The tested genes in this study were named according to the results of BLASTP searches.

In silico analysis of tea plant genes

Sequence identities were detected by performing clustal omega multiple alignment using EMBL-EBI. To analyse the phylogenetic tree of the tea plant and other plant amino acid sequences, the sequences were aligned using ClustalW, and trees were constructed by the neighbour-joining method and 1000 bootstraps using MEGA 5.0. The ProtParam tool (https://web.expasy.org/protparam/) was used to predict the molecular weights and theoretical pIs of the putative gene products.

Expression analyses of GA- and ABA-related genes

Total RNA was extracted from the tea plant samples according to the method of Yue et al. (2014). First-strand cDNA was synthesized using a PrimeScript™ RT reagent kit with the gDNA Eraser System (TAKARA Bio Inc., Dalian, China) according to the manufacturer's protocol. For quantitative real-time PCR (qRT-PCR) detection, specific primers were designed, and the *PTB* gene was selected as a housekeeping gene due to its stable expression level in plants that received different treatments and in various plant tissues (Hao et al. 2014). The primers used in qRT-PCR are listed in Table A1. The qRT-PCR assays were performed using a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) with SYBR Green reagents (TAKARA Bio Inc.) according to the product manual. 20-microlitre reaction mixtures that contained 1.0 µl of cDNA (equivalent to ~ 50 ng of cDNA), 0.4 µl of each primer, 0.4 µl of ROX Reference Dye II and 10 µl of SYBR Premix Ex Taq II reagent were used to determine the gene transcription levels. The PCR conditions were as follows: initial denaturation at 95 °C for 30 s, 40 cycles of dissociation at 95 °C for 5 s, annealing and extension at 60 °C for 34 s, and termination by melting curve analysis as recommended by the manufacturer. All assays were performed with three biological replicates, each of which consisted of three technical replicates, and quantified using the $2^{-\Delta\Delta Ct}$ or $2^{-\Delta Ct}$ method as described by Livak and Schmittgen (2001).

Results

Tea plant bud dormancy and meteorological analysis during the winter–spring season

Daily recording of the temperature from October to March of 2013–15 indicated that the temperature showed similar fluctuation patterns in the three consecutive years. In October, the majority of the average daily temperatures were above 15 °C. The temperature gradually decreased beginning in late October, and it reached its lowest point during the period from late December to January (during this period, the majority of the daily average temperatures were below 5 °C). The temperature then slowly increased, and the average temperature was maintained at 15 °C (Fig. A1). The growth of tea plant buds was arrested, and the plants became dormant, as the temperature decreased from November to December; dormancy then persisted throughout the cold season. In late February, the dormancy of the tea buds was released, and the buds started to grow in March (Fig. A1). In general, the tea plant buds that reached the stage of 'one bud and one leaf' or the stage of 'one bud and two leaves' during mid- and late March of each year are plucked to produce tea in Hangzhou city. Hence, in this study, the sample points from November to March were classified into active/dormant stages as follows: November was defined as dormancy stage 1 (DS-1), December was defined as dormancy stage 2 (DS-2), January was defined as dormancy stage 3 (DS-3), February was defined as active stage 1 (AS-1), and two time points in March were defined as active stages 2 and 3 (AS-2 and AS-3), respectively.

Genes involved in GA metabolism and signalling

GA is successively biosynthesized from geranylgeranyl diphosphate (GGDP) by terpene synthases (TPSs), cytochrome P-450 monooxygenases (P450s), and 2-oxoglutarate-dependent dioxygenases (2ODDs) (Yamaguchi 2008). The candidate genes encoding the key enzymes in the GA biosynthetic pathway (*CsKS, CsKO, CsKAO, CsGA20ox*, and *CsGA3ox*), as well as the catabolism-related gene *CsGA2ox* in the tea plant, were selected from our annotated transcriptome database and from other databases. Nine genes encoding these key enzymes were identified, and the full-length cDNA sequences of these genes were cloned using RACE-PCR (Table 1). *CsKS*, a member of the TPS superfamily, was highly similar (71%)

Table 1 The genes with the full-length cDNA involved in GAs and ABA metabolism and signaling identified from tea plant

Gene names	Accession number	ORF length (bp)	Deduced amino acid			Superfamilies	PCR
			Length (aa)	MW (kD)	PI		
CsKS	MF765778	2376	791	89.629	5.805	Terpene_synth superfamily	3′-RACE
CsKO	MF765779	1551	516	58.597	7.193	P450 superfamily	3′-RACE
CsKAO	MF765780	1470	489	56.272	9.179	P450 superfamily	/
CsGA20ox-1	KC193604	1152	383	42.944	6.329	PcbC superfamily	5′/3′-RACE
CsGA20ox-2	MF765781	1137	378	43.029	7.202	PcbC superfamily	
CsGA3ox-1	KF703743	1086	361	40.286	6.989	PcbC superfamily	5′/3′-RACE
CsGA3ox-2	MF765784	1122	373	41.077	8.092	PcbC superfamily	/
CsGA2ox-1	MF765782	1002	333	37.183	6.086	PcbC superfamily	/
CsGA2ox-2	MF765783	1008	335	38.529	5.317	PcbC superfamily	/
CsGID1a	JX235369	1026	341	38.529	5.705	Abhydrolase superfamily	5′-RACE
CsGID1b	AGU38487	1038	345	39.044	7.11	Abhydrolase superfamily	5′/3′-RACE
CsGID1c	AGU38488	1050	349	39.481	6.464	Abhydrolase superfamily	/
CsDELLA1	MF765785	1584	527	57.782	5.709	DELLA/GRAS superfamily	3′-RACE
CsDELLA2	MF765786	1599	532	58.434	5.257	DELLA/GRAS superfamily	5′/3′-RACE
CsDELLA3	MF765787	1785	594	65.113	5.219	DELLA/GRAS superfamily	3′-RACE
CsDELLA4	MF765788	1848	615	67.055	5.278	DELLA/GRAS superfamily	3′-RACE
CsZEP1	MF765766	2004	667	73.42	6.674	PRK068847 superfamily	/
CsZEP2	MF765767	1362	453	49.668	8.267	PRK068847 superfamily	/
CsZEP3	MF765768	1416	471	52.567	8.322	PRK068847 superfamily	/
CsNCED1	MF765769	1815	604	66.686	6.337	RPE65 superfamily	5′-RACE
CsNCED2	MF765770	1824	606	67.717	6.71	RPE65 superfamily	/
CsSDR	MF765765	834	277	29.303	6.589	FabI superfamily	/
CsAAO	MF765764	4092	1348	147.495	6.089	Ald_Xan_dh_C2 superfamily	/
CsCYP707A1	MF765771	1401	466	53.129	9.081	P450 superfamily	/
CsCYP707A2	MF765772	1437	478	54.963	9.278	P450 superfamily	/
CsCYP707A3	MF765773	1473	490	55.289	8.175	P450 superfamily	/
CsCYP707A4	MF765774	1416	471	53.613	9.592	P450 superfamily	/
CsCYP707A5	MF765775	1596	531	60.822	8.426	P450 superfamily	/
CsPP2C	MF765776	1587	528	56.955	4.847	PP2Cc superfamily	/
CsPYL8	MF765777	558	185	20.914	6.535	SRPBCC superfamily	/

and closely related to *CmKS* (AEF32083) from *Castanea mollissima* (Table 1 and Fig. A2). *CsKO* and *CsKAO*, which belong to the p450 superfamily, shared high similarity (greater than 70%) with grape *VvKO* (AFD54196) and *Sesamum indicum SiKAO* (XP_011085042), respectively.

CsGA20oxs, CsGA3oxs and CsGA2oxs are members of the 2ODD protein superfamily. Two *CsGA20ox* genes shared 54.2% identity in amino acid sequence. They were separated into different clades, and their sequences were shown to be closely related to the sequences of these proteins from other *Camellia* plants, including *Camellia lipogenesis* and *Camellia reticulata* (Fig. A2-d). *CsGA3ox-1* shared 79.8% amino acid identity with that of *CsGA3ox-2*, and both of these genes clustered into the same clade in the phylogenetic tree (Fig. A2-e). *CsGA2ox-1* and *CsGA2ox-2* had low similarity (48.1%) with each other but were classified into different groups (Fig.A1-f). All of the GA oxidase genes were regarded as members of the PcbC superfamily (Table 1).

