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Genome-wide identification and integrated analysis of gibberellin oxidase genes associated with branch angle and internode elongation in tea plants (*Camellia sinensis*)

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Introduction: Gibberellin oxidases (GAoxs) are key enzymes involved in maintaining gibberellin (GA) homeostasis and therefore play important roles in regulating plant architecture. However, the GAox genes associated with branch angle and internode elongation in tea plants (*Camellia sinensis*) remain largely uncharacterized.

Methods: In this study, 33 CsGAox genes were identified in the genome of the tea cultivar ‘Longjing 43’ and classified into four subfamilies. Comparative genomic analysis across eight *Camellia* genomes was performed, and phenotypic measurements were integrated with endogenous GA quantification, transcriptome profiling, qRT-PCR analysis, and exogenous GA₃ treatment.

Results: Whole-genome or segmental duplication contributed substantially to the expansion of the CsGAox family, with duplicated gene pairs in ‘Longjing 43’ predominantly undergoing purifying selection. The levels of GA₁, GA₉, and GA₂₀ were higher in the lateral buds of the draped cultivar than in those of the upright cultivars and were positively correlated with branch angle. CsGA20ox4.3 expression was positively associated with branch angle and the levels of those GAs, whereas CsGA2ox5.3 exhibited the opposite relationships. Exogenous GA₃ application significantly increased branch angle and was accompanied by the upregulation of CsGA20ox4.3 and CsGA20ox.un3 and the downregulation of CsGA2ox5.3. Integrated analyses across cultivars and developmental stages further showed that CsGA20ox4.3 and CsGA3ox3.3 were positively associated with internode elongation, whereas CsGA2ox1.1, CsGA2ox5.2, and CsGA2ox.un1 generally exhibited negative associations.

Discussion: These findings identify candidate CsGAox genes associated with branch angle and internode elongation, providing a basis for future functional studies and further evaluation in tea plant architecture breeding.

KEYWORDS

branch angle, gene family, gene pair, gibberellin, internode length, mechanical picking, new shoot, tea

1 Introduction

The tea plant (*Camellia sinensis*) is a perennial woody cash crop cultivated worldwide, and the manual harvesting of fresh tea leaves is the most labor-intensive work in tea production (Sun et al., 2018). With the increasingly severe problems of high labor costs and labor shortages, mechanical picking is an inevitable option for the tea industry in the future (Luo et al., 2023). Tree architecture is vital for tea mechanical harvesting. The branch angle and internode length in tea new shoots are two key traits that determine the suitability of tea plants for mechanical picking. However, the related genes and molecular mechanisms regulating tree architecture remain largely unknown.

Plant hormone gibberellins (GAs) play major roles in regulating plant branch angle and internode length (Liu et al., 2024; Dutt et al., 2022; Li Y. et al., 2021; Honi et al., 2020). For example, *OsGRF7* overexpression causes a narrower tiller angle through regulating the expression of the GA-biosynthetic gene *cytochrome P450* in rice (Zhu et al., 2025). Modification of GA signaling via DELLA proteins produces a narrower branch angle. *TAC1*-edited plants increased the branch angle through increasing GA₇ level in citrus leaves (Dutt et al., 2022). On the other hand, exogenous GA application promoted internode elongation of tea plants (Li W. et al., 2021), jute (Honi et al., 2020), and deep-water rice (Davière et al., 2014). Endogenous GA₃ levels significantly increase during new shoot elongation (Li W. et al., 2021) and are strongly associated with internode length of tea plants (Luo et al., 2023). Mutants defective in GA biosynthesis or perception typically have shorter stems (Davière et al., 2014). Compared with the tall cultivar, the decreased expression of GA biosynthetic genes resulted in a greater decrease in the accumulation of active GA₁ in the dwarf cultivar of *C. purpureus* (Yan et al., 2021). Similarly, the tea cultivars with longer internodes presented higher levels of GA₁ and GA₃, and a highly significant correlation was observed between GA₃ levels and internode length (Luo et al., 2023).

Bioactive GAs are signaling molecules essential for plant growth and development (Lange et al., 2020). The major bioactive GAs are GA₁, GA₃, GA₄, and GA₇, while the others are the precursors or deactivated forms of bioactive GAs (Luo et al., 2023). Gibberellin homeostasis is achieved via complex biosynthetic and catabolic pathways (Lange et al., 2020). Gibberellin oxidase (GAox) enzymes, including GA 20-oxidase (GA20ox), GA 3-oxidase (GA3ox), and GA 2-oxidase (GA2ox), belong to the 2-oxoglutarate-dependent dioxygenases (2-ODDs) family and catalyze the last steps of the GA metabolic pathway in higher plants (Pan et al., 2017; Hu et al., 2021; Lange et al., 2020). GA20ox and GA3ox enzymes are anabolic enzymes of bioactive GAs (Lange et al., 2020; He et al., 2019; Sun et al., 2023), whereas GA2oxs are catabolic enzymes (Li Y. et al., 2021; Hu et al., 2021; Lange et al., 2020; Ren et al., 2024).

Specifically, GA20ox enzymes catalyze successive oxidation at C-20, converting inactive GA₁₂ and GA₅₃ into GA₉ and GA₂₀, respectively (Lange et al., 2020; He et al., 2019; Sun et al., 2023). In the final step of bioactive GA formation, GA3ox enzymes catalyze the 3 β -hydroxylation of GA₂₀ and GA₉ to produce bioactive GA₁ and GA₄, respectively (Hu et al., 2021; He et al., 2019; Plackett et al., 2012; Song et al., 2011). GA2ox enzymes include two major groups, C₁₉-GA2ox and C₂₀-GA2ox, which act on C₁₉-GAs and C₂₀-GAs, respectively (Plackett et al., 2012). Through 2 β -hydroxylation, C₁₉-GA2ox enzymes inactivate C₁₉-GAs (GA₁ and GA₄) as well as their immediate precursors (GA₂₀ and GA₉), producing inactive GA₃₄, GA₈, GA₅₁, and GA₂₉, while C₂₀-GA2ox enzymes oxidize C₂₀-GAs (GA₁₂ and GA₅₃) to inactive GA₁₁₀ and GA₉₇ (Pan et al., 2017; Plackett et al., 2012; Song et al., 2011).

