Genome-wide identification, characterization, and expression analysis of tea plant autophagy-related genes (CsARGs) reveals diverse roles during development and abiotic stress

Wenjun Qian (qau-wenjunqian@qau.edu.cn)
Qingdao Agricultural University https://orcid.org/0000-0003-0530-361X
Huan Wang
Qingdao Agricultural University
ZhaoTang Ding
Qingdao Agricultural University
Mengjie Gou
Qingdao Agricultural University
Jianhui Hu
Qingdao Agricultural University
Yu Wang
Qingdao Agricultural University
Lu Wang
Chinese Academy of Agricultural Sciences Tea Research Institute
Yuchun Wang
Zhejiang A and F University
Taimei Di
Chinese Academy of Agricultural Sciences Tea Research Institute
Xinfu Zhang
Qingdao Agricultural University
Xinyuan Hao
Chinese Academy of Agricultural Sciences Tea Research Institute
Xinchao Wang
Chinese Academy of Agricultural Sciences Tea Research Institute
Yajun Yang
Chinese Academy of Agricultural Sciences Tea Research Institute

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Abstract

Background

Autophagy, meaning ‘self-eating’, is required for degradation and recycling of cytoplasmic constituents under stressful or non-stressful conditions, thereby contributing to maintaining cellular homeostasis, delaying aged and longevity in eukaryotes. So far, the functions of autophagy have been intensively studied in yeast, mammals and model plants, but few studies have focused on economic crops, especially for tea plants, the roles of autophagy in coping with different environment stimuluses have not yet been detailed. Therefore, exploring the functions of autophagy related genes in tea plant would contribute to further understanding the mechanism of autophagy in response to stresses in woody plants.

Results

Here, we totally identified 35 *CsARGs* in tea plant. Each CsARG is highly conserved with its homologues stemmed from other plant species, except for CsATG14. Tissue-specific expression analysis revealed that the abundances of *CsARGs* were varied with different tissues, but *CsATG8c/ i* showed a certain degree of tissue specificity, respectively. Under hormones and abiotic stress conditions, most of *CsARGs* were up-regulated at different treatment time points. In addition, the transcriptions of 10 *CsARGs* were higher in cold-resistance cultivar ‘Longjing43’ than the cold-susceptible cultivar ‘Damianbai’ during CA periods, however, *CsATG101* showed a contrary tendency.

Conclusions

We comprehensively analyzed the bioinformatics and physiological roles of *CsAR*Gs in tea plant, and these results provide the basis for deepen exploring the molecular mechanism of autophagy involved in tea plant growth and development and stress responses. Meanwhile, some *CsARGs* would be served as putative molecular markers for cold-resistance breeding of tea plant in future.

Background

Autophagy (ATG) is an evolutionarily conserved eukaryotic system that entails the degradation of cytoplasmic components, including many biological macromolecules (proteins, protein aggregates), and entire organelles in vacuole or lysosome [1]. Normally, autophagy occurs at basal levels in eukaryotic cells, but induced autophagic flux by specific developmental processes or stressful environments for degrading oxidative damaged proteins, damaged organelles, and other toxic compounds, so that the degradation products could be recycled and the cellular remolded in cells to sustain them survive adversity. Autophagy occurs through at least four pathways, classified as microautophagy [2], macroautophagy [3], chaperone-mediated autophagy [4], and selective autophagy [5]. Among of them, macroautophagy, which is manipulated by a special organelle known as autophagosome, is the most extensively characterized and is commonly referred to as autophagy. Hereafter, macroautophagy referred to as autophagy in our study. Accumulating evidences implicate that macroautophagy is derived from the formation of the cup-shaped double membranes named phagophore (or isolation membrane), which engulfs cytoplasmic material and then obturates to generate autophagosome. Following, the outer membrane of autophagosome fuses with the tonoplast and the rest forms an autophagic body, which will be degraded in the vacuolar lumen to release its cargoes for recycling [6].

The occurrence of the autophagy is mainly archived by a collection of *ATG* genes. Until now, more than 30 *ATG* genes have been identified in *yeast* and *Arabidopsis* respectively [7–11]. Among of them, ATG1-10, 12–14, 16–18, 29, 31 are served as key modulators to participate in the autophagy initiation, nucleation, elongation, maturation, and fusion of with vacuoles [12–14]. In *yeast* and *Arabidopsis*, the initiation of autophagy is mediated by target of rapamycin (TOR) kinase via the ATG1/13 association [15]; Then, PI3K complex, containing Vps34, Vps15, ATG6/Vps30 and ATG14, is activated to promotes vesicle nucleation; Followed by the expansion and enclosure of autophagy through the ATG5-12-16 and ATG8-PE conjugation systems; Later, the autophagosome is docked and fused to the tonoplast employing a vesicle trafficking system, called v-SNARE complex; Finally, the autophagic body in the vacuole is digested by a series of hydrolases including the lipase ATG15 and proteinases A (PEP4) and B (PRB1) [16]. Following the identification of the ATG protein families in *yeasts* and *Arabidopsis*, orthologs are constantly discovered in various plant and animal genomes, indicating that the core autophagic systems controlled by these proteins are conservative during evolution. In recent years,

ATG genes have been identified in a few plant species, such as 33 *OsATGs* in rice [17], 30 *NtATGs* in tobacco [18], 24 *SlATGs* in tomato [19], 30 *VaARGs* in grapevine [20], 32 *MaATGs* in banana [21], 37 *SiATGs* in foxtail millet [22], 29 *CaATGs* in pepper [23], etc. Based on the alignment of multiple ATG amino acid sequences, lots of the ATGs showed remarkable overall conservation in different plants, strongly suggesting that autophagic processes are mechanistically identical in different plants.

Along with the identification of *ATG* genes across the eukaryotic kingdoms, mounting evidences indicate that autophagy mechanism is involved in the whole life cycles of the plant, ranging from vegetative and reproductive development to environmental stress responses. Recently, reverse genetics approach effectively accelerated the functional analyses of *ATG* genes in plants, especially the mutation and overexpression techniques. Many research results found that most of the *ATG*s-mutated *Arabidopsis* lines performed premature senility phenotypes though they still owned intact life cycles, enlightening that autophagy contributes to leaf longevity during senescence [9, 11, 24, 25]. Autophagy deals with organ senescence and nutrient starvations (carbon or nitrogen starvation, sucrose deficiency, dark, etc.) through degrading damaged or unwanted proteins and organelles compounds to promote the recycling and remobilization of nutrients [1, 26–29]. There have been revealed that the biomass production and nitrogen remobilization efficiency both in *ATG*-mutated *Arabidopsis* and rice were remarkably lower relative to wild types, indicating that autophagy contributes to efficient nitrogen remobilization [29–31]. Besides, the synthesis of amino acids was reduced in autophagy mutants during carbon starvation, considering that autophagy machinery controls cellular homeostasis [32]. However, increased autophagic activity can promote yield and nitrogen use efficiency in plants. For example, overexpression of *OsATG8a* dramatically improved the level of autophagy and significantly improved nitrogen uptake efficiency in the transgenic rice under suboptimal N conditions [26]. Apart from responding to nutrient deficiency, autophagy can also be induced by diverse abiotic stresses, and the kinases SnRK1 and TOR may be the central regulators in these processing [27]. Under high salt and osmotic stress conditions, the expression of an autophagy-related gene, *AtATG18a*, was upregulated in *Arabidopsis*, and the *AtATG18a* mutants were more sensitive to salt and drought conditions than wild types [33]. However, overexpression of *ATG5* or *ATG7* promoted Atg8 lipidation, autophagosome formation and autophagic flux, thus increased the resistance of necrotrophic pathogens and oxidative stress, delayed senescence and improved growth, seed set, and seed oil content [34]. Moreover, autophagy could shape plant innate immune responses through a variety of ways, but there are three main ways for inducing autophagy, including a virulent or related pathogen-induced programmed cell death, salicylic acid and jasmonic acid, and virus-induced RNA silencing [35]. All in all, autophagy is involved in the regulation of the whole life cycles of plants during different growth phases under different growing environments.

As a special type of the evergreen plant, tea plant (*Camellia sinensis*) requires comfortable growing environments, such as acidic soil, high humidity, ordinary temperature, and so forth, thus it is mainly distributed in tropic and sub-tropic areas in Asia. However, being unable to move, tea plant is also regularly exposed to pathogen attack and herbivory, nutrient deficiency, and various types of abiotic challenges, such as extreme temperature, drought, salt, ozone, ion toxicity, etc. Under such adverse conditions, the morphology, physiology, and metabolism of tea plant were changed to survive. Accordingly, the studies on stress resistance of tea plant in responding to different stimulations have been greatly performed in recent years. For example, multiple omics techniques, including transcriptome, proteomic and metabonome have been widely used for exploring the dynamic changes of genes, proteins and the metabolites under different stress conditions [36–40]. In addition, many genes, which in responding to various stimulus, have been identified and analyzed by feat of the daft genome sequences [41–46], meanwhile, the functional studies of some difference expressed genes (DEGs), like Basic leucine zipper (bZIP) gene (*CsbZIP6*) [47], SWEET transporters gene (*CsSWEET16*) [48], vacuolar invertases gene (*CsINV5*) [49], and 12-Oxophytodienoate reductase gene (*CsOPR3*) [50], have been extensively studied. However, there has no one autophagy-related gene (*ARG*) been comprehensively analyzed in tea plant, and the roles of autophagy in coping with different environment stimuluses have also not yet been detailed in tea plant. To explore the in vivo roles of autophagy and the mechanistic genetics of *ARG* genes in tea plant, a genome-wide identification, characterization, and expression analysis of *CsARGs* were performed this study. The results will facilitate to deepen revealing the diverse roles of autophagy in response to different growth phases or environment stress conditions in tea plant.