In addition, three putative GID1 and four DELLA genes were isolated using the RACE-PCR method. Both CsGID1s belong to the abhydrolase superfamily (Ueguchi-Tanaka et al. 2005). The number of amino acid residues in CsGID1s ranges from 341 to 349. *CsGID1a* and *CsGID1c* were in the same cluster as *AtGID1a* (AT3G05120) and *AtGID1c* (AT5G27320), whereas *CsGID1b* and *AtGID1b* (AT3G63010) were classified into another clade (Fig. A2-g).

Four putative DELLA proteins contained a DELLA motif and a GRAS domain in the N-terminal and C-terminal regions, respectively, of their amino acid sequences. Both *CsDELLA3* and *CsDELLA4* were in the same clade as all five *AtDELLA*s, whereas *CsDELLA1* and *CsDELLA2* were grouped into another clade that contained other plant *DELLA*s (Fig. A2-h). All of these DELLA proteins possessed

conserved DELLA, THYNP, VHID and RVER motifs, which are critical for binding GID1s (Murase et al. 2008).

Genes involved in ABA metabolism and signalling

ABA is first biosynthesized from zeaxanthin by ZEP and is then successively catalysed by NCED, SDR, and AAO. ABA can be inactivated by hydroxylation by ABA 8′-hydroxylase (CYP707A) (Finkelstein 2013; Wang et al. 2015). In the present study, three putative full-length sequences of *CsZEP* genes were identified in our annotated transcriptome database. *CsZEP1* had a 2 004-bp ORF encoding 667 amino acid residues; this ORF was longer than those of *CsZEP2* and *CsZEP3* (Table 1). *CsZEP1* and *CsZEP3* were grouped into the same clade in the phylogenetic tree (Fig. A3-a).

Two chloroplast-like *CsNCED* genes, *CsNCED1* and *CsNCED2*, were identified in tea plants. They encoded proteins of equivalent length and molecular weight (Table 1). Both were closely related to the *AtNCED*s and were in the same clade; however, they were in different subgroups (Fig. A3-b).

CsSDR contained an 834-bp ORF encoding 277 amino acid residues (Table 1). It was closely related to the *SDR* of *Citrus sinensis* (NP_001275796) and *Bixa orellana* (AMJ39494) (Fig. A3-c). *CsAAO* had a 4 092-bp ORF (Table 1).

Five homologous genes of *CsCYP707As* were identified in the tea plant transcriptome database. The length of the proteins encoded by these genes ranges from 466 (*CsCYP707A1*) to 531 (*CsCYP707A5*) amino acid residues. All of the *CsCYP707As* genes belong to the P450 superfamily and encode proteins with relatively high isoelectric points (Table 1). These genes were separated into different clades in the phylogenetic tree (Fig. A3-e).

Two key components of ABA signalling, *CsPYL* and *CsPP2C*, were also selected and analysed in tea plants (Table 1).

Analysis of tissue-specific gene expression

The qRT-PCR approach was used to investigate the transcript abundance of the identified genes in the roots, stems, leaves, flowers and buds of tea plants to determine the tissue-specific expression levels of the individual genes. Although expression of all 30 genes was detected in all tissues, the transcript levels of individual genes varied widely among tissues (Fig. 1). In general, almost all of the studied genes were expressed at lower levels in stems than in roots, leaves, flowers and buds. Interestingly, the GA-related genes such as *CsGA20ox-1/-2, CsGA3ox-1/-2, CsGID1a/b/c* and *CsDELLA1/2/3/4* showed preferential expression in flowers and/or buds, whereas *CsKO, CsKAO*, and *CsGA3ox-2* were relatively highly expressed in roots. In contrast, the

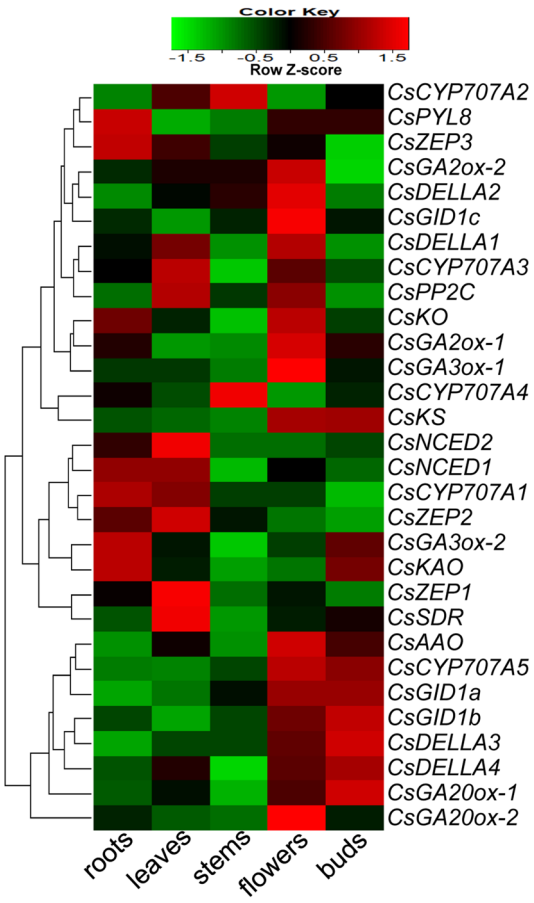

Fig. 1 Tissue-specific expression analysis of GA- and ABA-related genes. The transcript levels of each gene in the roots, leaves, stems, flowers and buds were determined using qRT-PCR. The results are presented as the average of three replicates and were calculated using the $2^{-\Delta Ct}$ method; *CsPTB* served as a housekeeping gene. The average log2 values of three replicates were used to generate the heat map using *R* software. Green represents low expression, and red denotes high expression

ABA-related genes such as *CsZEP1/2/3, CsNCED1/2*, and *CsCYP707A1* were mainly expressed in both roots and leaves. The expression levels of both *CsSDR* and *CsAAO* were higher in leaves, flowers and buds than in stems. *CsCYP707As, CsPP2C* and *CsPYL8* were also differentially expressed among the tested tissues.

Expression analysis of GA-related genes in response to GA_3 treatment

The expression patterns of the GA-related genes in plants subjected to GA_3 treatment were analysed. In plants treated with GA_3 at various concentrations ranging from 50 to 150 µM, the expression levels of *CsKAO, CsKO* and *CsKS* were up-regulated. Under these conditions, *CsGA20ox-2* expression was also induced. The key genes involved in the catabolism of bioactive GA (*CsGA2ox-1/-2*) were

dramatically upregulated in response to GA_3 treatment; however, the expression of *CsGA3ox-1*/*-2* and *CsGA20ox-1* was repressed. *CsGID1b* and *CsGID1c* were differentially expressed after treatment with GA_3 at various concentrations, whereas *CsGID1a* expression was not affected. With the exception of the induction of *CsDELLA3* expression, the expression of other *CsDELLA*s decreased in response to GA_3 treatment.

Analysis of the time course of gene expression in response to 100 μM GA_3 showed that the expression of *CsKAO, CsKO, CsGA20ox-2, CsGA2ox-1*/*-2* and especially *CsGA2ox-1* and *CsGA3ox-1* was upregulated during the 24-h exposure of the plants to GA_3 (Fig. 2b). The expression of *CsKS* did not change significantly during the 24-h GA_3 treatment. However, the expression levels of *CsDELLA1, CsDELLA2* and *CsDELLA4* and of three *CsGID1*s were repressed during the 24-h treatment.