Previous studies showed that GAoxs regulate plant angle at the transcriptional level. For example, the asymmetric expression of *OsGA3ox1* regulated the gravitropic growth of rice leaf sheath through triggering cell wall loosening (Zhu et al., 2025). GA2ox overexpression increased the branch angle by reducing the levels of bioactive GAs (Dutt et al., 2022). On the other hand, GAoxs also play key regulatory roles in plant internode elongation. For example, *AtGA20ox1* and *AtGA20ox2* act redundantly to promote internode elongation (Fukazawa et al., 2017). Mutations in *GA20ox* or *GA3ox* genes generated dwarf or semi-dwarf phenotypes in *Arabidopsis*, *Zea mays*, and rice (Sun et al., 2023; He et al., 2025). The *ga20ox1*, *ga20ox2*, and *ga20ox3* mutants all showed a severe dwarfing phenotype (Plackett et al., 2012). Overexpression of *GA2ox* genes results in a dwarf phenotype in *Arabidopsis*, *Jatropha*, and *C. lipoensis* (Honi et al., 2020; He et al., 2019; Ren et al., 2024), while silencing of the *GA2ox* gene in transgenic tobacco leads to increased plant height and xylem cell number (Honi et al., 2020).

After the release of the genome of tea cultivar Yunkang10 (YK10), Pan et al. performed a genome-wide study of the CsGAox gene family in YK10 (Pan et al., 2017). At present, multiple *Camellia* genomes have been released, including those of the tea cultivars Biyun (BY), Huangdan (HD), Shuchazao (SCZ), Tieguanyin (TGY), LJ43, Yunkang10 (YK10), the related species *C. oleifera* (COL), *C. chekiangoleosa* (CCH), and the wild tea plant DASZ. Given the important roles of GAox genes in GA metabolism and plant development, a genome-wide survey of the CsGAox gene family was conducted using eight *Camellia* genomes. The bioinformatic characteristics of CsGAox genes in LJ43 and their associations with branch angle and internode length in machine-picked tea plants were investigated. Furthermore, to gain insights into the evolutionary diversity of CsGAox genes, the syntenic relationships and gene duplication patterns between LJ43 and the other seven genomes were comparatively analyzed.

In this study, 33 CsGAox genes were identified from the genome of tea cv. 'LJ43' and classified into four subfamilies based on phylogenetic analysis, gene structure, and conserved motifs. Synteny analysis indicated that whole-genome duplication (WGD) or segmental duplication (SD) events played major roles in the expansion of CsGAox members. Comparative syntenic analysis combined with phylogenetic analysis provided deep insight into the phylogenetic relationships of CsGAox genes. Integrated analyses of phenotypes, GA contents, and gene expression levels indicated CsGA20ox4.3, CsGA20ox.un3, and CsGA20ox5.3 as candidate genes associated with branch angle, while CsGA20ox4.3, CsGA3ox3.3, CsGA20ox1.1, CsGA20ox5.2, and CsGA20ox.un1 were identified as candidate genes associated with internode elongation in tea plants. The expression responses of CsGAox genes to exogenous GA₃ treatment further supported the association of the above three candidate genes with GA-responsive branch angle formation. Meanwhile, exogenous GA₃ treatment altered the expression of several CsGAox genes, consistent with transcriptional feedback responses in GA metabolism. Taken together, this study identified candidate CsGAox genes associated with tea plant architecture, providing candidates for future functional studies and further evaluation in tea plant architecture breeding for mechanical harvesting.

2 Materials and methods

2.1 Identification of GAox family members in tea plants

To identify CsGAox family genes in the tea plant genome, a BLASTP search was performed using the sequences of 16 *Arabidopsis* AtGAox proteins as queries against eight *Camellia* genome databases (e-value $\leq 1 \times 10^{-5}$) (<https://tpia.teaplants.cn/index.html#>). Furthermore, the Hidden Markov Model (HMM) profiles of the 2OG-FeII_Oxy (PF03171) and DIOX_N (PF14226) domains were obtained from the Pfam database (<http://pfam.janelia.org>). Candidate proteins containing both domains were identified using HMMER v.3.2 with an E-value cutoff of 1×10^{-10} (Sun et al., 2018; Ren et al., 2024). The putative CsGAox genes were further validated by the NCBI Conserved Domain Database (CDD) and online Simple Modular Architecture Research Tool (SMART) (<http://smart.embl.de>). All of the 33 putative CsGAox genes from tea cv. LJ43 were shown in Supplementary Table 1; the sequences of the 33 CsGAox and 16 *Arabidopsis* AtGAox proteins were listed in Supplementary Table 2. For the eight *Camellia* genomes, the numbers of identified CsGAox genes were listed in Supplementary Table 3.

2.2 Physicochemical property analysis of LJ43 CsGAox proteins

The physicochemical properties, including amino acid (AA) number, isoelectric point (pI), molecular weight (MW), grand average of hydropathicity (GRAVY), instability index, and aliphatic index, were predicted using the ExPASy website tool (<https://www.expasy.org/>).

The subcellular localization was predicted using DeepLoc-2.0.

2.3 Multiple sequence alignment, phylogenetic analysis, conserved motif analysis, and gene structure analysis of LJ43 CsGAox proteins

The amino acid sequences of 33 putative CsGAox proteins from LJ43 and 16 AtGAox proteins from *Arabidopsis thaliana* were aligned using MUSCLE implemented in TBtools. The multiple sequence alignment was visualized using Jalview. A maximum-likelihood (ML) phylogenetic tree was constructed using MEGA 7.0 under the Poisson amino acid substitution model with 1,000 bootstrap replicates. No separate model-selection procedure was performed. The resulting phylogenetic tree was visualized using FigTree. The conserved motifs were predicted using the MEME online software (<http://meme-suite.org/tools/meme>) (maximum motif = 10, width = 6–50). The gene structures (exon and intron) were analyzed and visualized using the online tool Gene Structure Display Server (GSDS v.2.0, <http://gsds.cbi.pku.edu.cn/>). The gene structure information of 27 LJ43 CsGAox genes located on chromosomes was also listed in Supplementary Table 3.