Results

Identification of CsARGs in tea plant

Based on three different identification paths, a total of 35 *CsARGs* were identified from the two published tea plant genomes ('ShuChaZao' and 'YunKang10'). Among of them, four genes (*CsATG1s*, *CsATG8s*, *CsATG18s* and *CsVTI13s*) were detected to have isoforms (Table 1). Here, we identified a UV radiation resistance protein/autophagy-related protein 14, named as *CsATG14*, which was

not yet fully studied in plants and may be deficit in *Arabidopsis*. In addition, as there has no one strict criterion to identify all paralogues of these four genes, and also as the genome assembly and gene annotation of the two reported tea plant genomes have not yet fully completed as compared to *Arabidopsis*, rice and tobacco genomes, which result from partial paralogues of these four genes as mentioned in *Arabidopsis*, rice, tobacco were not identified in the tea plant genomes. Bioinformatics analysis results showed that as a type of biological macromolecules, the *CsARGs* ORF lengths are varied from 285 to 7410 bp, the corresponding numbers of deduced amino acids are ranged from 94 to 2469 aa, and the molecular weights are away from 10.51 to 276.87 kD. The theoretical isoelectric points (pI) were predicted from 4.52 to 9.41. The prediction of subcellular location results found that most of CsARGs were predicted to locate in nuclear, and some of them were also predicted to locate in cytoplasm, chloroplast and mitochondria. Signal peptides predicting results showed that no one of these CsARGs contains signal peptide. In addition, CsATG9 was predicted to contain 5 TMHs, CsATG18b and three vesical transport vesicle-Soluble NSF Attachment Receptor (v-SNARE) proteins, CsVTI12, CsVTI13a and CsVTI13b, were predicted to contain 1 TMHs respectively.

Table 1

Basic information of CsARGs. ORF, opening reading fame; AA, the numbers of amino acid residues; pI, Theoretical isoelectric point; MW, Molecule weight; Loc, Subcellular location; TMHs, Transmembrane helices.

Gene name	Accession number	ORF (bp)	AA	MW (kDa)	pI	instability index	Aliphatic index	Loc	SignalP	TMHs
CsATG1c	XP_028071137.1	2193	730	80.78	6.69	unstable	83.37	Nucleus	NO	NO
CsATG1t	XM_028254805.1	852	283	31.70	6.72	unstable	100.21	Cytoplasm	NO	NO
CsATG2	XM_028214951.1	6039	2012	220.59	5.69	unstable	87.78	Nucleus	NO	NO
CsATG3	XM_028269562.1	945	314	35.72	4.72	unstable	78.82	Cytoplasm	NO	NO
CsATG4	XM_028205167.1	1473	490	54.16	5.54	unstable	73.45	Nucleus	NO	NO
CsATG5	XM_028239072.1	1047	367	41.29	4.77	unstable	98.26	Cytoplasm	NO	NO
CsATG6	XM_028209545.1	1581	526	59.43	5.87	unstable	71.77	Nucleus	NO	NO
CsATG7	XM_028207488.1	2121	706	77.95	5.63	unstable	91.20	Cytoplasm	NO	NO
CsATG8a	XM_028204481.1	354	117	13.65	6.60	unstable	84.19	Cytoplasm	NO	NO
CsATG8c	XM_028257959.1	360	119	13.65	8.78	unstable	83.61	Nucleus	NO	NO
CsATG8f	XM_028237334.1	369	122	14.00	8.75	stable	95.08	Nucleus	NO	NO
CsATG8g	XM_028213593.1	354	117	13.64	8.73	stable	86.67	Nucleus	NO	NO
CsATG8i	XM_028202806.1	393	130	14.96	7.58	unstable	64.38	Nucleus	NO	NO
CsATG9	XM_028219288.1	2610	869	99.93	6.25	unstable	79.55	plasmid	NO	5
CsATG10	XM_028214294.1	717	238	27.19	4.96	unstable	82.73	Nucleus	NO	NO
CsATG11	XM_028237709.1	3471	1156	129.98	5.57	unstable	82.95	Nucleus	NO	NO
CsATG12	XM_028206145.1	285	94	10.51	9.41	unstable	88.19	Cytoplasm	NO	NO
CsATG13	XM_028260289.1	1863	620	68.75	8.90	unstable	65.27	Nucleus	NO	NO
CsATG14	XM_028265144.1	1440	479	53.78	8.85	unstable	77.37	Chloroplast	NO	NO
CsATG16	XM_028206301.1	1527	508	55.74	6.08	unstable	91.44	Cytoplasm	NO	NO
CsATG18a	XM_028253213.1	1296	431	47.63	6.62	stable	77.82	Nucleus	NO	NO
CsATG18b	XP_028071781.1	1107	368	40.18	7.15	unstable	96.49	Cytoplasm	NO	1
CsATG18c	XM_028196882.1	1257	418	46.36	8.01	unstable	84.40	Chloroplast	NO	NO
CsATG18f	XM_028238387.1	2697	898	97.13	8.47	unstable	75.46	Mitochondria	NO	NO
CsATG18g	XM_028202982.1	2988	995	108.70	5.78	unstable	80.87	Chloroplast	NO	NO
CsATG18h	XM_028252480.1	3069	1022	111.90	5.79	unstable	76.91	Chloroplast	NO	NO
CsATG20	XM_028265532.1	1206	401	46.15	8.20	unstable	83.47	Chloroplast	NO	NO
CsATG101	XM_028236970.1	657	218	25.43	6.46	stable	88.03	Nucleus	NO	NO
CsATI	XP_028079241.1	948	315	35.00	4.52	unstable	64.13	Nucleus	NO	1
CsVTI12	CSA033576	669	222	25.26	9.22	unstable	105.81	Nucleus	NO	1
CsVTI13a	XM_028240825.1	666	221	25.12	9.30	unstable	102.81	Cytoplasm	NO	1
CsVTI13b	XM_028226760.1	666	221	24.89	9.41	unstable	101.95	Cytoplasm	NO	1
CsVPS15	XM_028202873.1	4632	1543	172.13	6.19	unstable	87.43	Nucleus	NO	NO

Gene name	Accession number	ORF (bp)	AA	MW (kDa)	pI	instability index	Aliphatic index	Loc	SignalP	TMHs
CsVPS34	XM_028243914.1	2361	814	93.36	6.39	unstable	92.46	Cytoplasm	NO	NO
CsTOR	XM_028205854.1	7410	2469	276.87	6.22	unstable	102.06	Cytoplasm	NO	NO

Phylogenetic analysis of CsARGs in tea plant

To explore the evolutionary relationships and the classification of CsARGs in tea plant, total of 177 ARG proteins from tea plant, *Arabidopsis*, *Setaria italic*, *Oryza sativa*, and *Nicotiana tabacum* were aligned to construct phylogenetic tree. As shown in Fig. 1, except for CsATG14 that had only been identified in tea plant, each of the other CsARG proteins was highly clusted together with the homologues proteins stemmed from the other four species, and almost all of the CsARGs showed a closest relationship with NtARGs. Meanwhile, we found the bootstrap values among the different ARGs proteins in each subtree was nearly 100% except for ATG8s subfamily, which suggest that ARGs protein sequences are highly conserved, and they may share similar functions among different species.

Gene structure, protein domain distribution and cis-acting element analysis

Understanding the exon-intron structure is benefit to exploring the evolution of multiple gene families [57]. To investigate how the differences in exon-intron structure were generated, both the genomic and ORF sequences of *CsARGs* were uploaded into GSDS v2.0 to predict the exon-intron structure. As shown in Fig. 2a, the numbers of exons in *CsARGs* family are varied from each other, with members within *CsATG8s* or *CsVTI13s* subfamilies having similar exon-intron structures respectively.

To further dissect the functions of CsARG proteins, the protein domains of each CsARG was analyzed by SMART program. As shown in Fig. 2b, CsATG1s encode Serine/Threonine protein kinases, which contain catalytic domain involved in protein phosphorylation in the progress of autophagy [58]. CsATG9 contains 5 transmembrane helix regions (88–110, 155–177, 320–342, 403–425 and 438–457) as detected by the TMHMM v2.0 program, which play a unique role in autophagosome formation derived from the endoplasmic reticulum (ER) in plants [59]. CsATG16 contains a coiled coil region and 7 WD40 domains, which formed a conserved Atg12-Atg5-Atg16 complex during autophagy process. Each member of CsATG18s subfamily is basically a β-propeller and formed by 2 or 3 WD40 domains. CsATG20, also known as Snx42, contains a PX domain, which plays a central role in efficiently inducing the non-selective autophagy. CsVPS15 encodes a Serine/Threonine protein kinase, which is formed by 5 WD40 domains and is regulated by a PI 3-kinase (PI3K), CsVPS34. CsVPS34 is now characterized as a central regulator in mediating vesicular trafficking and cellular homeostasis [60]. As an ATG8-interacting protein, CsATI1 contains a transmembrane region that may contribute the protein complex to moving on the ER net-work and reaching the lytic vacuole. CsVTI1s, including CsVTI12, CsVTI13a andCsVTI13b, all contain a coiled coil region, a t_SNARE domain and a transmembrane region respectively, and these domains may be involved in trafficking of cargo to the vacuole. CsTOR as a conserved phosphatidylinositol kinase-related protein kinase, contains a specific rapamycin binding domain, a P13Kc catalyzing domain and a FATC domain, which suggests it participate in redox-dependent structural and cellular stability.

To decipher the regulatory mechanisms of *CsARG*s in responding to growth and development, stress defenses, and hormone signaling. 2000 bp 5′-upstream non-coding region sequences of each *CsARG* was isolated to predict *cis*-elements. As Fig. 2c shown, the distributions, numbers and types of *cis*-elements are varied from different promoter sequences. Nevertheless, most of the promoters contain numbers of MYB- and MYC-binding sites, except for the promoter of *CsATG12* that lacks of MYC-binding sites. Besides, most of the promoters of *CsARGs* contain ABA-, MeJA-responsive elements, and partial of them contain GA, SA, auxin, cold, drought, defense and stress (TC-rich repeats) responsive elements. In addition, all of the promoters of *CsARGs* contain many light-responsive elements, including G-box, MRE, Box-4, AE-box, etc. (not shown in Fig. 2c). All in all, each *CsARG* may play vital role in responding to circadian variation, hormones, and biotic and abiotic stresses.