Expression analysis of GA-related genes in response to ABA treatment

On exposure of the plants to various concentrations of ABA, the expression of *CsKS, CsKAO*, and *CsKO* was significantly up-regulated (Fig. 2c). *CsGA20ox-2* was induced by 100 and 150 μM ABA, whereas the expression level of *CsGA20ox-1* was dramatically elevated by 100 μM ABA. *CsGA3ox-1* was significantly upregulated by ABA treatment, whereas *CsGA3ox-2* was considerably downregulated by 50 and 150 μM ABA (Fig. 2c). In contrast, high ABA levels (100 and 150 μM) significantly repressed *CsGA2ox-2* transcription, whereas the expression level of *CsGA2ox-1* increased in response to 50 and 150 μM ABA (Fig. 2c). *CsGID1*s were somewhat repressed by ABA; *CsGID1b* in particular exhibited significantly reduced expression. In contrast, with the exception of *CsDELLA4*, which exhibited no significant change in response to ABA treatment, the expression levels of *CsDELLA1, CsDELLA2* and *CsDELLA3* markedly increased after exposure of the plants to several ABA concentration conditions (Fig. 2c).

The expression of *CsKO, CsKS* and *CsKAO* was consistently upregulated during 24 h of treatment with 100 μM ABA (Fig. 2d). In addition, transcription of *CsGA3ox-1, CsGA20ox-1* and *CsGA20ox-2* was markedly induced during the 24-h treatment, especially in the samples collected at the 24-h time point. Nevertheless, the expression of *CsGA2ox-1* and *CsGA2ox-2* was clearly repressed as the treatment time progressed for 24 h. *CsGA3ox-2* also exhibited reduced expression at the 6- and 12-h time points. During the 24-h ABA treatment, the expression of *CsGID1*s was notably repressed, especially after 12 h. Conversely, the majority of the *CsDELLA* genes were induced after short-term treatment (3–6 h), but their expression was suppressed after longer treatment (12–24 h).

Analysis of the expression of ABA-related genes in response to GA_3 treatment

The majority of the genes involved in the ABA metabolic pathway, including *CsAAO, CsSDR, CsZEP-1*/*-2*/*-3, CsCYP707A4, CsCYP707A5* were significantly repressed by 50 and/or 100 μM GA_3 treatment after 3 h. Interestingly, the different expression patterns of *CsCYP707A2* and *CsCYP707A3* that were observed showed that *CsCYP707A2* was markedly downregulated but *CsCYP707A3* was markedly upregulated in response to various ABA concentrations. In contrast, both *CsNCED1* and *CsNCED2* were significantly upregulated by 50 and 150 μM GA_3, whereas their expression did not change significantly when the plants were treated with 100 μM GA_3. The expression of *CsPP2C* was induced and that of *CsPYL8* was repressed in response to different GA_3 concentrations, but these changes were not significant (Fig. 3a).

During 24-h treatment with 100 μM GA_3, the expression of the majority of the studied genes, including *CsZEP*s, *CsSDR, CsCYP707A2, CsCYP707A4, CsPP2C* and *CsPYL8*, was repressed. In contrast, the transcript abundance of *CsAAO, CsCYP707A1* and *CsNCED2* increased to a maximum at the 12-h time point (Fig. 3b).

Analysis of the expression of ABA-related genes in response to ABA treatment

The expression of ABA-related genes in response to ABA treatment was also investigated. As shown in Fig. 3c, the *CsAAO, CYP707A3, CYP707A5, CsPP2C, CsNCED1* and *CsNCED2* genes were upregulated under different ABA concentrations. The transcript abundance of *CsNCED1, CsNCED2* and *CYP707A5* was dramatically induced by treatment with 50 and 150 μM ABA. In contrast, the transcription of *CsSDR* and *CYP707A2* was significantly suppressed by ABA. The transcription of *CsZEP1, CsZEP2, CsZEP3* and *CsCYP707A4* did not change significantly in response to ABA treatment.

The expression levels of *CsAAO, CsCYP707A1, CsCYP707A5, CsNCED2* and *CsNCED1* were upregulated and persisted at significantly high levels during the 24-h treatment with 100 μM ABA (Fig. 3d). In contrast, the expression of *CsSDR* and *CsCYP707A2* was repressed after 12 h of treatment, but several genes (*CsPYL8, CsZEP1, CsZEP2, CsCYP707A4*, and *CsPP2C*) exhibited low transcript abundance at the 12-h time point. The transcript abundance of *CsZEP3* was not affected by ABA treatment in the 24-h time course assay (Fig. 3d).

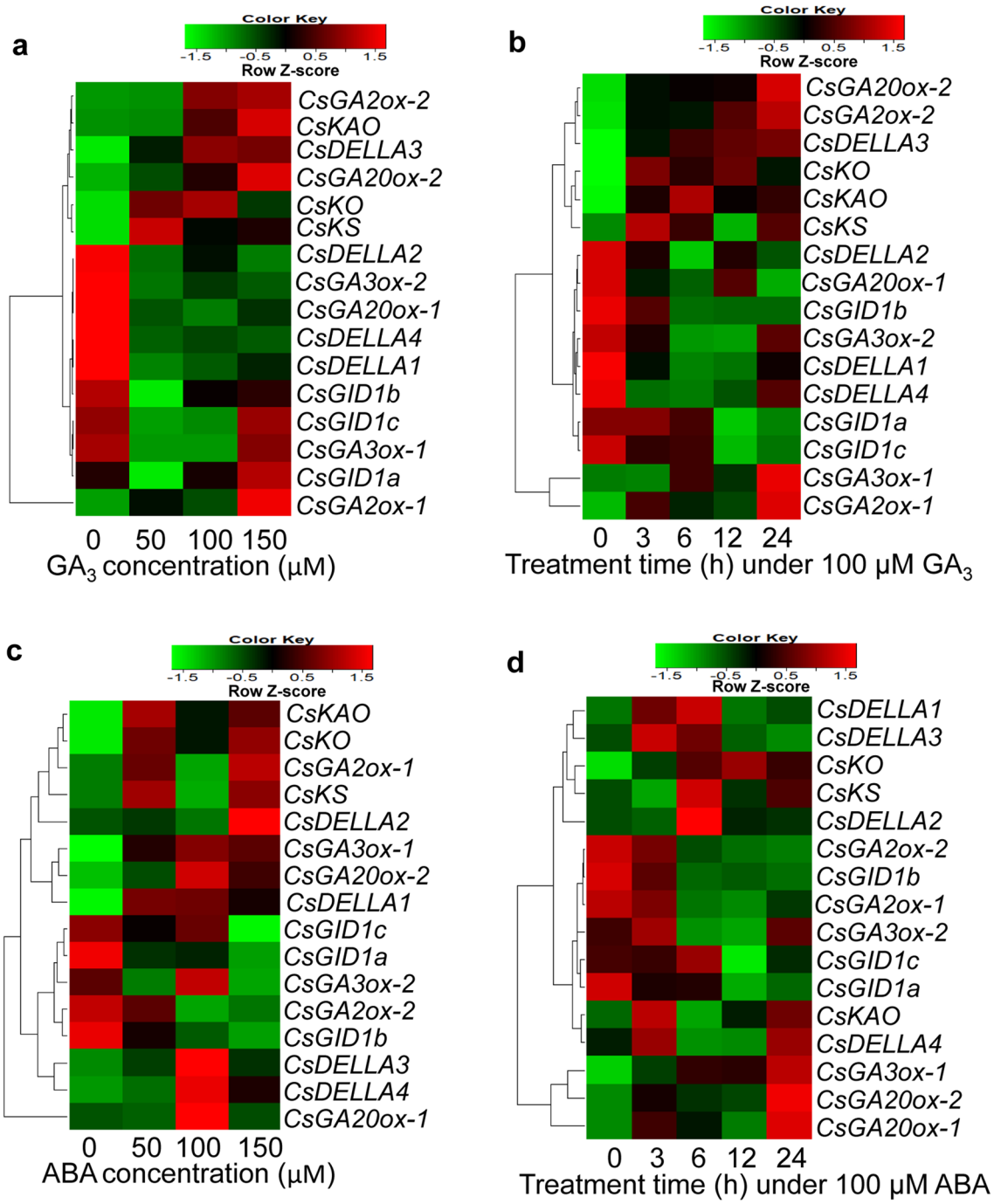

Fig. 2 Analysis of the expression of GA-related genes in response to GA_3 and ABA treatment. The expression patterns of GA-related genes in the leaves 3 h after treatment with 0, 50, 100, and 150 μM GA_3 (**a**) and ABA (**c**) were analysed using qRT-PCR. In addition, the expression profiles of the tested genes during the 24-h treatment in response to 100 μM GA_3 (**b**) and ABA (**d**) were determined using qRT-PCR. The results are presented as the average of three replicates and were calculated using the $2^{-\Delta\Delta Ct}$ method; the heat map was generated using *R* software. *CsPTB* served as a housekeeping gene. Green represents low expression, and red denotes high expression