2.4 Chromosome mapping, gene duplication analysis, and selective pressure analysis

The chromosomal location maps of CsGAox genes were constructed based on the genome annotation file of eight *Camellia* genomes using TBtools. The duplication events of CsGAox genes, including the gene collinearity relationships, were analyzed using MCScanX (<https://chibba.agtec.uga.edu/mcscanx-web>) and visualized with TBtools. Tandem duplication events were determined according to the following two conditions: (1) the relatively constant short sequences that are more than 70% of the length of the long sequences; (2) the similarity of two aligned sequences is greater than 70%. Tandemly duplicated genes were defined as homologous genes located within a 100-kb region on the same chromosome and separated by fewer than five intervening genes (Jiao et al., 2024). The synonymous (*Ks*) and non-synonymous (*Ka*) values as well as the *Ka/Ks* ratios for each gene pair were calculated using the KaKs Calculator 2.0 (<https://sourceforge.net/projects/kakscalculator2/>).

2.5 Cis-acting element analysis

The promoter sequences (1,000 bp) of 33 CsGAox genes in LJ43 were extracted using TBtools, and the promoter elements were predicted and analyzed using the PlantCARE online tool (<http://bioinformatics.psb.ugent.be/>).

2.6 Gene expression analysis of CsGAox genes associated with tea plant architecture

To investigate the associations between CsGAox gene expression and tea plant architecture, we analyzed their FPKM values

using transcriptome datasets from our research group and a publicly available dataset. Our own RNA-seq results were further verified by quantitative real-time PCR (qRT-PCR).

2.7 Gene expression analysis of CsGAox genes associated with branch angle formation in tea plants

Two upright tea cultivars (*C. sinensis* L.), CT2009-0025 (upright tea 1, UT1) and CT2013-0114 (UT2), and one draped tea cultivar, CT2009-0160 (DT), were selected as experimental materials. The draped cv. DT was defined as the control. The branch angle of the three cultivars can be seen in [Supplementary Figure 2](#), and the cultivation conditions, decapitation treatment, sampling, and measurement of branch angles were shown in Section 4.1 of our recent study. The lateral buds were sampled for transcriptome sequencing, qRT-PCR testing, and hormone measurement (Zhu et al., 2025). As shown in Section 4.6 of the recent study, exogenous GA₃ treatment was conducted using the upright tea cv. Zhongcha 108 (ZC108). The cultivation conditions and pruning treatment were described in the same study (Zhu et al., 2025). GA₃ solutions at concentrations of 0.5, 5, 50, and 100 mg/L were sprayed onto the tea plants, with an equal volume of pure water serving as the control. On the 20th day after GA₃ spraying, the branch angle between the shoot stem and the first lateral bud was measured. The lateral buds were sampled for hormone measurement and qRT-PCR testing (Zhu et al., 2025).

Here, the FPKM values of CsGAox genes were analyzed based on these transcriptome data. Total RNA was extracted using the CTAB method. The extracted RNA was treated with DNase I (Invitrogen, CA, USA). The integrity, concentration, and purity of all the RNA samples were analyzed and monitored. The reverse transcription was carried out using a PrimeScriptTM RT-PCR Kit (RR047A, TaKaRa, China), and cDNA was diluted 10-fold. The gene-specific primers were designed using Primer 5.0, and the primer sequences and T_m were listed in [Table 1](#). CsGAPDH was selected as the reference gene. The qRT-PCR assay was carried out using an ABI 7500 Real-Time PCR System (Applied Biosystems,

Foster City, CA, USA) with a SYBR Green I Master kit (Roche, Basel, Switzerland). Each reaction was performed in a total volume of 20 μL, with each forward and reverse primer at a final concentration of 0.2 μM. The thermal cycling program consisted of an initial denaturation at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 30 s for annealing and extension. Three independent biological replicates were analyzed for each sample. The relative expression levels of CsGAox genes were calculated using the 2^{-ΔΔCT} method.

2.8 Gene expression analysis of CsGAox genes associated with internode elongation in tea plants

Mature plants of two tea cultivars, Longjing 43 (LJ43) and Zhongcha 102 (ZC102), were used in this study. These two cultivars have significantly different internode lengths, and both were selectively bred from the Longjing population. Therefore, the two cultivars have a similar genetic background. The cv. LJ43, with a longer internode, was defined as the control. The tea plants were lightly pruned after spring tea picking. At the 2-, 3-, and 5-leaf stages, the internodes in the new shoots on the first node emerging just below the trimmed cuts were selected as research objects. The internode length of each node was measured, respectively, and the average internode length was calculated. The samples of the mixed internodes were collected for transcriptome sequencing and measurement of GA contents (Zhang et al., 2026). Here, the FPKM values of CsGAox genes were analyzed based on these transcriptome data. Total RNA extraction, reverse transcription, and qRT-PCR assay protocols were the same as described in Section 2.7.

In a previous report, transcriptome sequencing was conducted to study the regulatory mechanism of internode length in three tea cultivars, LJ43, Qiancha 1 (QC1), and Feiyan (FY), whose internode lengths increased successively. The cv. LJ43 was defined as the control. When the tea plants reached the stage of one bud and two leaves, the internode length was measured, and the stem tissues were sampled for transcriptome sequencing. Here, the raw data of

TABLE 1 Primers of CsGAox genes used for qRT-PCR.