Protein-protein interaction networks of CsARGs

To investigate the interaction among of the CsARGs in tea plant, *Arabidopsis* used as the reference species to construct PPINs. As Fig. 2d shown, 34 CsARGs were matched to 33 AtARGs, and those 34 CsARG proteins formed 333 protein-protein association patterns, each ARG is closely related to each other except for ATG14 which has not been fully studied in *Arabidopsis*. Among of them,

23 ARGs, including ATG9, ATG7, ATG1c, etc., were reported or predicted to interact with more than 20 ARGs respectively, suggesting that the occurrence of autophagy requires the protein interactions of numerous ARG proteins.

Conserved domain and motif distribution analysis of CsATG8s

As the member of UBQ superfamily, ATG8s coupled with their conjugation system are key components for autophagy. In our study, to clearly understand their regulatory mechanisms, the bioinformatic characteristics of CsATG8s subfamily proteins were further explored. As Fig. 3 shown, total of 5 CsATG8s were identified in tea plant based on the homologous alignment analysis. Phylogenetic analysis result showed that CsATG8s were subdivided into 3 clades. Among of them, CsATG8a/c and CsATG8g/f were respectively clusted into one group, and CsATG8i was aligned closely with MdATG8i (Fig. 3). Motif distribution analysis result showed that CsATG8s contain motif 1–4, and CsATG8i contains an additional motif 7 at C-terminal (Additional file2). All of these CsATG8s proteins contain the conserved GABARAP domains, four putative tubulin binding sites, three ATG7 binding sites, and a conserved glycine (G) residue. In addition, we found the conserved G residue both in CsATG8a and CsATG8g was directly exposed at C-terminal (Fig. 3), suggesting the functions of CsATG8a and CsATG8g involved in autophagy may distinct from the other three proteins, they may be no need cysteine protease, Atg4, to cleave C-terminal, but directly bound to E1-like enzyme, Atg7.

Expression profiles of CsARGs in different tea plant tissues

To confirm the tissue specificity of *CsARGs*, the root, stem, mature leaf, tender leaf, and seed of tea plant were chose for qRT-PCR analysis. The results showed that the transcriptions of all *CsARGs* were detected among the above tissues though the mRNA level of each *CsARG* was varied in different tissues (Fig. 4). Besides, we found most of *CsARGs* exhibited higher transcription abundances in stem and seed, suggesting that the autophagy plays important roles in the development of stem and seed in tea plant. Moreover, we found *CsATG3/ 7/ 101*, *CsVPS15/ 34*, *CsATI*, *CsVTI12/ 13b*, *CsATG8s* and *CsATG18s* subfamilies genes were highly expressed in different tissues. Remarkably, *CsATG8c* was significantly expressed in mature leaf and seed, and *CsATG8i* was dramatically expressed in stem and seed. In brief, our results found that the expression patterns of each *CsARGs* are varied in different tissues, but partial of them showed a certain degree of tissue specificity.

Differential expressions of CsARGs in response to hormone treatments

To elucidate the comprehensive roles of *CsARGs* under ABA and GA treatment conditions, we analyzed the expression pattern of each *CsARG*. Under ABA treatment condition, we found multiple numbers of *CsARGs* were highly induced after 12 h and/or 2 d of ABA treatment. Among of them, *CsATG6/ 11/ 12/ 14/ 16/ 18b/ 18c/ 18f/ 20/ 101*, and *CsVTI12/ VTI13b* expressions were induced more than 2 folds at least at one time point. Meanwhile, partial genes, like *CsATG3/ 11/ 18c/ 101/*V*PS15/ TOR*, were straightly up-regulated during entire ABA treatment periods (Fig. 5). In contrast, *CsARGs* showed contrary expression profiles under GA stress as compared to ABA stress. Most of *CsARGs* initially decreased but significantly increased after 2 d of GA treatment. Among of them, the expressions of 13 genes, including *CsATG12/ 14/ 16/ 18a/ 18b/ 18c/ 18 g/ 18 h/ 20/ ATI/ VTI12/ VTI13a/ VTI13b*, were increased more than 2 folds after 2 d of GA treatment. Furthermore, there have 6 genes, *CsATG7/ 8c/ 8f/ 101/ VPS15/ VPS34*, were negatively down-regulated throughout GA stress periods. These results indicated that the autophagy is required for responding to hormone treatments in tea plant.

Expression patterns of CsARGs in responding to different abiotic stresses

Similarly, for exploring the temporal expression patterns of *CsARGs* under abiotic stress conditions, the related expression level of each *CsARG* was performed by qRT-PCR. As the Fig. 6 shown, the expressions of all *CsARGs* were regulated to different degrees under various abiotic stress conditions.

During CT periods, the expressions of almost all of *CsARGs* were up-regulated at different processing time points, except for 2 genes, *CsATG18c/ h*, which were down-regulated throughout the CT periods. In addition, there are 20 induced *CsARGs* showed highest expression levels after 12 h of CT respectively. Specifically, the expression levels of *CsATG3/ 4/ 6/ 8i/ 10/ 11/ 18a/ 18 g/ 101/ VTI12/ VTI13a/ VTI13b/ VPS34* were more than 5 folds as compared to 0 h of CT, respectively. Besides, *CsATG5/ ATG18g/* ATI were gradually induced within the 2 days of CT. Under DT condition, there have 15 *CsARGs* were induced at different DT time points, and most of these genes showed highest expression levels after 12 h of DT. The expression levels of *CsATG2/ 3/ 6/ 8i/ 11/ 16/ VPS34* were more than 2 folds as compared to 0 h of DT, respectively. Moreover, *CsATG18h* and *CsATG101* were gradually up-regulated as the DT time extended. Conversely, the expressions of the remaining *CsARGs*, such as *CsATG4/ 7/ 8a/ 10/ 14*, etc., were slightly deduced or not affected by DT. Within NT periods, lots of the *CsARGs* were also induced in

different degrees. In contrary to CT, most of the induced *CsARGs* showed highest expressions levels after 1 d of NT, but deduced following the treatment time extended. Especially, the expression levels of *CsATG9/ 10/ 18a/ 18 g/ 101/ VTI13* were more than 2 folds after 1 d of NT as compared to control, respectively. However, *CsATG8g/ 12/ 16/ ATI* were significantly up-regulated after 12 h of NT. Taken together, these results demonstrated that autophagy plays central roles in responding to abiotic stress in tea plant.

Differential expressions of CsARGs **in different tea plant cultivars during CA periods**

To compare the differential expression patterns of *CsARGs* in different tea plant cultivars (cold-resistance cultivar 'Longjing43' and cold-susceptible cultivar 'DaMianBai') within CA periods. 11 *CsARGs*, which have been confirmed to be remarkably induced under CT condition as shown in Fig. 7, were selected to perform qRT-PCR analysis. The results found that these 11 genes presented different expression patterns in 'Longjing43' and 'DaMianBai' during CA periods in 2018–2019. Specifically, these 11 genes showed contrary expression patterns from Nov 14th to Dec 13th in 'Longjing43' and 'DaMianBai'. With the exception of *CsVPS34*, the other *CsARGs* were continuously up-regulated in 'Longjing43', but all of them were gradually deduced in 'DaMianBai' from Nov 14th to Dec 13th periods. In contrast, the transcription levels of these 11 genes were all increased in 'DaMianBai' from Dec 13th to Jan 17th, but many *CsARGs*, such as *CsATG16/ 18 g/ 101/ VTI12* were decreased in 'Longjing43'. Notably, we found the transcription level of *CsATG101* was lower in 'LongJing43' than 'DaMianBai' throughout CA periods. These results indicated that those *CsARGs* play important role in response to cold resistance of tea plant, but their regulation mechanisms may be varied in different cultivars.

Discussion

CsARGs involved in different occurrence stages of autophagy in tea plant

Autophagy is a catabolic degradation pathway essential for degrading long-lived proteins, protein aggregates, and damaged organelles [61]. It has been proved that autophagy is highly conserved from *yeast* to humans, which is the result of the interaction of many proteins. So far, there have more than 30 ATG-related genes been identified in many eukaryotes, and those *ATGs* encode many core proteins that involved in the entire process of autophagy from the induction to the degradation, recovery and recycling of autophagosome. As a type of evergreen wooden plant, the recycling of some broken or discarded macromolecular substances plays an important role in the special development period or in the resistance to stresses in tea plant. At present study, a total of 35 *CsARGs* were identified in tea plant genome. Some *CsARGs*, like *CsATG8* and *CsATG18*, exist multiple copies in tea plant genome, and the same results were also observed in many other species, such as *Oryza sativa* [17], *Nicotiana tabacum* [18], *Vitis vinifera* [20], *Musa acuminate*[21], and *Setaria italic* [22], etc. In addition, the results of phylogenetic and protein domain analysis further confirmed that ATGs are highly homologous among different plant species. In *yeast*, ATG proteins were divided into four functional groups based on their roles involved in autophagy process [62]. Similarly, the identified CsARGs also constituted a relatively complete autophagic machinery, where they function in forming ATG1 kinase complex (CsATG1s/13, CsTOR), Class ⅢPI3K complex (CsATG6/14, CsVPS15/34), ATG9 recycling complex (CsATG2/9/18 s), and Atg8-lipidation system (CsATG3/4/7/8 s) and Atg12-conjugation system (CsATG5/7/10/12/16).