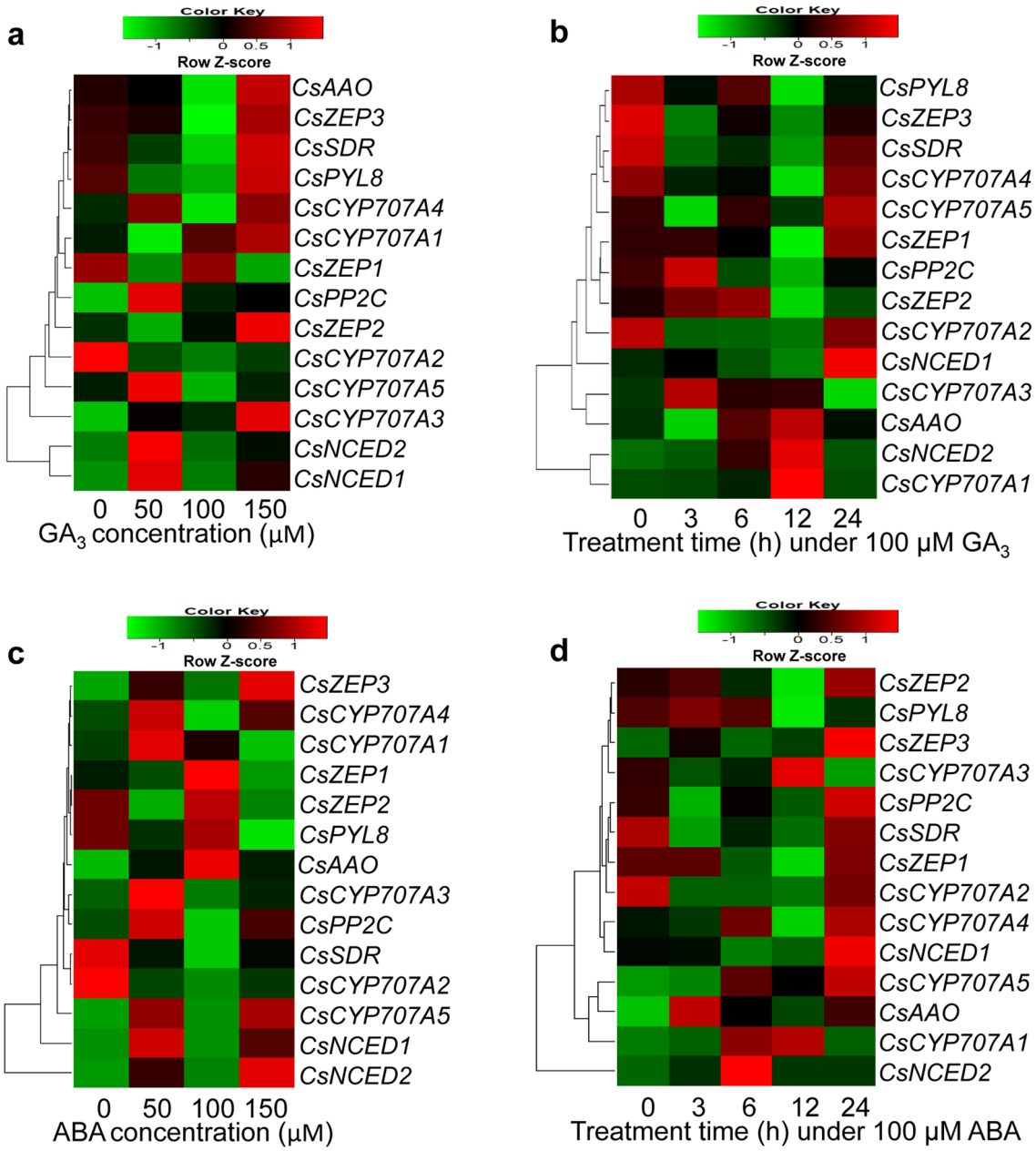

Fig. 3 Analysis of the expression of ABA-related genes in response to GA_3 and ABA treatment. The expression patterns of ABA-related genes in the leaves 3 h after treatment with 0, 50, 100, and 150 μM GA_3 (**a**) and ABA (**c**) were analysed using qRT-PCR. In addition, the expression profiles of the tested genes during the 24-h treatment in response to 100 μM GA_3 (**b**) and ABA (**d**) were determined using qRT-PCR. The results are presented as the average of three replicates and were calculated using the $2^{-\Delta\Delta Ct}$ method; the heat map was generated using *R* software. *CsPTB* served as a housekeeping gene. Green represents low expression, and red denotes high expression

Analysis of the expression of GA-related genes during the activity-dormancy cycle of the winter–spring season

Throughout the activity-dormancy cycle of 2013–14, the expression levels of genes that regulate the synthesis of bioactive GA, including *CsKAO* and *CsKO*, were dramatically downregulated during bud dormancy (from DS-1 to DS-3). When the buds began to be active (AS-1 to AS-3), these genes were significantly upregulated (Fig. 4a). Interestingly, the expression of *CsKS* decreased considerably at AS-3 (27 Mar 2014). An opposite transcription profile was observed for *CsGA20oxs* and *CsGA3oxs*. The genes of *CsGA20ox-1* and *CsGA3ox-2* were induced and their expression was maintained at high levels in the period of bud dormancy stages (DS-1 to DS-3); however, the