Gene names	Forward primer sequence (5'–3')	Reverse primer sequence (5'–3')	T _m (°C)
CsGA20ox4.4	GAGCCAATCGTCCGAGTCCA	CGGTCTCTGGTGGCGATTCT	59.0, 59.0
CsGA20ox4.1	GGGTGAGAACTGTCCGACT	GCTCGGTTCTCTCCGAACA	58.3, 58.9
CsGA20ox5.3	CCGAGATTGATGGCGGAGAT	GAGCACCAGGGACACAGGAG	59.1, 59.2
CsGA3ox15	CTCGATCGCCAGAGGGTGTT	GGGCCAAAGTTGGCAAGCAT	59.0, 59.1
CsGA20ox4.3	ACCGAAGCCTGTGAGACCTG	CGACTCGATTGCTCGGCTCT	58.9, 59.5
CsGA20ox7.3	TGAAGGTGTTCAAGAGGCCGTT	CAAGTCGCCGACGGTGTTTC	59.1, 59.0
CsGA20ox.un3	GCAAAGAACCTGGCCTCACC	TCGAGGCCTCCAACCTCGTC	58.6, 58.9
CsGA20ox1.1	CCGAGTCGACCGAGTTGTCA	GTTACCCGGAGACCCTTCCG	59.0, 59.0
CsGA20ox5.2	CGATGCGAGCCAAGAATGGG	TACCCACTTCCGGCGATGAC	58.9, 59.0
CsGA20ox5.4	CCGACACGAAGACCCTCCTC	TGAGTTCTGTGGGACGCCAT	59.0, 58.8
CsGA20ox.un1	AACCTGCAAGCTCACCACCA	AGTTGGGACGCCATGGTTGA	59.3, 59.1
CsGA3ox3.3	TAGCTGCTCACACGACTCG	GGGTTGTGGAGAACCGTGA	59.0, 59.1
CsGAPDH	GAGACTGGAGCCGAATTCATT	GATCTGGCTTGAATCCTTCTCA	59.0, 59.0

the internode length in the three cultivars were downloaded from the previous study (Mi et al., 2025). The raw transcriptome data were downloaded from the National Genomics Data Center (<https://ngdc.cncb.ac.cn>, PRJCA036838) and analyzed. The reads in the downloaded transcriptome data were mapped back onto the sequences of representative transcripts in LJ43 using the 'Kallisto Super GUI Wrapper' Plugin in TBtools. The FPKM values of all the representative transcripts in LJ43 were obtained, and the FPKM values of CsGAox genes were extracted subsequently.

2.9 Determination of GA contents

The measurement of endogenous GA contents was carried out by Nanjing Ruiyuan Biotechnology Co., Ltd., China. Three independent biological replicates were analyzed for each cultivar or treatment. Briefly, 50 mg of fresh lateral buds and 1.0 g of fresh internode samples were collected for the branch-angle and internode-length experiments, respectively, and ground in liquid nitrogen. Fifty microliters of an internal-standard solution containing isotope-labeled GA standards and 1 mL of a water-acetonitrile solution containing 1% formic acid were added sequentially. The samples were vortex-mixed, extracted, and centrifuged. The supernatant (800 μ L) was collected and dried under a stream of nitrogen gas. The dried extracts were reconstituted in 100 μ L of water-acetonitrile (1:1, v/v), followed by centrifugation. GA contents were determined using an Agilent 1290 Infinity UHPLC system (Agilent Technologies) coupled to a 5500 QTRAP mass spectrometer (AB SCIEX). GA contents were quantified according to the validated UHPLC-MS/MS quantification procedure of Nanjing Ruiyuan Biotechnology Co., Ltd.

2.10 Statistical analysis

All data were analyzed using the SPSS 21.0 (SPSS, Inc., Chicago, IL, USA) software. Quantitative data were presented as mean $\pm$ SE. The significance of differences between control and treatment groups was assessed by Student's *t*-test. Differences among the three branch-angle cultivars DT, UT1, and UT2, among all gene-treatment combinations in the exogenous GA₃ experiment, among the six cultivar-developmental stage combinations in LJ43 and ZC102, and among the three tea cultivars LJ43, QC1, and FY were analyzed by one-way ANOVA followed by Duncan's multiple range test. Differences were considered significant at $P < 0.05$.

3 Results

3.1 Genome-wide identification and bioinformatics analysis of CsGAox gene family in tea plants

To identify CsGAox family genes, a BLASTP search of nine *Camellia* genome databases was conducted using the sequences of *Arabidopsis* AtGAox proteins as queries (Supplementary Table 2).

Because representative transcripts could not be extracted from the DASZ genome, CsGAox genes were not identified from DASZ. The CsGAox members were identified after manual screening, and the obtained protein sequences were further aligned to the Swiss-Prot database. Subsequently, the protein sequences containing 'DIOX_N (PF14226) and 2OG-FeII_Oxy (PF03171)' domains were screened from all the proteins of tea plants using the HMMER method. Based on the intersection of the BLASTP and HMMER search results, a total of 33, 42, 45, 17, 50, 42, 53, and 35 putative candidate CsGAox proteins were retained in LJ43, SCZ, BY, YK10, TGY, COL, CCH, and HD, respectively (Supplementary Table 3). Furthermore, two CsGAox proteins (ChaUn1649.3 and Cha03g006450) obtained from LJ43 in our recent study (Zhu et al., 2025), 17 CsGAox proteins identified from YK10 by Pan et al. (2017) (Supplementary Table 3), as well as the CsGAox proteins identified from the other seven genomes, were all aligned to the LJ43 genome. Overall, a total of 33 unique CsGAox proteins were obtained from the LJ43 genome (Supplementary Table 1) and used for further analysis here.

Based on the genome data of LJ43, the genetic mapping of CsGAox genes on the 15 chromosomes was investigated. Among 33 putative CsGAox genes, 27 genes were found to be unevenly located on twelve chromosomes, and six CsGAox genes were not assembled onto the chromosomes. The number of CsGAox genes on each chromosome ranged from one to five. Specifically, only one CsGAox gene was located on chromosomes 6, 9, 10, 11, 14, and 15, respectively. Two CsGAox genes were on chromosome 1. Three CsGAox genes were on chromosomes 3 and 7, respectively. Four CsGAox genes were on chromosomes 4 and 8, respectively. Five CsGAox genes were on chromosome 5 (Supplementary Figure 1).