Until now, there have lots of reports on the functional analysis of ATG14 in mammals, but few in plants. In mammals, the human homologs ATG14, hAtg14/Barkor/Atg14L, has been shown to be the sole specific subunit in phosphatidylinositol 3-kinase (PI3-kinase) complex. Atg14L could be interacted with Beclin-1/2 through their coiled-coil domains, and proved to be the targeting factor for PI3KC3 to autophagosome membrane [63, 64]. Similarly, ATG14 is only integrated into phosphatidylinositol 3-kinase complex I to direct association of complex I to the pre-autophagosomal structure (PAS) in *yeast* [65]. Here, we identified an ATG14 homologous protein, referred to as CsATG14, in tea plant. Bioinformatics analysis results predicted that CsATG14 is a hydrophilic protein, and contains a coiled-coil motif at the N-terminus region from 10 to 367 aa, suggesting that CsATG14 may be interacted with Beclin-1 (named as CsATG6 in our study) to serve as a scaffold for recruiting the class III phosphatidylinositol-3-kinase (PIK3C3).

As a highly conserved ubiquitin-like protein, ATG8 is activated by conjugation to the lipid phosphatidylethanolamine (PE) to form ATG8-PE adduct, thereby participates to the autophagosome formation and phagophore expansion [66, 67]. During conjugation, the C-terminal of ATG8 must be cleaved by a cysteine protease, Atg4, to expose a glycine residue [68, 69]. In present study, five ATG8 isoforms were identified, and they displayed high sequence similarity to AtATG8s, NtATG8s and OsATG8s. However, a conserved glycine residue for lipidation was directly exposed at the C-terminal of CsATG8a/g respectively, similar phenomena were also observed in MdATG8g/i, AtATG8h/i and OsATG8e, suggesting that ATG4 may be not necessary to the conjugation of ATG8-PE.

Besides, ATG8 also can interact with their specific substrates or receptors via an Atg8 Interacting Motif (AIM) in the target proteins during selective autophagy. Recently, two plant-specific proteins in *Arabidopsis*, termed ATI1 and ATI2, were identified and proved to be interact with AtATG8f and AtATG8h [70]. In our study, however, only one unique ATI homologous gene, CsATI, was identified. Sequences alignment analysis found that CsATI also contains two putative AIMs (17–20, 267–270) and a predicted transmembrane domain (242–259) (Additional file2), suggesting that CsATI also can bind one of five CsAtg8 isoforms in tea plant.

During macroautophagy, a v-SNARE complex, including v-SNARE VTI1, Rab-like GTP-binding protein (YKT6), and syntaxin (VAM3), was contributed to the maturation and fusion of autophagosome to lysosome/vacuole [71]. In plants, there are three VTI1-type SNAREs members (VTI11, VTI12, VTI13) have been identified [72]. Sanmartin et al. (2007) suggested that VTI12 and VTI11 might involve in trafficking to storage and lytic vacuoles in vegetative and seed tissues in *Arabidopsis*, respectively [73]. Moreover, VTI13 participates in trafficking of cargo to the vacuole within root hairs and also plays an essential role in maintenance of cell wall organization in *Arabidopsis* [74]. In our study, CsVTI12 and CsVTI13a/b were all predicted contain the typical v-SNARE domains (Fig. 2b), and phylogenetic analysis result showed a close relationship with other VTI1s (Fig. 1), suggesting that CsVTI1s are essential for autophagy and may mediate different protein transport pathways.

CsARGs mediated the growth and development of tea plant

Under normal growth conditions, autophagy served as a housekeeping process to degrade unwanted proteins, organelles and damaged cytoplasmic contents. In *Arabidopsis*, to explore the functions of *ATG* genes, the corresponding *ATG*-mutants and *ATG*-overexpression lines were popularly used. Numerous studies have showed that almost all of *ATG*-mutants could complete their entire life cycles but coupled with early senescence phenotypes under normal growth condition, suggesting that autophagy mediates plant senescence [75]. Indeed, autophagy is necessary to anther and seed development [26, 30, 76], root elongation [77], chloroplast recycling [78]. In present study, we found the transcription abundances of most *CsARGs* were higher in stem and seed than other tissues, which indicate that autophagy may mediates the nutrients allocation or recycling from source tissues to sink tissues in tea plant. Specifically, *CsATG8s* subfamily genes showed high transcription levels in all detected tissues, which demonstrate they play a great role in modulating tea plant growth and development. A similar result was also observed in *Arabidopsis*, where *AtATG8s* were distinctly expressed throughout the plant [79]. As core ATG proteins, ATG8s have been used as very convenient markers to monitor autophagic activity, and also play vital role in regulating the nitrogen remobilization efficiency and grain quality in plants [80]. For instance, overexpression of *OsATG8b* increased the nitrogen recycling efficiency to grains in transgenic plants, while reduced nitrogen recycling efficiency and grain quality in *OsATG8b*-RNAi transgenic plants [81]. Similarly, *ATG8a*, *ATG8e*, *ATG8f* and *ATG8g* overexpressed in *Arabidopsis* could promote autophagic activity and improve nitrogen remobilization efficiency and grain filling in transgenic plants [82]. In our study, both *CsATG8c* and *CsATG8i* were strongly expressed in tea seed, suggesting that the high mRNA levels of these two genes may promote nitrogen remobilization efficiency to tea seeds. From this point of view, selecting tea plant germplasms with higher *CsATG8s* transcription abundances may attribute to improving the tea seed quality so that guarantee the seedling emergence rate and survival rate.

Chloroplasts are specific energy converters of higher plants and photoautotrophs, which are not only participating in photosynthesis, but also performing plant metabolism. It has been well established that nearly 80% of the total leaf nitrogen was stored in chloroplasts in C_3 plants [83]. In addition, more recent evidences found that chloroplasts degraded by autophagy in RCBs and whole organelles forms respectively during leaf senescence, so that chloroplasts could be served as a principal nitrogen source for recycling and remobilization. Indeed, it has been reported that autophagy also contributed to leaf starch degradation [84]. Tea plant is evergreen and C_3 plants, the numbers of chloroplasts in leaves are gradually increased from the tender leaves to mature leaves, and then decreased with leaf senescence. In the present study, we analyzed *CsARGs* expressions both in mature and tender leaves, the results found that there have 11 *CsARGs*, especailly *CsATG1c/ 4/ 5/ 8c/ 8f/ 10/ 13/ 18 h*, exhibited more than 3-folds higher expression levels in mature leaves than in tender leaves, indicating that autophagic activity is changed following the maturation of leaves, a higher autophagic activity in mature leaves may be attribute to prolonging leaf longevity so that maintaining the evergreen of tea plant for a long time.

CsARGs improved abiotic stress tolerance in tea plant

In addition to mediate plant growth and development, autophagy also plays a critical role in plant resistance to various stresses, such as nutrient deficiency, oxidation stress, cold, drought, salt, wounding, heavy metal, pathogen attack, etc. Under favorable condition, autophagy is maintained at basal level, but it is relative quickly stimulated under stress conditions. Overexpression and mutation

methods have been widely used to explore the functions of *ATG* genes in different species. For example, *AtATG18a* was dramatically induced after a few hours of NaCl and mannitol treatments, and the roots of *AtATG18a*pro: *GUS* transgenic plants were evenly stained under nutrient starvation, oxidative stress (MV), NaCl, mannitol stress conditions. However, the growth of RNAi-*AtATG18a* plants were retarded and the seed germination was also delayed as compared to WT under different stress conditions, indicated that *AtATG18a* may function in the response of plants to these stresses [33]. Similarly, a homologous gene, *MdATG18a*, overexpressed in apple plants could enhance drought resistance probably by inducing a greater autophagosome production and a higher autophagic activity [85]. Overexpression of *MdATG18a* also regulated the expressions of many genes that involved in anthocyanin biosynthesis, sugar metabolism, and nitrate uptake and assimilation, and finally promoted the soluble sugar and anthocyanin accumulation, starch degradation and nitrate utilization improvement in response to N-depletion [86]. Apart from *ATG18a*, *ATG3/ 5/ 7/ 8 s/ 10* were also been reported had critical roles in dealing with different stresses. Overexpression of *MdATG3s* enhanced the tolerance to multiple abiotic stresses both in transgenic *Arabidopsis* and apple plants [87]. Overexpression of *AtATG5* or *AtATG7* in transgenic *Arabidopsis* activated AtAtg8-PE conjugation, autophagosome formation, and autophagic flux, thus increased the tolerance of necrotrophic pathogens and oxidative stress, also retarded aging and improved growth and seed yields [34]. Indeed, MdATG8i proved to interact with MdATG7a and MdATG7b, overexpression of *MdATG8i* enhanced tolerance to nutrient-starvation both in transgenic *Arabidopsis* and apple plants [22]. In the present study, the expressions of *CsATG3/ 5/ 7/ 8 g/ 8i/ 18a/ 18 g* were quickly induced by cold, drought, NaCl treatments. Furthermore, the promoters of these genes contains series of *cis*-acting elements that potentially involved in responding to environmental stresses or hormones, indicating that autophagy would be induced in tea plants under adverse environmental conditions, and lots of *CsATG* genes participate in dealing with different stresses.