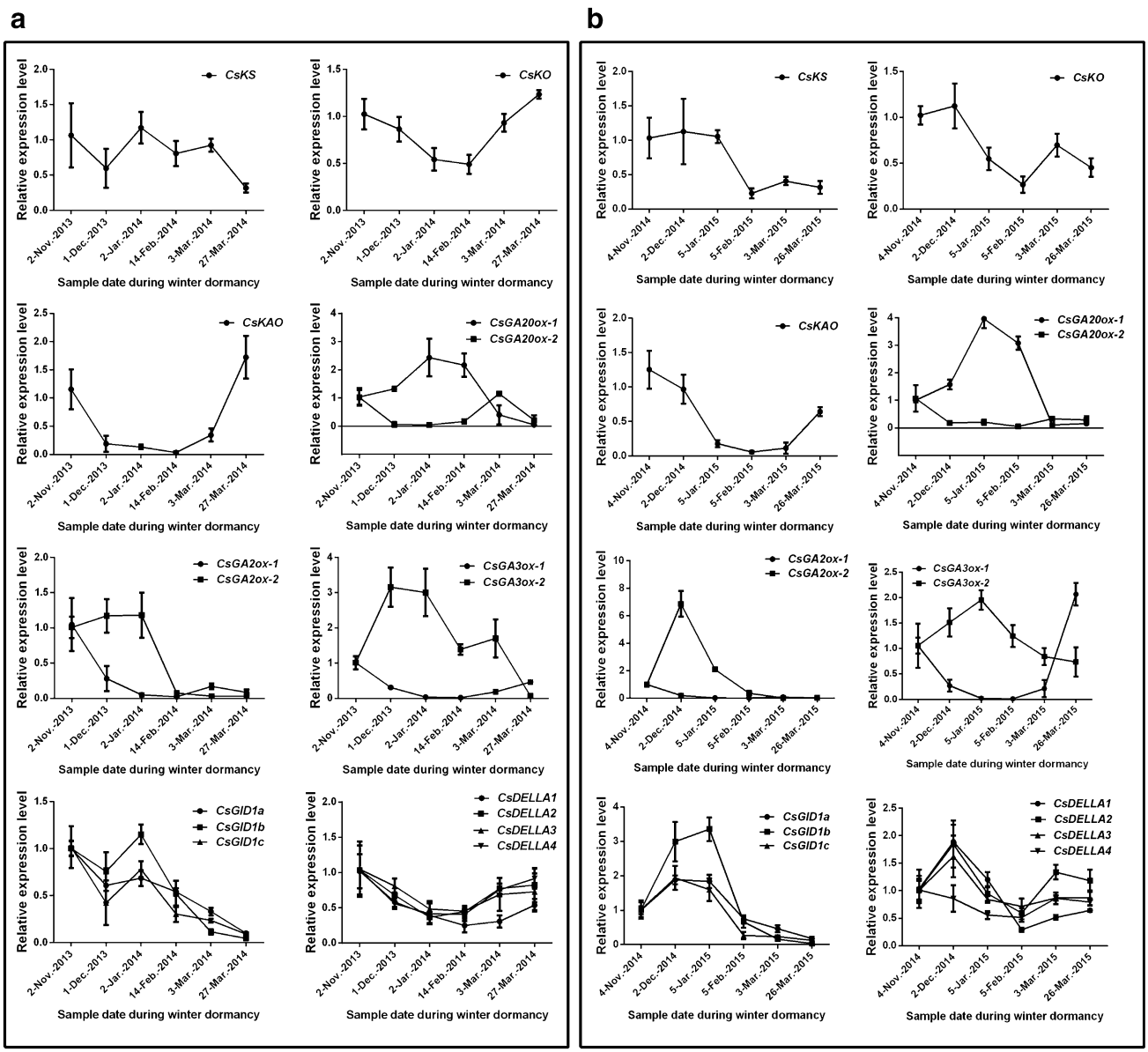

Fig. 4 Expression patterns of GA-related genes in activity-dormancy during the winter–spring seasons of 2013–2014 (**b**) and 2014–2015 (**c**). A schematic of the GA metabolic pathway in higher plants is shown in (**a**). The dates of 2 Nov 2013, 1 Dec 2013 and 2 Jan 2014 were defined as bud dormancy stage 1 (DS-1), DS-2 and DS-3. 14 Feb 2014, 3 Mar 2014 and 27 Mar 2014 were designed as bud active stage 1 (AS-1), AS-2 and AS-3, respectively. Correspondingly, the sampling dates of 4 Nov 2014, 2 Dec 2014 and 5 Jan 2015 were expressed as DS-1, DS-2 and DS-3, and 5-Feb 2015, 3 Mar 2015 and 26 Mar 2015 were defined as AS-1, AS-2 and AS-3, respectively

expression levels of *CsGA20ox-2* and *CsGA3ox-1* were dramatically repressed and were low at these stages. Regarding the expression of bioactive GA catabolic genes, *CsGA2ox-1* was considerably suppressed, but the expression of *CsGA2ox-2* did not change significantly during DS-1 to DS-3; however, these genes displayed low levels of expression during AS-1 to AS-3 (Fig. 4a). Interestingly, the expression of three *CsGID1*s was downregulated, and the lowest level was recorded during AS-3 (27 Mar 2014). The expression of *CsDELLA*s gradually decreased during bud dormancy (DS-1 to DS-3) but then increased during bud active stages (AS-1 to AS-3) (Fig. 4a). With the exception of *CsKAO, CsKO* and *CsDELLA1*, the transcription of the studied genes was significantly repressed at bud sprouting (AS-3).

As expected, the expression patterns of *CsKAO, CsGA3ox-1/-2, CsGA20ox-1/-2, CsGA2ox-1/-2, CsGID1a/b/c* and *CsDELLA1/2/3/4* observed in 2014–15 were somewhat similar to the patterns observed in 2013–14 (Fig. 4b). Nevertheless, the expression levels of

CsGA20ox-2, CsGID1a/b/c, CsDELLA1/2 increased during the bud dormancy stages (DS-1–DS-3) (Fig. 4b).

Analysis of the expression of ABA-related genes in the activity-dormancy cycle of the winter–spring season

The expression of ABA-related genes in tea plant lateral buds exhibited two different patterns to some extent based on the expression profiles in both 2013–14 and 2014–15. The expression of the majority of genes, including *CsNCED2, CsZEP1/2/3, CsCYP707A-2/3/4/5, CsPP2C* and *CsPYL8*, increased during the bud dormancy period (DS-1 to DS-3). During the bud active stages (AS-1–AS-3), the transcript abundance of these genes decreased (Fig. 5a, b). In contrast, the expression of *CsSDR* and *CsAAO* was repressed during the bud dormancy-active stages in both two-year cycles. Different expression profiles of *CsNCED1* and *CsCYP707A-1* during the bud dormancy-active cycle were observed in 2013–14 and 2014–15 (Fig. 5a, b).

Discussion

GA and ABA are broadly involved in plant growth and development, including shoot growth, flowering, and seed and bud dormancy. Numerous studies based on physiological and biochemical detection and molecular biology have shown that bud dormancy-active cycles are controlled by GA and ABA in many plants. In tea plants, GA and/or ABA were shown to participate in bud dormancy using content detection and transcriptome analyses (Hao et al. 2017; Paul et al. 2014). To explore how these two phytohormones regulate tea bud dormancy, the genetic information and detailed expression patterns of GA- and ABA-related genes during the dormancy-active cycle of the winter–spring season should be preferentially taken into account.