To further investigate the evolutionary relationships of the CsGAox family genes, the ML phylogenetic tree was constructed using the sequences of 33 CsGAox proteins from LJ43 together with 16 AtGAox proteins from *Arabidopsis*. According to the four AtGAox subfamilies in *Arabidopsis*, the CsGAox proteins in LJ43 were clustered into four subfamilies, namely CsGA20ox, CsGA3ox, C₁₉-CsGA2ox, and C₂₀-CsGA2ox. Specifically, the CsGA20ox subfamily contained four CsGA20ox proteins with five *Arabidopsis* members (I), the GA3ox subfamily contained eight CsGA3ox proteins with four AtGA3ox members (II), the C₂₀-GA2ox subfamily contained six C₂₀-CsGA2ox proteins with two C₂₀-AtGA2ox members (III), and the C₁₉-GA2ox subfamily contained 15 C₁₉-CsGA2ox proteins with five *Arabidopsis* members (IV), respectively (Figure 1; Supplementary Table 3). The gene structure information of the 27 CsGAox genes that were located on chromosomes can be seen in Supplementary Table 3. These classifications were further supported by gene structure and conserved motif analyses. The CsGAox genes were named based on their chromosomal locations and the subfamilies to which they belong. The identified CsGAox genes for which loci could not be determined were named as CsGAox.un genes. Thus, the names of the 33 CsGAox genes in LJ43 were determined, which represent the location on the chromosome and their subfamilies. The numbers of CsGAox genes identified from the eight *Camellia* genomes are shown in Supplementary Table 3.

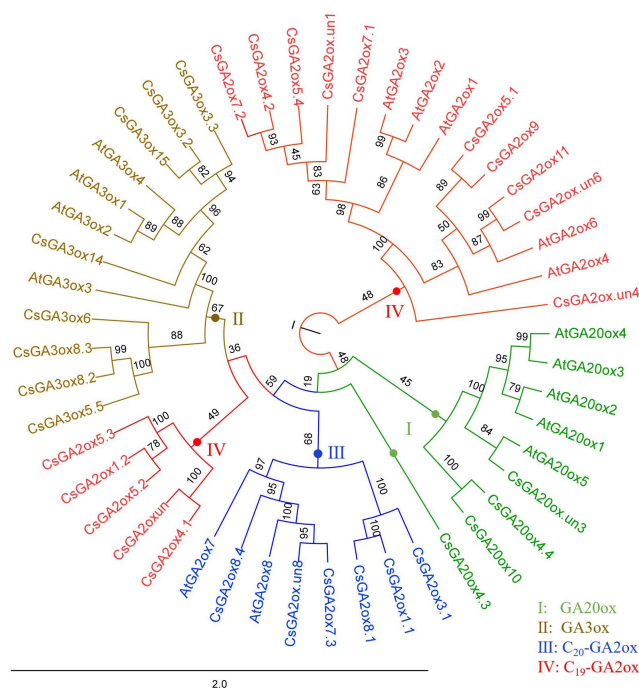


FIGURE 1

Phylogenetic relationships of CsGAox proteins from tea cv. LJ43 and AtGAox proteins from *Arabidopsis thaliana*. The maximum-likelihood (ML) tree was constructed using MEGA 7.0 under the Poisson amino acid substitution model with 1,000 bootstrap replicates and visualized using FigTree. The four subfamilies, GA20ox, GA3ox, C20-GA2ox, and C19-GA2ox, are indicated by different colors.

3.2 Physicochemical property analysis of CsGAox proteins in LJ43

The physicochemical properties were predicted. The detailed information and the physicochemical properties of the putative CsGAox proteins in LJ43 were listed in [Supplementary Table 1](#). The CDS lengths ranged from 351 bp (*CsGA2ox7.2*) to 1326 bp (*CsGA3ox15*), encoding proteins ranging from 116 to 441 AA. The expected MW ranged from 13.40 to 49.11 kDa. The theoretical pI showed a wide range, varying from 4.76 (*CsGA2ox1.1*) to 9.92 (*CsGA3ox3.3*). Among them, 26 are acidic and seven are basic in nature. The GRAVY values of the CsGAox proteins ranged from -0.626 to -0.117, indicating that they are hydrophilic in nature. Twelve CsGAox proteins had instability indices below 40, indicating that they were relatively stable. However, the instability indices of the other 21 CsGAox proteins exceeded 40 (40.03–56.25), indicating that they were unstable.

The subcellular localization of the proteins could represent their variable functions. It was predicted that *CsGA2ox7.3* might be located in the cytoplasm, nucleus and plastid, while *CsGA20ox.un3*, *CsGA3ox3.2*, as well as *CsGA2ox11*, *CsGA2ox.un1*, *CsGA2ox.un6*, and *CsGA2ox3.1* might be located in the nucleus. Except for the above seven CsGAox proteins, the remaining CsGAox proteins were all localized in the cytoplasm and nucleus ([Supplementary Table 4](#)). It should be noted that 23 CsGA2ox proteins had nuclear localization signals, *CsGA3ox8.2* had a peroxisomal targeting signal, while another nine CsGAox proteins had no localization signals.

3.3 Multiple sequence alignment and phylogenetic analysis of CsGAox family proteins in LJ43

Multiple alignment analysis was performed to investigate the homologous domain sequence features of 33 CsGAox proteins in LJ43. The results showed that two conserved domains, 2OG-FeII_Oxy (PF03171) and DIOX_N (PF14226), were common in all 33 CsGAox sequences. The proposed consensus sequence NXYPXC of 2-ODDs was also highly conserved in all CsGAox members. Four D residues were identified, and it was found that the second D residue only existed in the sequences of three C₁₉-GA2ox proteins (*CsGA2ox7.1*, *CsGA2ox.un1*, and *CsGA2ox5.4*). Three H residues were conserved in all putative CsGAox proteins. On the other hand, three R residues and four S residues were identified in the sequences of CsGAox proteins ([Figure 2](#)).