It is well known that sugars are not only important osmoprotectants and ROS scavengers, but also act as core signaling molecules in plants under adverse conditions [49]. The occurring of autophagy also closely related to sugar signaling. The central energy-sensing SnRK1 acts as a positive regulator, which acts upstream of TOR on sugar-phosphate perception to activate autophagy, and TOR kinase acts as a negative factor to inhibit autophagy [88]. Accumulating evidences proved that sugar contents increased, and many genes involved in sugar metabolism, transport and signaling were differentially expressed in tea plant during abiotic stress conditions [53, 89]. Among of these genes, *CsSnRK1.2* was induced, but *CsSnRK1.1*was not influenced and *CsSnRK1.3* was sharply reduced during CA periods [89]. Combined with our results, the expression profile of *CsTOR* was not strictly showed a contrary tendency to *CsSnRK1*, where we found the expression of *CsTOR* was slightly induced under cold condition, but reduced under drought and NaCl conditions, suggesting that autophagy could be activated by TOR-independent pathways, and SnRK1 could also mediate autophagy through a TOR independent mechanism in tea plant under certain stress conditions. Autophagy is also regulated by phytohormones. Under normal conditions, TOR kinase phosphorylates PYLs receptors and represses ABA signaling, whereas ABA signaling represses TOR kinase activity through the phosphorylation of Raptor B mediated by SnRK2 under stress conditions [90]. In the present study, however, we found the expression of *CsTOR* was slightly induced under ABA treatment condition, which indicate that the inhibition of TOR is not simply affected by the transcription level, but mainly influenced at post-translation level during stress conditions. At present, there are few studies explored the relationship between autophagy and GA. Kurusu et al. (2017) found that *OsATG7* mutated in rice could reduce the endogenous level of active-forms of gibberellins (GAs) in anthers of autophagy-defective mutant, Osatg7-1 during flowering stage, which suggested that autophagy mediated the biosynthesis of GAs in rice [91]. In the present study, we found two-thirds of *CsARGs* were induced after 2 d of GA treatment, which indicates that there is a close relationship between the GA metabolism and autophagy, but the specific regulatory mechanism needs to be further investigated.

Cold acclimation (CA) is an indispensable process to increase cold tolerance of tea plant. During CA, gene expressions, protein activities, and metabolic contents are altered and varied between cold-resistant cultivar ‘Longjing43’ and cold-susceptible cultivars ‘DaMianBai’ [51]. Specifically, a higher ROS contents and a lower SOD activity were observed in ‘DaMianBai’ as compared to ‘Longjing43’, and many genes related to ROS production and scavenging were also induced and deduced, respectively, in ‘DaMianBai’ under CA condition, demonstrated that the stimulation of ROS-scavenging genes was a principal strategy for tea plants in response to cold stress. In addition to ROS-scavenging genes, there has also been reported that the production of ROS could induce autophagy, which in turn inhibits ROS production [27]. In our study, we performed 11 *CsATGs* expressions during CA periods (from Dec.13 to Jan.17), and their expressions were higher in cold-resistance cultivar ‘Longjing43’ than the cold-susceptible cultivar ‘DaMianBai’, except for *CsATG101*, which indicate that autophagic activity may higher in cold-resistance cultivar ‘Longjing43’ than the cold-susceptible cultivar ‘DaMianBai’ during CA periods. Strangely, we found the transcription of *CsATG101* was higher in cold-susceptible cultivar ‘DaMianBai’ than the cold-resistance cultivar ‘Longjing43’ throughout CA periods in winter season. It is well known that CsATG101 is a component of the ULK1 complex, which served as a stabilizer of ATG13 in cells. In mammals, ATG101 is required for

maintaining tissue homeostasis in both adult brains and midguts. In plants, however, the physiological role of ATG101 has not been fully understood. The specific regulatory mechanism of *CsATG101* responding to CA needs to be further studied in future. In a word, based on the differential expressions patterns in different cultivars, we believe that these 11 *CsATG* genes would be served as putative molecular markers for cold-resistance breeding of tea plant in future.

Conclusions

In the present study, a total of 35 *CsARGs* were identified, each of CsARG showed a closer relationship to its homologues stemmed from other plant species. The transcription abundances of *CsARGs* are varied in different tissues, but partial of them showed a certain degree of tissue specificity. Under various abiotic stress conditions, most of *CsARGs* were induced at different treatment time points, which indicated autophagy plays central roles in responding to abiotic stress in tea plant. In addition, 10 *CsARGs* were highly expressed in cold-resistance cultivar than cold-susceptible cultivar during CA periods, however, *CsATG101* showed a contrary tendency, suggesting that these genes would be served as putative molecular markers for cold-resistance breeding of tea plant. All in all, we comprehensively analyzed the bioinformatics and physiological roles of CsARGs in tea plant, and these results would be provide the basis for further study on the molecular mechanism of autophagy involved in tea plant growth and development and stress responses.

Methods

Plant materials and stress treatments

3-year-old clonal potted seedlings of ‘LongJing43’ cultivar, which planted in the greenhouse of the Tea Research Institute of the Chinese Academy of Agricultural Sciences (TRI, CAAS, N30°10', E120°5'), were used for tissue-specific analysis. The tissues including roots, stems, mature leaves, tender leaves, and seeds were sampled and quickly frozen in liquid nitrogen to store at -80 °C. Three independent biological replicates were performed, and each replicate contains three seedlings with similar growth states.

1-year-old clonal potted seedlings of ‘LongJing43’ cultivar, which cultivated in experimental base of Qingdao Agricultural University (QAU, N36°33', E120°4'), were used for performing cold, drought, salt, and hormones treatments. Before treatments, all seedlings were cultured in growth chamber under the following growth conditions: temperature, 23 ± 0.5 °C; lighting time, 14 h/10 h (light/dark); and humidity, 75%. The cuttings with the same growth potential were used to process different treatments as described by Qian et al (2016) [46] with some modified. For cold treatment (CT), the temperature of the growth chamber was plummeted to 4 °C without changing any other growth conditions. PEG-6000 (10% (w/v)) and 250 mmol·L^{-1} NaCl were used to imitate drought (DT) and salt treatment respectively. To proceed hormones treatments, 100 μmol·L^{-1} ABA and 100 μmol·L^{-1} GA were sprayed on the surface of tea leaves respectively. During stress treatments periods, the other aspects of the growth conditions were kept the same as the control. All of these treatments were carried out for 2 days, with samples of the third and/ or fourth mature leaves from the terminal bud taken at 0, 12, 24 and 48 h post treatment. For each stress treatment, the samples collected at the 0 h time point were taken as control, respectively. All samples were quickly frozen in liquid nitrogen and stored at -80 °C. Each stress treatment contains three biological replicates.

A cold-resistance cultivar ‘LongJing43’ and a cold- susceptible cultivar ‘DaMianBai’ as reported by Wang et al. (2019) [51] were used for natural cold acclimation (CA) analysis in 2018–2019. Both of the two-tea cultivars were 18-year-old and cultivated at the Tea Research Institute of the Chinese Academy of Agricultural Sciences (TRI, CAAS, N30°10', E120°5'). The sampling methods were performed as described by Qian et al. (2018) [49].

Identification of CsARGs in tea plant

To identify putative *CsARGs* in tea plant, three ways were used to search the homology sequences of *ARGs* in published tea plant genomes and transcriptomes. Firstly, ‘autophagy’ or ‘ATG’ as a keyword was searched in Tea Plant Information Archive (TPIA, http://tpia.teaplant.org/index.html) database [52]. Secondly, ‘autophagy’ or ‘ATG’ as a keyword was searched in published transcriptome data [53]. Thirdly, the nucleotide and protein sequences of ATG-related genes in *Arabidopsis* (44), rice (33), banana (31), and grapevine (35), tobacco (29) were retrieved from Phytozome v12.1 database (JGI, https://phytozome.jgi.doe.gov/pz/portal.html), and then they were all used as references to perform Blastn and Blastx against the TPIA database respectively. After removing redundant sequences, all of the retained protein sequences were searched in the NCBI conserved domain database

(https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi)[54] to verify the presence of ATG-related domains. In addition, partial proteins, which have confirmed to be interacted with ATGs, were also identified and analyzed with the same methods in this study.

Bioinformatics analysis of CsARGs in tea plant

The open reading frame (ORF) and potential amino acids were searched using ORF finder web (https://www.ncbi.nlm.nih.gov/orffinder/). Molecular weights and theoretical pI were calculated using the ProtParam tool (http://web.expasy.org/protparam/). Signal peptides and transmembrane regions were predicted using The SignalP 4.1 Server (http://www.cbs.dtu.dk/services/SignalP/) and the TMHMM Server v.2.0 (http://www.cbs.dtu.dk/services/TMHMM/), respectively. Plant-mPLoc (http://www.csbio. sjtu.edu.cn/bioinf/plant-multi/), WoLF PSORT (http:// wolfpsort.org/), TargetP 1.1 Server (http://www.cbs. dtu.dk/services/TargetP/), MitoProt (https://ihg.gsf.de/ihg/mitoprot.html), and YLoc (http://abi.inf.unituebingen.de/Services/YLoc/webloc.cgi) were used to predict the subcellular locations of CsARGs.

Phylogenetic analysis

To explore the evolutionary relationships among the ARG proteins in various plant species, an unrooted phylogenetic tree of the ARGs, identified in *Arabidopsis*, rice, tobacco, and foxtail millet, were constructed. Simply, multiple ARG proteins were aligned together using ClustX2.1 with the default settings. Then, the phylogenetic tree was generated by the neighbor-joining method using MEGA 5.0 with 1000 repeated bootstrap tests, p-distance, and pairwise deletion. Finally, the obtained original tree was managed using ITOL website (https://itol.embl.de/).

Gene structure, protein domain distribution and cis**-acting elements analysis**

The exon-intron structures of *CsARGs* were visualized by GSDS 2.0 website (http://gsds.cbi.pku.edu.cn/), according to comparing the coding sequences (CDS) with their corresponding genomic sequences. Protein domains of CsARGs were performed using SMART online tools (http://smart.embl-heidelberg.de/). 2000 bp up-stream non-coding region sequences of each gene were used to predict *cis*-acting elements involved in responding to stresses and hormone by using PlantCARE software (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

Conserved domains and motifs analysis

Multiple amino acids of ATG8s and ATI originated from different species were used to align conserved domains according to Clustlx2.0 software respectively, and the results were exported using Genedoc software. The conserved domains of CsATG8s were identified using MEME website (http://memesuite.org/) with optimum motif $\geq$ 5 bp and $\leq$ 50 bp and maximum number of motifs 15.