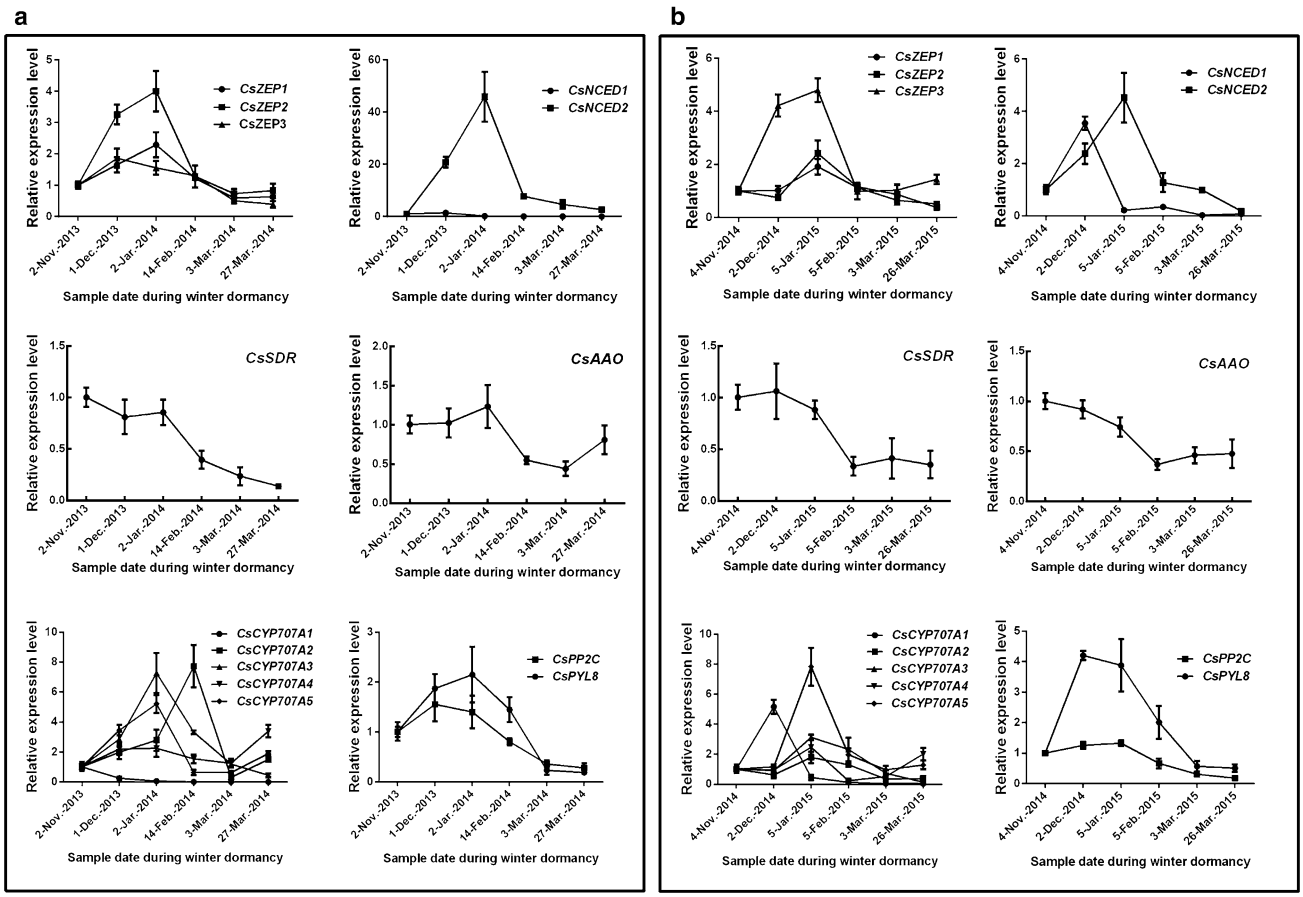

Fig. 5 Expression patterns of ABA-related genes in activity-dormancy during the winter–spring seasons of 2013–2014 (**a**) and 2014–2015 (**b**). The dates of 2 Nov 2013, 1 Dec 2013 and 2 Jar 2014 were defined as bud dormancy stage 1 (DS-1), DS-2 and DS-3. 14 Feb 2014, 3 Mar 2014 and 27 Mar 2014 were designed as bud active stage 1 (AS-1), AS-2 and AS-3, respectively. Correspondingly, the sampling dates of 4 Nov 2014, 2 Dec 2014 and 5 Jan 2015 were expressed as DS-1, DS-2 and DS-3, and 5 Feb 2015, 3 and 26 Mar 2015 were defined as AS-1, AS-2 and AS-3, respectively

Genes involved in the metabolism and signalling of GA and ABA in tea plants

In this study, 30 genes involved in the metabolism and signalling pathways of GA and ABA were identified in tea plants (Table 1). Sixteen of the identified genes were predicted to encode key regulatory enzymes involved in GA biosynthesis (*CsKS, CsKO, CsKAO, CsGA20oxs*, and *CsGA3oxs*) and deactivation (*CsGA2oxs*), GA receptors (GID1s) and major signal repressors of DELLAs (Ueguchi-Tanaka et al. 2007; Yamaguchi 2008). Fourteen of the genes encoded enzymes in the ABA metabolism pathway, including *CsZEPs, CsNCEDs, CsSDR, CsAAO* and *CsCYP707As* and the ABA receptor complex of *CsPP2C* and *CsPYL8*.

Among these genes, *KS, KO* and *SDR* might be transcribed from a single gene in tea plants because no additional sequences were found in the transcriptome data in the NCBI database. Similarly, it was shown that only one *KS* and one *KO* gene are present in *Arabidopsis* and that only one *KAO* gene is present in maize (Song et al. 2011). It is suggested that these single genes not only play critical roles in GA and ABA metabolism but that they have been conserved during the evolution of GA and ABA in plants. In contrast, the majority of proteins involved in GA and ABA metabolism and signalling are encoded by multigene families. For instance, there are five *GA20oxs* and *NCEDs* genes in *Arabidopsis*, indicating that the genetic regulation of the downstream pathway of GA and ABA metabolism might be more complicated than that of the upstream pathway. Considering its large genome size (3.02 Gb, 25 times larger than the genome of *Arabidopsis*), it is likely that additional genes could be isolated from the tea plant. In addition, the proteins involved in GA and ABA signalling are mainly encoded by multigene families. In *Arabidopsis*, there are 3 *GID1s*, 5 *DELLAs*, and 80 *PP2Cs* (Wang et al. 2011; Xu et al. 2008), suggesting that these genes have redundant roles and partially specialized functions. For instance, RGA and GAI may act synergistically to repress GA-mediated internode elongation, abaxial trichome initiation and leaf expansion, while both RGL1 and RGL2 participate in seed germination regulation (Wang et al. 2011). Many of the GA- and ABA-related genes of tea plants have not previously been identified. The tea plant genome has recently been reported (Xia et al. 2017). However, we searched this genome data and discovered that many genes do not contain a full-length coding sequence. Since both GA and ABA have fundamental functions in various growth and developmental processes and stress responses, the key genes and gene family members, including *CsGA20ox*s, *CsGA3oxs, CsNCED*s and *CsPP2Cs*, should be identified and subjected to systematic investigation.

Differential expression patterns of target genes in multiple organs

In this study, the expression profiles of target genes in roots, leaves, stems, flowers and buds were evaluated. The genes expressed in all five organs indicate that GA and ABA are needed to ensure the vegetative growth and reproductive development of tea plants. Many genes, especially GA-related genes, were preferentially expressed in flowers and buds, whereas several genes were expressed at low levels in roots and stems (Fig. 1). The growth and development of plants, especially in actively dividing and elongating tissues, are regulated by GA. Phytohormone responses facilitate the majority of the physiological and biochemical reactions that occur in growing tissues such as flowers and buds to keep the cell rapidly dividing and elongating (Carles and Fletcher 2003; Regnault et al. 2014). In addition, bioactive GA are primarily produced at the site of their action (Kaneko et al. 2003; Yamaguchi 2008), resulting in higher transcript abundance of the majority of GA-related genes in elongating and dividing tissues. In addition, the expression of GA-related genes, especially *GA20ox*s, *GA3ox*s and *GA2ox*s, exhibits tissue-specific patterns within a single tissue and at specific developmental stages (Pearce et al. 2015; Roumeliotis et al. 2013; Ye et al. 2015), indicating that flowering and bud development in tea plants is also controlled by GA.

Interestingly, the majority of ABA-related genes were generally highly expressed in roots and leaves, whereas a few genes, such as *CsSDR, CsAAO* and *CsCYP707A-5*, were highly expressed in flowers and/or buds (Fig. 1). ABA is synthesized predominantly in the vascular parenchyma cells of leaves and root tips and is then translocated to its site of action in plants (Antoni et al. 2011; Boursiac et al. 2013; Merilo et al. 2015; Umezawa et al. 2010). This was confirmed by our results based on the tissue-specific expression patterns of the key genes associated with ABA metabolism in tea plants. ABA is an essential signalling molecule under stress conditions and regulates stomatal opening and closure to control transpirational water loss as a defence against various stress stimuli (Bomke et al. 2008; Huerta et al. 2009; Xiao et al. 2010), and leaves and roots are major organs for these defence mechanisms. Tan et al. (2003) reported that five *AtNCED*s localized to different organs, including roots, flowers and seeds, in *Arabidopsis* and suggested that the developmental control of ABA synthesis involves localized patterns of *NCED* gene expression. Therefore, the difference between the tissue-specific expression profiles of GA- and ABA-related genes indicates that GA and ABA play distinct roles in the regulation of tea plant growth and development. Although they belong to the same gene family, these genes were differentially expressed. For instance, *CsGA20ox-1* and *CsGA20ox-2* were predominantly expressed in buds and flowers, respectively, whereas both *CsDELLA-3* and

CsDELLA-4 displayed high transcription levels in flowers and buds. These differences suggest that single genes in the same gene family might play distinct physiological roles and/or have redundant functions in the same tissues or organs.