3.4 Phylogenetic, conserved motifs, and gene structure analyses of CsGAox proteins in LJ43

A phylogenetic tree was constructed, and ten conserved motifs were identified among the 33 putative CsGAox proteins of LJ43. First, the CsGA20ox subfamily (subgroup I) contained four members, of which *CsGA20ox10* and *CsGA20ox4.4* each contained nine motifs (motifs 2, 8, 10, 7, 3, 4, 1, 6, and 5). Compared with the above two CsGA20ox proteins, *CsGA20ox4.3* and *CsGA20ox.un3* lack one (motif 8) and two motifs (motifs 6 and 5), respectively. Firstly, it

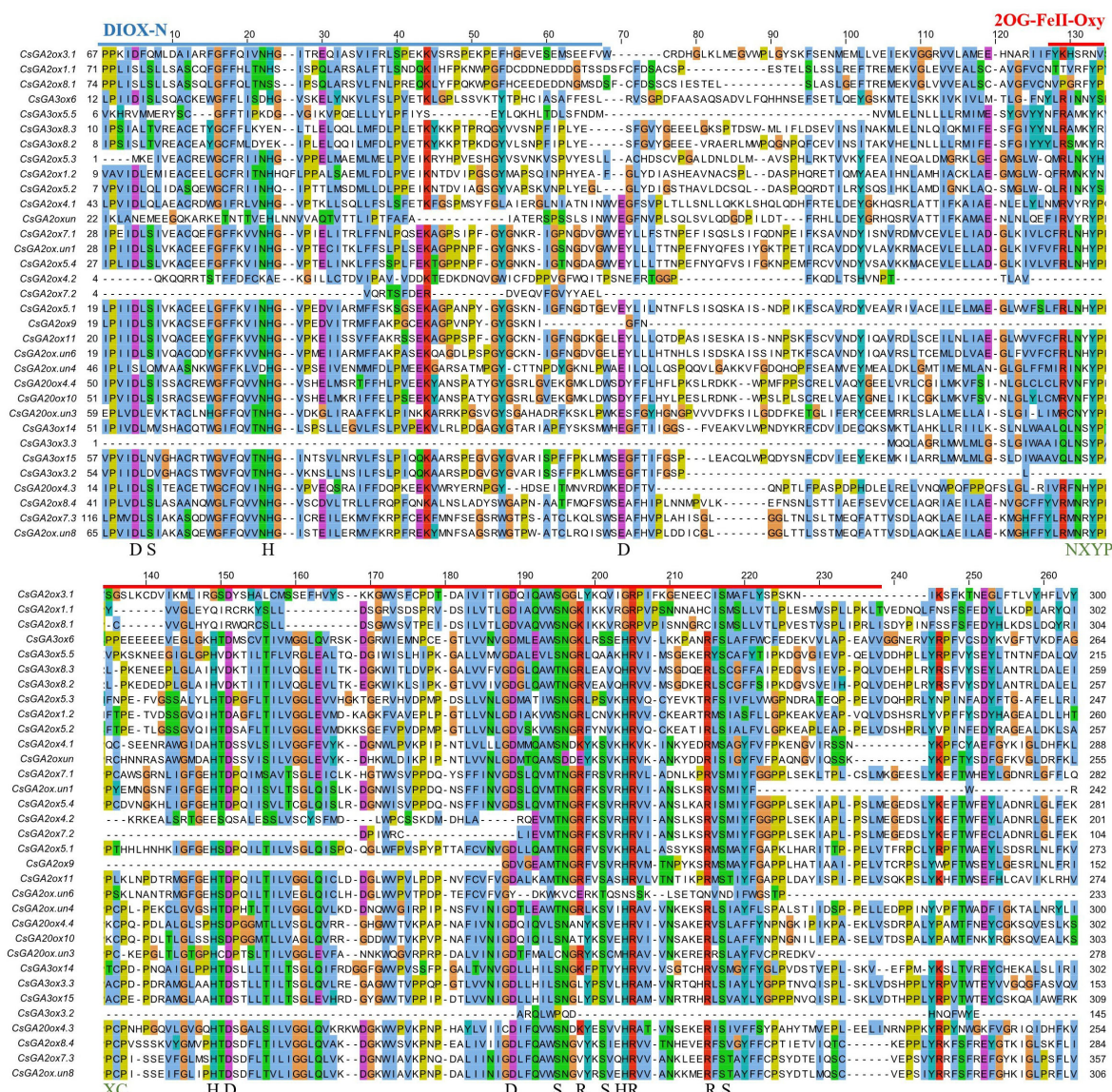


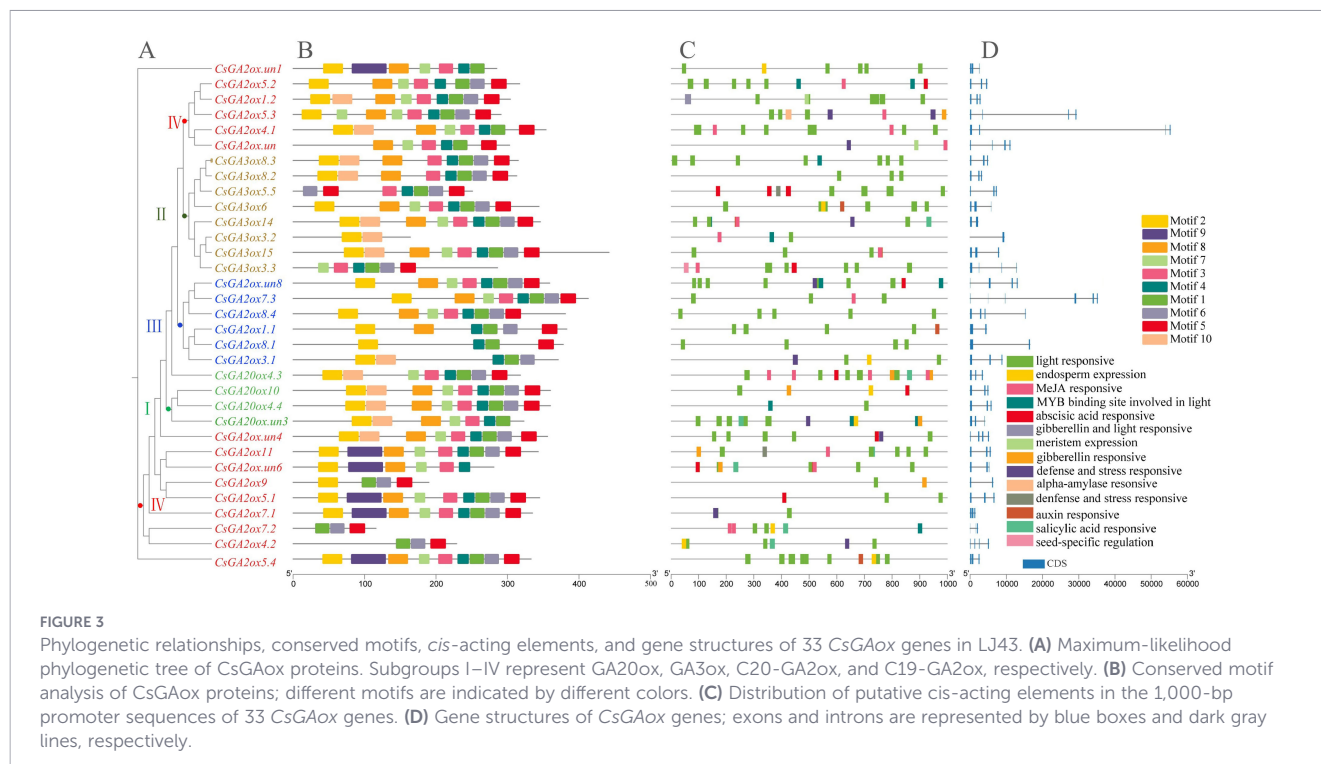
FIGURE 2

Multiple sequence alignment of 33 predicted CsGAox proteins in LJ43. The DIOX_N (PF14226) and 2OG-Fell_Oxy (PF03171) domains and the proposed consensus sequence NXYPC are highlighted with bold lines. Three H, three R and four S residues are conserved in all CsGAox proteins, whereas four D residues are identified, with the second D residue present only in CsGA2ox7.1, CsGA2ox.un1, and CsGA2ox5.4. These conserved residues are indicated below the alignment.

can be seen that CsGA20ox proteins in subgroup I all contain motifs 2, 10, 7, 3, 4, and 1. Secondly, CsGA3ox proteins in subgroup II have eight members. To be specific, CsGA3ox14 and CsGA3ox15 have nine motifs (motifs 2, 8, 10, 7, 3, 4, 1, 6, and 5); CsGA3ox6 has eight motifs, which lacks motif 10 compared with CsGA3ox14 and CsGA3ox15; CsGA3ox8.2 and CsGA3ox8.3 contain eight motifs, both lacking motif 7 compared with CsGA3ox14 and CsGA3ox15; CsGA3ox5.5 and CsGA3ox3.3 contain seven and six motifs, respectively, and the identity and order of the motifs in these proteins are also quite different from the other CsGA3ox proteins. CsGA3ox3.2 only contains two motifs, which may be due to the incomplete genome assembly in LJ43 (Figures 3A, B).