Construction of protein interaction networks

The protein-protein interaction networks (PPINs) were constructed with STRING (https://string-db.org, Ver10.5). Simply, the amino acid sequences of the CsARGs family were uploaded into the Multiple Sequences column, and the *Arabidopsis thaliana* database was used for blast searching. Finally, the highest homologous AtARGs proteins as compared to CsARGs were used to build PPINs.

qRT-PCR analysis

Total RNA isolation and first-strand cDNA synthesis of all samples were performed as described by Qian et al. (2018) [49]. For qRT-PCR analysis, 20 μL reaction volumes including 10 μL SYBR Premix Ex Taq, 1.6 μL forward/reverse primers, 2 μL cDNA and 6.4 μL distilled water, was performed on a Roche 384 real-time PCR machine (Roche). The qRT-PCR program began with 95 °C for 10 min, followed by 45 cycles of 94 °C for 10 s, 60 °C for 15 s and 72 °C for 12 s, thus a melting curve was added finally. *CsPTB* [55] as actin gene was used to quantify the relative expression levels of each *CsARGs* according to the method of $2^{-\Delta Ct}$ or $2^{-\Delta\Delta Ct}$ [56]. Three replications were generated for each RNA sample to quantitative analysis, and the representative data of each *CsARGs* was presented by the mean values ± standard error (± SE). The qRT-PCR primers are listed in Additional file1.

Abbreviations

ATG: Autophagy; ARG:Autophagy-related gene; CA:Cold acclimation; CT:Cold treatment; DT:Drought treatment; ORF:Open reading frame; PI3K:Phosphoinositide 3-kinase; qRT-PCR:quantitative real-time PCR; TOR:Target of rapamycin kinase.

Declarations

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Author Contributions

QW and WX conceived and designed the experiments. QW and WH wrote the original draft. WH and GM performed the experiments. WL, HX, WY and DT sampled the materials. HJ and ZX analyzed the qRT-PCR results and performed figures. WY, DZ and YY reviewed and edited manuscript. All authors read and approved the final manuscript.

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Figures

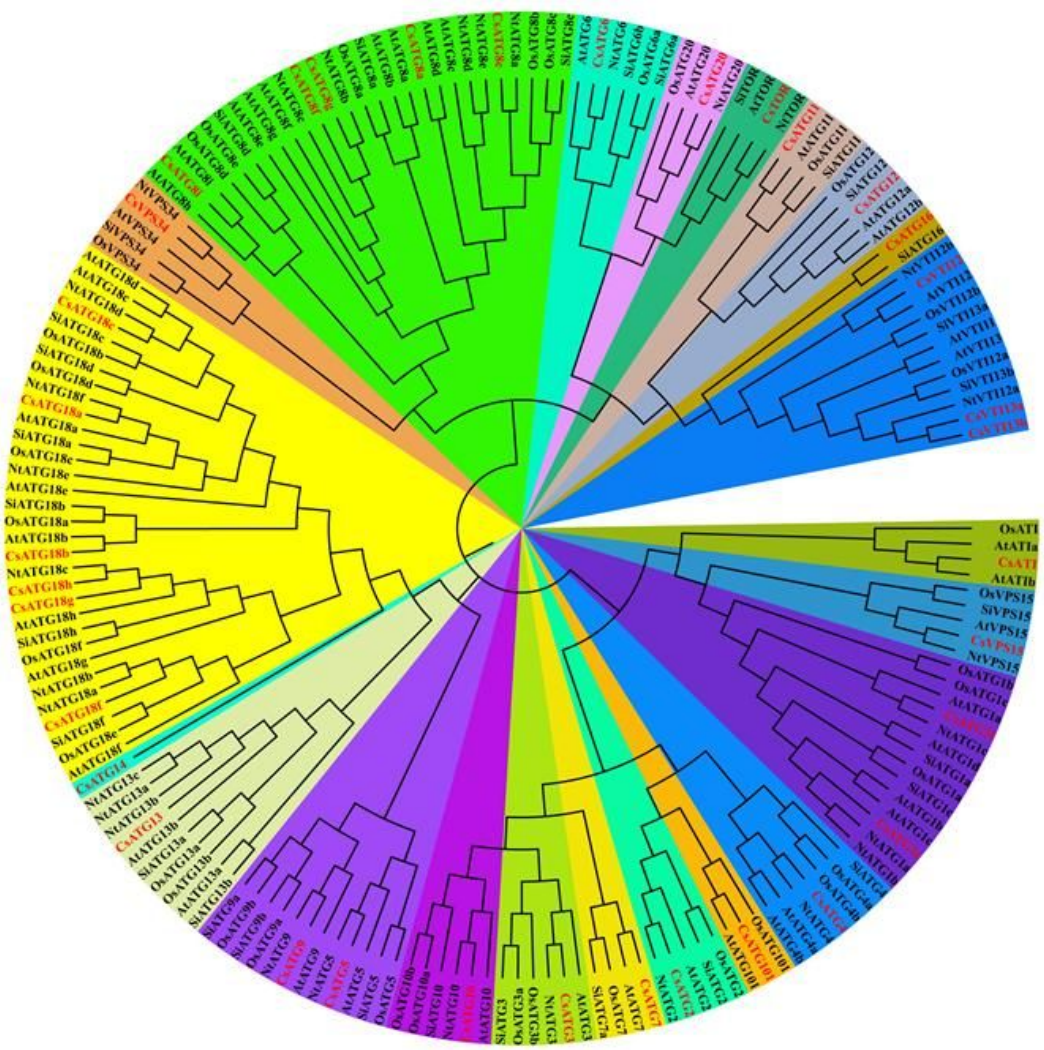

Figure 1

Phylogenetic analysis of CsARGs and known ARGs in Arabidopsis, Setaria italic, Oryza sativa and Nicotiana tabacum. A total of 177 ARG protein sequences were used to construct phylogenetic tree throughout the neighbor-joining method with 1000 repeated bootstrap tests, p-distance, and pairwise deletion in MEGA 5.0 software. CsARGs are highlighted with red color, and different ARG subfamilies were covered with different colors.

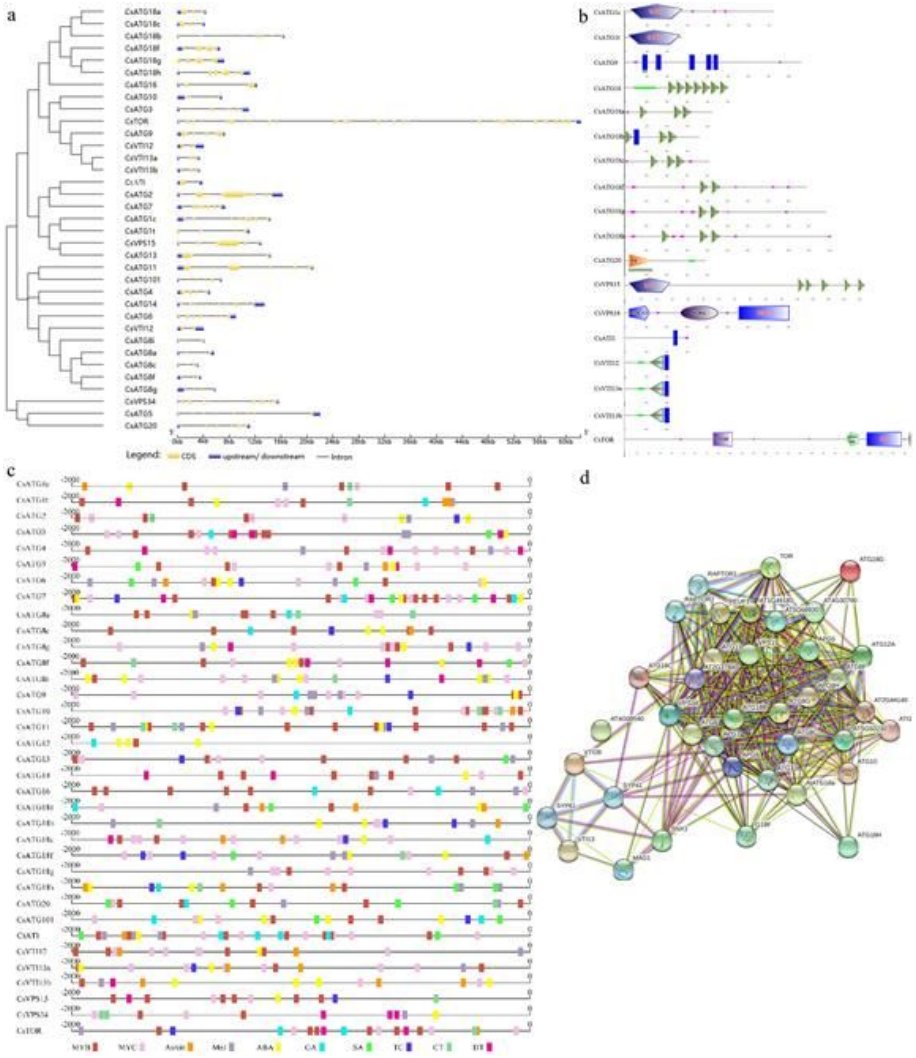

Figure 2

The exon-intron structures, protein domains, cis-acting elements and protein-protein interaction networks of CsARGs. a. Exon-intron structure of CsATG genes. The coding sequence and the corresponding genomic sequence of each CsARG were compared by using the Gene Structure Display Server (GSDS) program. Blue boxes represent untranslated upstream/downstream regions, yellow boxes represent exons, and lines indicate introns. b. Protein domains of CsARGs. c. The cis-acting regulatory elements of CsARGs. 2 000 bp up-stream non-coding region sequences of each CsARG gene was used to predict cis-acting elements, and different colored blocks represent different elements. (D) Protein-protein interaction networks of CsARGs. 34 CsARGs were matched to 33 AtARGs of Arabidopsis, and those 34 CsARG proteins formed 333 protein-protein association patterns.