Feedback regulation of target genes in response to GA_3 and ABA treatment

The biosynthesis of bioactive GA is feedback-regulated by exogenous GA_3 treatment mainly due to the control of the expression of 2ODD-type genes. For instance, after GA_3 treatment, certain *GA20oxs* and *GA3oxs* genes were repressed (also known as negative feedback regulation), and some *GA2oxs* genes were induced (also known as positive forward regulation) (Carrera et al. 1999; Du et al. 2015; Griffiths et al. 2006; Mitchum et al. 2006; Thomas et al. 1999). The expression of *CsGA3ox-2* and *CsGA20ox-1* was consistently downregulated, whereas *CsGA2ox-1* and *CsGA2ox-2* expressions were up-regulated at specific GA_3 concentrations and during the 24-h treatment (Fig. 2a, b). However, both *CsGA3ox-1* and *CsGA20ox-2* exhibited increased transcription in leaves in response to GA_3, which suggested that the feedback regulation of certain genes involved in GA metabolism might vary by gene family member (Carrera et al. 1999; Du et al. 2015; Yamaguchi et al. 2001). In contrast to *CsGA20oxs, CsGA3oxs* and *CsGA2oxs*, all *CsKO, CsKAO* and *CsKS* genes were upregulated by GA_3 treatment, indicating that these types of genes might not be controlled by feedback regulation, as was previously reported in wheat (Huang et al. 2012) and *Salvia miltiorrhiza* (Du et al. 2015). These results also reflect the complexity of the underlying mechanism of GA biosynthesis in plants. Hence, exogenous GA_3 treatment could compensate for the effect of bioactive GA by upregulation of the expression levels of *CsGA3oxs* and *CsGA20oxs* and by downregulation of *CsGA2oxs*. In contrast, aside from the increased expression of *CsDELLA3* after GA_3 treatment, both *CsGID1*s and the remaining *CsDELLA* genes were downregulated, similar to previously reported results (Gao et al. 2012; Griffiths et al. 2006; Liu et al. 2016; Shen et al. 2015; Yano et al. 2015). The difference in the gene expression patterns suggested that different members of the *GID1*s and *DELLA*s gene families fulfil distinct roles in the plant developmental process (Gallego-Giraldo et al. 2014; Voegele et al. 2011). In addition, GA signalling might also be feedback-regulated by exogenous GA_3 (Griffiths et al. 2006; Li et al. 2013; Zentella et al. 2007). GA promote plant growth by inducing the degradation of the growth-repressing DELLA proteins; DELLAs both upregulate GA synthesis genes and downregulate GA deactivation (*GA2oxs*) genes (O'Neill et al. 2010; Zentella et al. 2007). DELLA proteins are transcription factors that regulate the expression of numerous genes during plant growth and development (de Lucas et al. 2008). Ravindran et al. (2017) showed that increased GA levels cause degradation of RGL2, a DELLA protein, repressing *GATA12* expression and thereby releasing dormancy. Furthermore, with respect to GA signalling, decreases in the transcript abundance of *GID1*s and *DELLA*s repress the formation of the GA-GID1-DELLA complex.

ABA modulates GA biosynthesis and responses by controlling the expression of genes involved in the GA biosynthesis pathway and signal transduction. We found that the majority of bioactive GA synthesis-related genes were upregulated upon ABA treatment. For instance, the expression of *CsGA20ox-1, CsGA20ox-1*, and *CsGA3ox-1*, and especially of *CsGA20ox-1*, was significantly induced. ABA also upregulated *CsKS, CsKAO* and *CsKO* transcription but repressed the expression of *CsGA2oxs* genes (Fig. 2c, d). Similar results have been widely observed in other plants. However, GA and ABA are mutually antagonistic. Transcriptome analysis results show that ABA treatment inhibits GA-related genes (Li et al. 2015; Yang et al. 2014). Moreover, overexpression of some ABA-related genes (e.g., *ABI* and *ABF*) lowered the transcript abundance of GA metabolism genes (Muniz Garcia et al. 2014; Shu et al. 2013, 2016). In addition, both *CsGID1*s were downregulated under ABA conditions, suggesting that ABA also inhibits the GA response partly by suppressing the transcript abundance of GA receptors, whereas promotion and/or inhibition of *CsDELLA*s were observed in response to ABA treatment. It has recently been demonstrated that DELLA acts as a hub that integrates multiple signal responses to regulate plant growth and development by linking the cross-talk between GA, ABA, ethylene and environmental stimuli (Achard et al. 2003; Golldack et al. 2013; Jiang and Fu 2007). The cross-talk functions of DELLA between GA and ABA suggest that the expression of various *CsDELLA*s plays a distinct role in GA and/or ABA signalling.

In contrast to the effects of ABA on the expression of GA-related genes, most of the genes involved in the ABA synthesis pathway were repressed by GA_3 treatment (Fig. 3a, b). Surprisingly, *CsNCED*s were upregulated by both low (50 μM) and high (150 μM) concentrations of GA_3; however, the opposite expression pattern was observed in response to moderate GA_3 concentrations during the 24-h treatment, suggesting that the regulation of transcription of GA- and ABA-related genes depends not only on the concentration of GA_3 but also on the duration of treatment. NCEDs catalyse the key step of ABA biosynthesis, which results in enhanced ABA action. In *Arabidopsis*, the induction or mutation of *AtNCED* genes controls endogenous ABA accumulation, which in turn influences GA-dependent seed germination (Lefebvre et al. 2006; Martinez-Andujar et al. 2011; Seo et al. 2016), suggesting that the antagonism between GA_3 and ABA is partially based on the fact that

each transcriptionally regulates the biosynthetic pathway of the other. However, endogenous ABA levels are controlled by multiple factors. Whether or not GA_3 treatment affects the metabolism of ABA requires further investigation.

ABA controls the expression of numerous genes involved in its metabolic and signal transduction pathways. In this study, we observed that several genes, including *CsSDR, CsZEP1, CsZEP2, CsCYP707A2, CsPP2C* and *CsPYL8*, displayed downregulated expression profiles in response to different ABA concentrations and/or during the 24-h treatment period, whereas *CsAAO, CsCYP707A1, CsCYP707A5*, and *CsNCED2* showed increased transcript abundance (Fig. 3c, d). This diversity of expression patterns has been previously reported (Lou et al. 2017; Saito et al. 2004; Wang et al. 2009; Zhang et al. 2016). It is largely due to the fact that each enzyme is encoded by multiple genes, and one type of gene may even have distinct roles in different tissues/organs (Lefebvre et al. 2006; Nonogaki et al. 2014; Schwarz et al. 2015; Tan et al. 2003). Interestingly, we found that *CsNCED1* and *CsNCED2* had similar expression patterns; however, the inconsistencies between these patterns in response to some ABA treatments suggested that these genes might play different roles in the ABA response. ABA can amplify ABA biosynthesis and signalling via a positive feedback mechanism that is mainly mediated by *NCED* expression (Nonogaki and Nonogaki 2017; Nonogaki et al. 2014). Our results showed that exogenous ABA treatment induces *CsNCED* expression. Although the content of endogenous ABA was not measured, we infer that *CsNCED2* plays an important role in ABA-positive feedback regulation based on the upregulation of *CsNCED* gene expression.

Expression of GA- and ABA-related genes is correlated with the activity-dormancy transition of the winter–spring season

The antagonism of GA and ABA modulates the bud activity-dormancy cycle. In general, increasing ABA and decreasing GA induce the onset of dormancy; the opposite conditions facilitate the breaking of dormancy (Cooke et al. 2012; Horvath et al. 2003; Meier et al. 2012; Paul and Kumar 2011; Rohde and Bhalerao 2007; Wang et al. 2015). Similar changing trends in ABA and GA expression have been detected in dormant buds during the winter–spring season in different species of tea plants (Nagar and Kumar 2000). In the present study, we investigated the expression patterns of GA- and ABA-related genes during winter bud activity-dormancy changes in two consecutive years. We hypothesized that the expression profiles of GA- and ABA-related genes would be upregulated and/or downregulated in a manner consistent with the plant's activity-dormancy cycles.