Thirdly, C₂₀-CsGA2ox proteins in subgroup III contain six members, among which three CsGA2ox proteins (CsGA2ox.un8, CsGA2ox7.3, and CsGA2ox8.4) all have eight motifs (motifs 2, 8, 7,

3, 4, 1, 6, and 5). Compared with these three proteins, CsGA2ox1.1, CsGA2ox8.1, and CsGA2ox3.1 have six motifs (lacking motifs 7 and 3), four motifs (lacking motifs 8, 7, 3, and 6), and five motifs (lacking motifs 7, 3, 8, and 5, but containing motif 10), respectively. In conclusion, C₂₀-CsGA2ox proteins in subgroup III all contain motifs 1, 2, and 4. Fourthly, C₁₉-CsGA2ox proteins in subgroup IV have 15 members, among which four members (CsGA2ox11, CsGA2ox5.1, CsGA2ox7.1, and CsGA2ox5.4) all have nine motifs (motifs 2, 9, 8, 7, 3, 4, 1, 6, and 5); CsGA2ox.un4 and CsGA2ox1.2 also contain nine motifs, with motif 10 present but motif 9 absent compared with the above four proteins; Compared with CsGA2ox.un4 and CsGA2ox1.2, CsGA2ox4.1 and CsGA2ox5.2 both contain eight motifs, which lack motif 6 and 10, respectively; CsGA2ox5.3 has one more motif 7 compared with CsGA2ox5.2; CsGA2ox.un1 and CsGA2ox.un6 have seven and six motifs, respectively, lacking the last two motifs (motifs 6



and 5) and three motifs (motifs 1, 6, and 5), respectively; *CsGA2ox.un* lacks motifs 2 and 10 compared with *CsGA2ox4.1*; *CsGA2ox7.2* and *CsGA2ox4.2* both have three motifs (motifs 1, 6, and 5), and *CsGA2ox9* has one more motif (motif 2) compared with *CsGA2ox7.2* and *CsGA2ox4.2* (Figures 3A, B). These motifs are typical for GAox family proteins.

It was shown that the coding sequences of 33 *CsGAox* genes in LJ43 are all disrupted by introns, and the number of exons varies from two to six. Overall, the number of exons in most *CsGAox* genes is between two and three. Furthermore, it can be seen that nine *CsGAox* genes, including five *C19*-GA2ox, three *C20*-GA2ox, and one *GA3ox*, contain more than three exons. Some of the *C20*-GA2ox and *C19*-GA2ox members have relatively longer introns (Figures 3A, D).