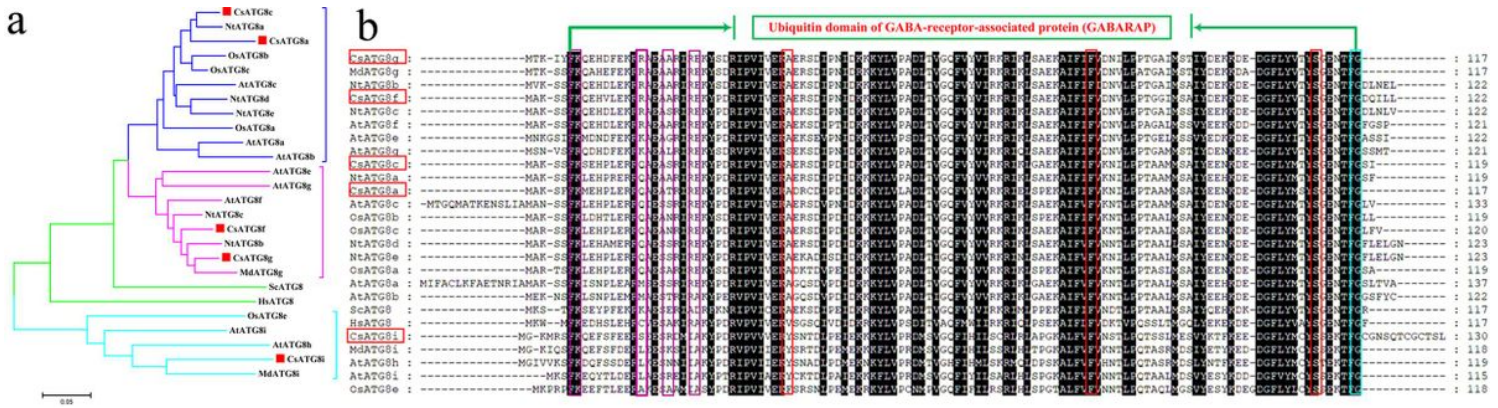

Figure 3

Conserved domains analysis of CsATG8s. a. Phylogenetic analysis of CsATG8s and known ATG8s in Arabidopsis, Oryza sativa, Malus domestica, Saccharomyces cerevisiae and Humans. CsATG8s were highlighted with red boxes. b. Amino acids alignment analysis of CsATG8s and known ATG8s in Arabidopsis, Oryza sativa and Malus domestica. Three putative ATG7 binding sites were contained in the red boxes respectively, four putative tubulin binding sites were contained in the pink boxes may be the respectively, and a conserved glycine (G) residue was framed in the bright blue box.

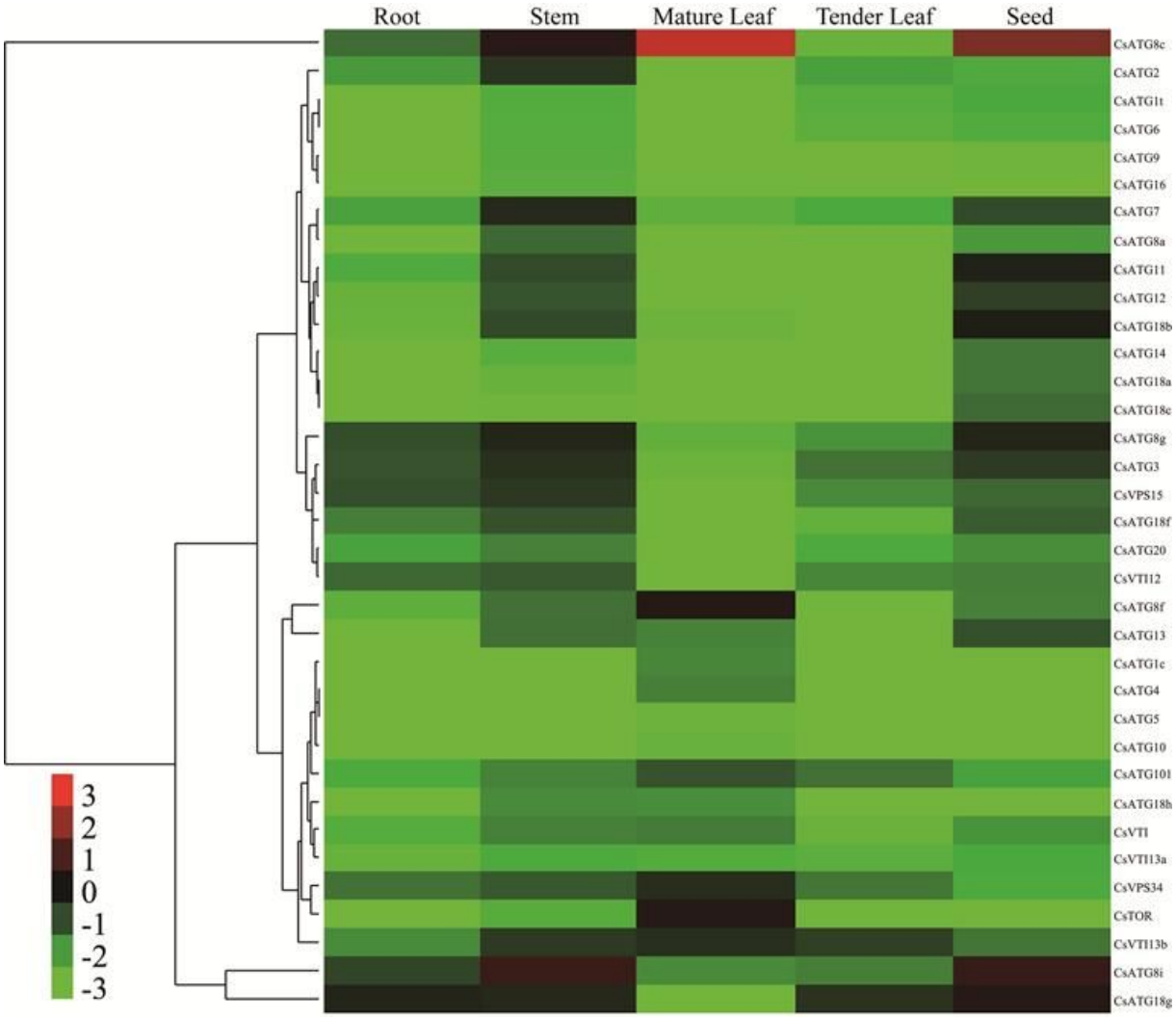

Figure 4

Expression profiles of CsARGs in different tea plant tissues. The transcription abundances of CsARGs in different tissues were monitored by using qRT-PCR technique, and the results were calculated by using 2−ΔCt method. CsPTB was chose as actin gene. The

heat map was generated by using Cluster 3.0 software. The colorbar was displayed on the lower-left of the heat map, red and green colors represent higher and lower expression levels, respectively.

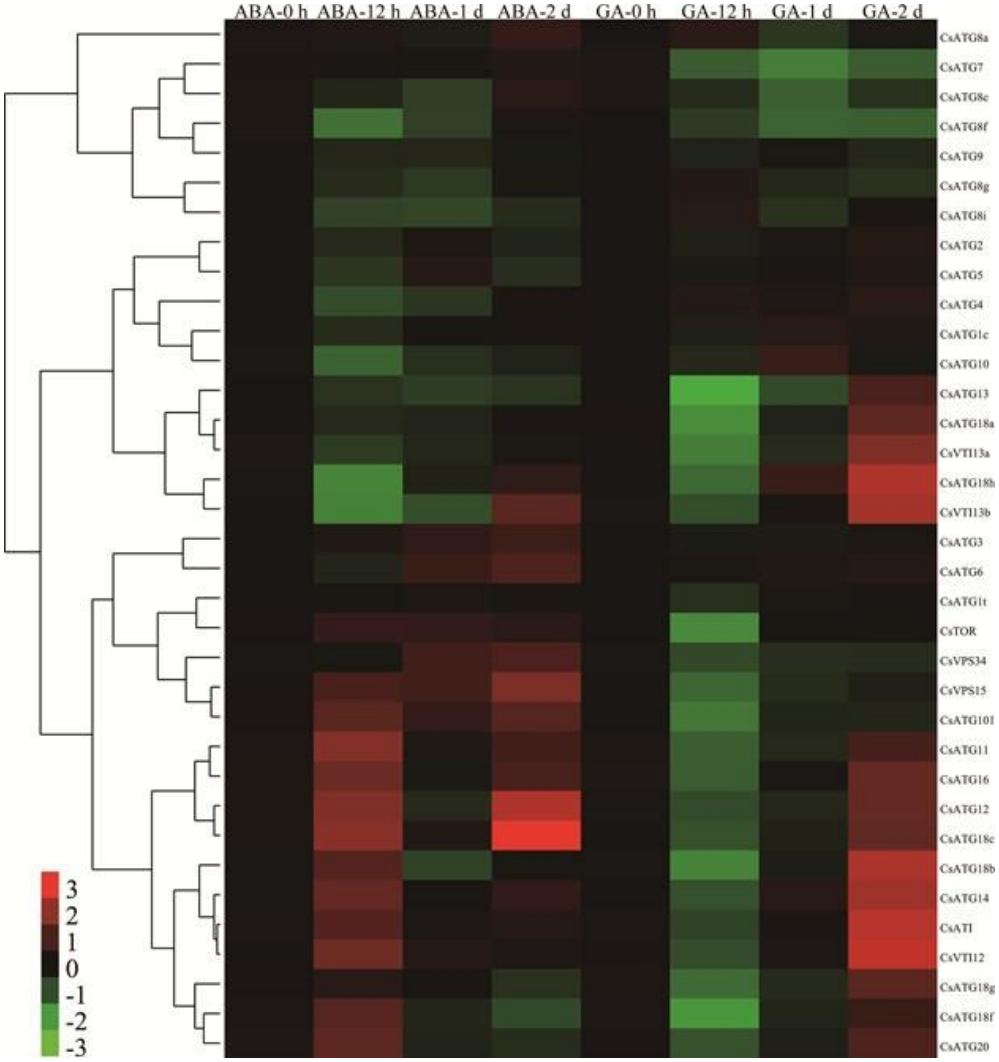

Figure 5

Temporal-spatial expression patterns of CsARGs in response to hormone treatments. The expressions of each CsARG gene within 2 d of ABA and GA treatments were performed by using qRT-PCR technique respectively. CsPTB was chose as actin gene. The final results were calculated with 2−ΔΔCt method, and the samples that collected at 0 h were set as control. The heat map was generated by using Cluster 3.0 software. The colorbar was displayed on the lower-left of the heat map, red and green colors represent higher and lower expression levels, respectively.