Compared with the expression patterns of GA-related genes, the expression patterns of ABA-related genes were highly correlated with the activity-dormancy cycle, suggesting that ABA might be more closely related to bud dormancy than GA (Khalil-Ur-Rehman et al. 2017; Li et al. 2003). Additional studies have recently shown that ABA controls bud activity-dormancy by modifying metabolic processes. The expression patterns of several genes, including *NCEDs* and *CYP707As*, in various species such as pear, grape and peach are similar to those in tea plants (Parada et al. 2016; Tuan et al. 2017; Vergara et al. 2017; Wang et al. 2015; Zheng et al. 2015). Exogenous application of ABA promotes bud dormancy and inhibits its release, whereas overexpression of *NCED* genes contributes to ABA accumulation and inhibits seed germination (Lefebvre et al. 2006; Martinez-Andujar et al. 2011; Seo et al. 2016; Wang et al. 2009). Based on the results, we obtained by studying tea plant gene expression in plants treated with ABA and GA_3, we conclude that ABA accumulation may be predominantly controlled by the ABA synthesis gene *CsNCED2* and the catabolic genes *CsCYP707As* (Fig. 5). Upregulation of *CsNCED2* from November (DS-1) to the following January (DS-3) might contribute to the accumulation of ABA to high levels and promote the ABA response. Although this process does not trigger bud dormancy initiation and maintain the dormant state, it may be beneficial for withstanding cold temperature stress in winter.

In addition to the critical role played by GA in seed dormancy, GA also modulate bud dormancy by integrating metabolic processes and signal transduction (Cooke et al. 2012). The content of GA_3 decreases during the dormancy period but increases dramatically at the bud flush stage, and exogenously applied GA_3 can promote dormancy release during tea production (Barua 1969; Nagar and Kumar 2000). Exogenous application of bioactive GA promotes bud dormancy release by mediating changes in energy metabolism, protein metabolism, cell structure, cell division, and signalling and transcription pathways (Hansen et al. 1999; Zhuang et al. 2013). We found that the majority of GA-related genes were differentially expressed during the winter dormancy period and that the expression of these genes was maintained at a low level during the dormancy release period (Fig. 4), similar to the results of the transcriptome analysis by Hao et al. (2017). Similarly, Barros et al. (2012) reported that flower bud break in almond is accompanied by decreased expression of *PdGA20ox* under natural conditions, suggesting that repressed transcription of *GA20ox* plays an important role in bud break. However, it has been reported that increases in *GA20oxs* and *GA30xs* expression are correlated with bud burst (Choubane et al. 2012). These inconsistent results might be due to interspecies differences. The tea plant is an adeciduous plant, whereas species such as pear, peach and poplar are deciduous. Tea plant bud dormancy is strongly influenced by many environmental factors, and

the regulation of bud dormancy among these plants differs (Hao et al. 2017).

Interestingly, Nagar and Kumar (2000) reported that the content of free GA decreased significantly during bud dormancy and that the content of conjugated GA dramatically increased; however, in the bud break stage, the content of free GA markedly increased, but conjugated GA markedly decreased. These results suggest that a reversal in the levels of conjugated and free GA plays an essential role in tea plant bud break. Moreover, phytohormones, especially GA, are closely related to the release of tea bud dormancy (Barua 1969; Jeyaraj et al. 2014; Thirugnanasambantham et al. 2013; Wang et al. 2014). Based on our analysis of the expression patterns of GA-related genes in the present study (Fig. 5), we hypothesized that the accumulation of GA that causes the dormancy break of tea plant buds might ultimately originate from GA biosynthesis but that dormancy break is mainly due to the release of conjugated GA within the tea plant. ABA can also be inactivated by conjugation to another molecule, such as by esterification of ABA to ABA-glucose ester, for storage or transport in plants (Finkelstein 2013). Moreover, we postulate that the dynamic changes in ABA during the activity-dormancy cycle mainly result from the biosynthesis and catabolic regulation of ABA. Finally, a potential model of bud active/dormancy controlled by GA and ABA is proposed (Fig. 6). The activity-dormancy transition primarily depends on the balance in the fluctuations of GA and ABA levels resulting from the differential expression of their related genes.

Conclusion

Although numerous studies have demonstrated that GA and ABA play crucial roles in the regulation of plant growth and developmental, the genes involved in their metabolism and signalling pathways, as well as their expression patterns, are unclear. In the current study, 30 genes involved in the metabolism and signalling pathways of GA and ABA were first cloned and characterized in tea plants. Tissue-specific expression revealed that GA-related genes were somewhat predominantly expressed in the flowers and tea buds, whereas high transcript levels of ABA-related genes were present in the roots and leaves. Under GA_3 and ABA treatments, the expression levels of *CsKS, CsKAO, CsKO, CsGA20ox-2*, and *CsNECD2* were up-regulated, whereas the expression levels of *CsGID1b, CsSDR, CsPYL8*, and *CsCYP707A2* were repressed. The changes in *CsGA20oxs, CsGA3oxs, CsGA2oxs* and *CsNCED*s showed feedback regulation in response to GA_3 and ABA. Analysis of gene expression during the active growth-dormancy-sprouting transitions showed that some genes, including *CsKAO, CsKO, CsGA20ox*s, *CsGA3ox*s, *CsGA2ox*s, *CsDELLA*s, *CsZEP*s, *CsNCED*s, *CsCYP707A*s, *CsPP2C* and *CsPYL8*, were closely related to tea plant bud dormancy, indicating that bud dormancy in tea plants is regulated by GA and ABA. In summary, the important roles of GA and ABA signalling in the activity-dormancy cycles of tea plants were elucidated, and several genes involved in the regulation of bud dormancy were identified. These genes warrant further investigation.

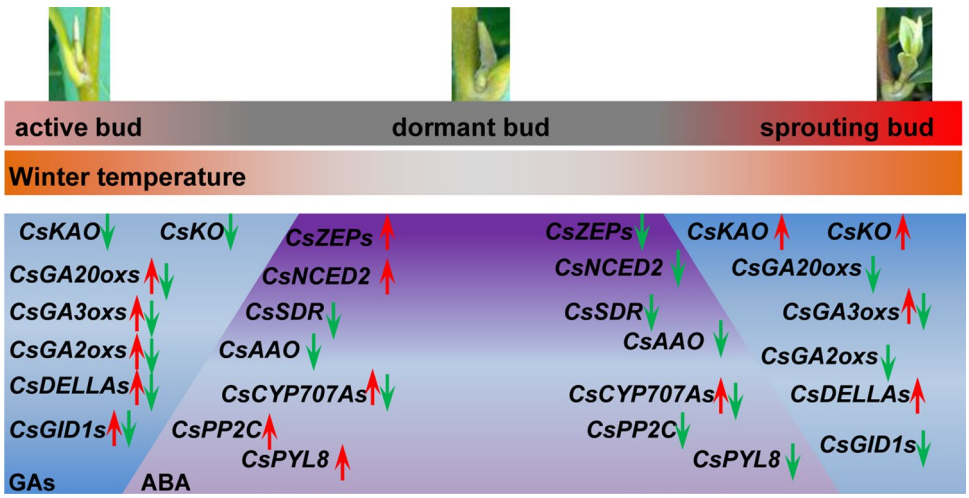

Fig. 6 Potential model of bud activity/dormancy controlled by GA and ABA balance. The bud activity-dormancy transition of tea plant during winter–spring season (red) was controlled by a series of environment factors such as the temperature fluctuations (orange). In this shift, GA (blue)- and ABA (purple)-related genes were differentially expressed that results in the alterations of metabolism and siganalling of GA and ABA, thereby regulating the bud dormancy. The expression patterns of GA- and ABA-related genes identified in this study were summarized. Red and green arrows indicated the upregulated expression and downregulated expression, respectively

Author contribution statement XW and YY conceived and designed the experiments. CY, HC, XH, JZ, WQ, YG and NY performed the experiments and analysed the data. CY and HC wrote the paper. XW and YY revised and approved the final manuscript. All authors reviewed the manuscript.

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