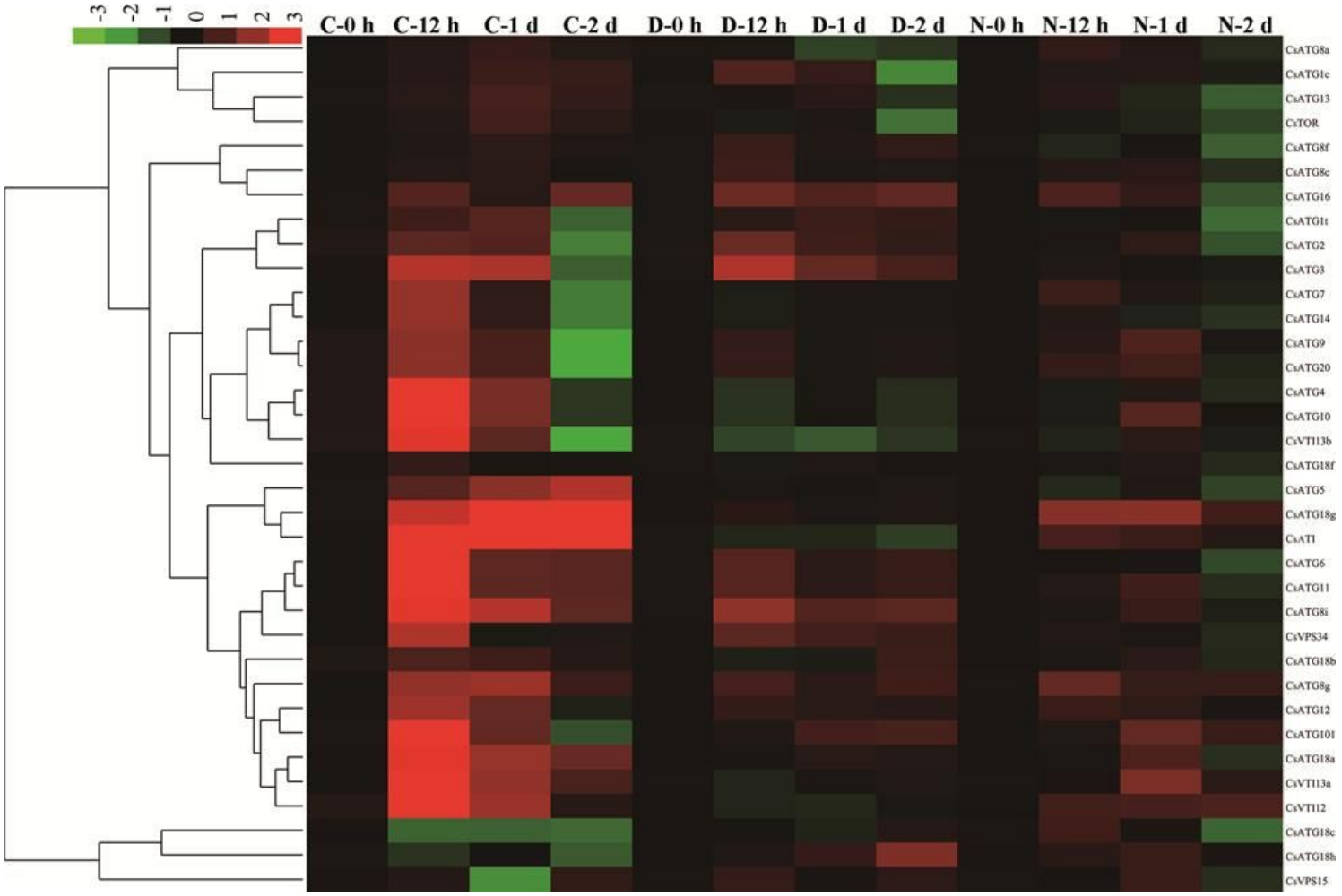

Figure 6

Temporal-spatial expression patterns of CsARGs in tea plant under various abiotic stresses. The expressions of each CsARG gene within 2 d of cold, drought, NaCl treatments were performed by using qRT-PCR technique respectively. CsPTB was chose as actin gene. The final results were calculated with $2-\Delta\Delta Ct$ method, and the samples that collected at 0 h were set as control. The heat map was generated by using Cluster 3.0 software. The colorbar was displayed on the upper- left of the heat map, red and green colors represent higher and lower expression levels, respectively.

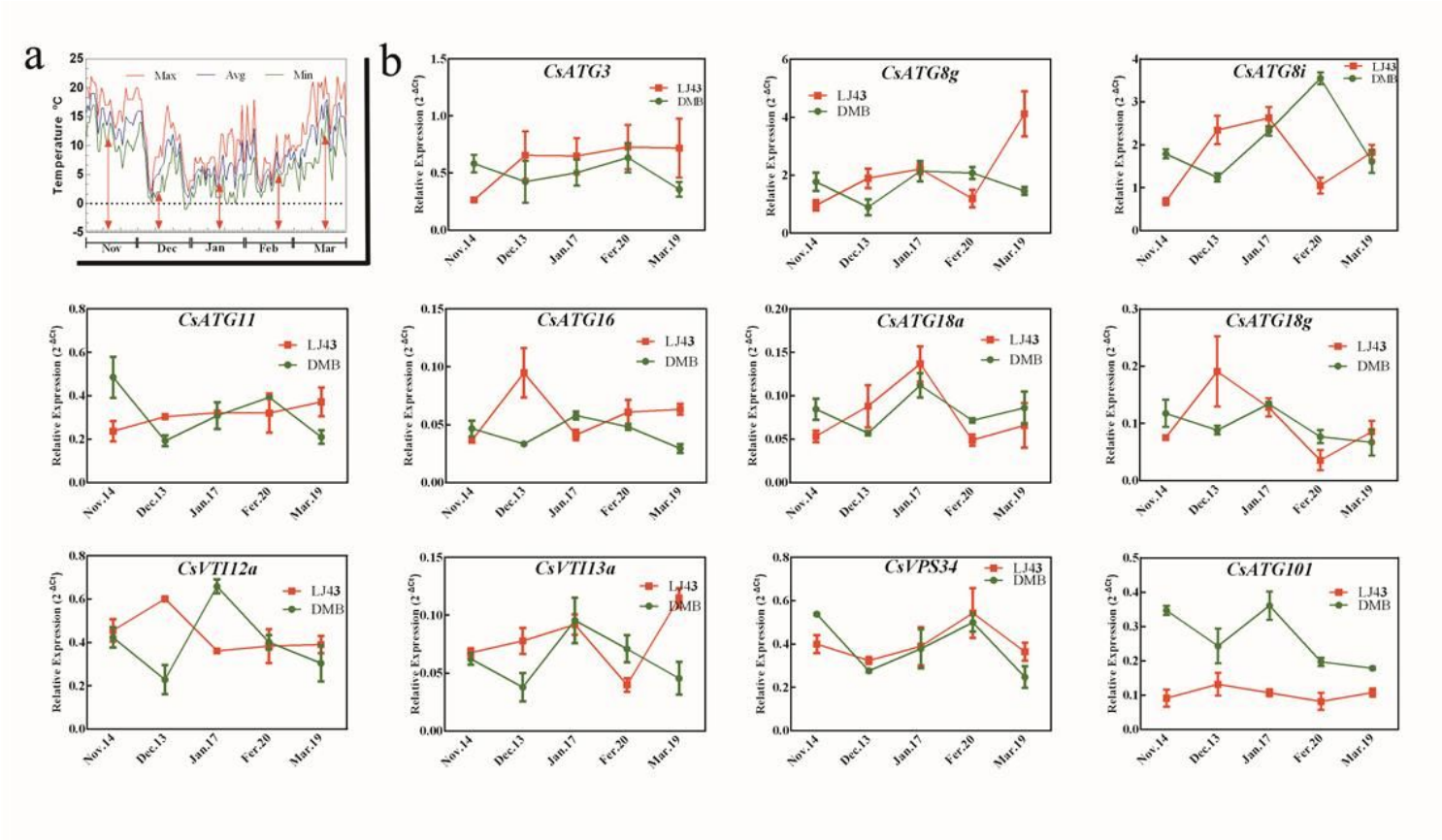

Figure 7

Expression analysis of CsARGs during CA periods in 2018-2019. a. Changes in air temperature from November 2018 to March 2019. The maximum (Max), average (Avg) and minimum (Min) daily temperatures were respectively indicated by red, blue and green colored lines. The red arrows represent sampling days. b. The relative expression levels of 11 CsARGs in two-tea cultivars during CA periods in 2018-2019. The expression profiles of CsARGs in two-tea cultivars were displayed with different colored lines. The results were calculated by using the 2−ΔCt method with CsPTB as actin gene. Data are shown as the means ± SE (n = 3).

Supplementary Files

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