3.5 *Cis*-acting element analysis of *CsGAox* genes in LJ43

To reveal the biological processes in which *CsGAox* genes might be involved, the *cis*-acting elements were analyzed. After eliminating some elements with unknown functions, 14 kinds of important elements were selected for further analysis. The distribution of the promoter elements in LJ43 *CsGAox* genes was shown in Figures 3A, C. Firstly, the light-responsive elements were ubiquitous and most abundant. Secondly, some hormone-responsive elements were also widely distributed, such as the response elements of GA, methyl jasmonate (MeJA), auxin, abscisic acid (ABA), and salicylic acid (SA). Notably, the *CsGAox* family genes had 38 MeJA-responsive elements, which was greater than the number of any of the other four types of hormone-responsive elements. Thirdly, ten defense- and stress-responsive elements were identified. Fourthly, some development-related elements

were also identified, including endosperm expression-related elements, meristem expression-related elements, α -amylase responsive elements, and seed-specific regulatory elements. Fifthly, MYB-binding sites involved in light responsiveness were relatively abundant (Figures 3A, C; Supplementary Table 5).

3.6 Gene duplication of *CsGAox* genes in tea plants

To explore the genetic diversity and formation mode of the *CsGAox* gene family, the gene duplication events of *CsGAox* family members in eight *Camellia* genomes were investigated. The results showed that all 17 *CsGAox* genes in YK10 were classified as dispersed duplications. Except for YK10, the *CsGAox* genes in the other seven *Camellia* genomes showed four duplication types, including WGD/SD, dispersed, tandem, and proximal duplication. *CsGAox* family members are involved in 13, 15, 15, 20, 15, 16, and 14 SD/WGD events in LJ43, SCZ, BY, TGY, COL, CCH, and HD, respectively. Specifically, among the 33 identified *CsGAox* genes in LJ43, 15 genes belong to dispersed, 13 genes belong to WGD/SD, four genes belong to proximal, and only one gene belongs to tandem duplication (Supplementary Table 6).

Among the 27 *CsGAox* genes located on the 12 chromosomes of LJ43, nine duplicated gene pairs were identified, and the genes in these pairs were all associated with WGD/SD events. *CsGA2ox4.1* and *CsGA2ox4.4*, which form a gene pair, are both located on chromosome 4. However, except for this gene pair, the two genes within each of the other gene pairs are located on different chromosomes. Notably, three duplicated gene pairs are formed between any two genes of *CsGA2ox11*, *CsGA2ox5.1* and *CsGA2ox9*; *CsGA3ox15* formed two gene pairs with *CsGA3ox3.2* and

CsGA3ox14, respectively (Figure 4A; Supplementary Table 7). To gain insights into the evolutionary constraints acting on the *CsGAox* gene family in LJ43, the *Ka/Ks* ratios of the syntenic gene pairs were calculated. The results showed that, except for BY and YK10, for which *Ka/Ks* ratios could not be obtained, all successfully calculated *Ka/Ks* ratios in the other six genomes were less than one (Supplementary Table 8).

3.7 Collinearity analysis of *CsGAox* genes between LJ43 and other *Camellia* genomes

To further explore genome synteny, comparative syntenic maps were constructed between the LJ43 genome and the other six *Camellia* genomes. The results suggested that a total of 22, 23, 21, 23, 22, and 23 *CsGAox* genes in LJ43 had syntenic genes in the genomes of SCZ, BY, TGY, COL, CCH, and HD, respectively. A total of 42, 41, 45, 46, 38, and 46 orthologous gene pairs were detected between LJ43 and SCZ/BY/TGY/COL/CCH/HD, respectively (Figure 4B; Supplementary Table 9). Notably, *CsGA20ox4.4* and *CsGA20ox5.1* in LJ43 were both found to have three or four syntenic gene pairs with the other six *Camellia* genomes, respectively; *CsGA2ox9*, *CsGA2ox8.1*, *CsGA2ox1.1*, *CsGA2ox11*, *CsGA2ox4.1*, *CsGA2ox7.3*, *CsGA20ox10*, and *CsGA3ox15* in LJ43 all have two, three, or four gene pairs with the other six *Camellia* genomes, respectively; *CsGA2ox8.4*, *CsGA2ox5.2*, *CsGA2ox5.4*, *CsGA3ox3.2*, *CsGA3ox14*, *CsGA3ox5.5*, and *CsGA3ox6* genes in LJ43 all have one, two, or three gene pairs with the other six *Camellia* genomes, respectively; *CsGA20ox4.3*, *CsGA2ox3.1*, *CsGA2ox7.1*, and *CsGA3ox8.2* genes in LJ43 all have one gene pair with the other six *Camellia* genomes, respectively (see the summary in Supplementary Table 9).

3.8 Association of *CsGAox* gene expression with branch angle formation in tea plants

In our recent report, three tea cultivars with different branch angles, including UT1, UT2, and DT, were selected to investigate branch angle formation through transcriptome and plant hormone analysis. Among the detected 18 kinds of GAs, GA_1 , GA_3 , GA_4 , and GA_7 are bioactive GAs; the contents of GA_{51} and GA_3 were not detected, and the contents of GA_{12} -ald and GA_7 were very low. It was found that there were higher levels of GA_1 , GA_{24} , GA_{19} , GA_{20} , and GA_9 in the lateral buds of the draped cv. DT when compared with two upright cvs. UT1 and UT2 (Zhu et al., 2025). Here, the results of correlation analysis showed that branch angle was highly positively correlated with the contents of GA_1 , GA_9 , and GA_{20} (Figure 5A; Supplementary Table 10). Based on the above results, three GAs, including GA_1 , GA_9 , and GA_{20} , were selected for further screening of candidate *CsGAox* genes by analyzing the correlations of the branch angle or GA contents with the FPKM values of *CsGAox* genes. The results of correlation analysis suggested that, firstly, branch angle, GA_9 content, and GA_{20} content were all highly positively correlated with the FPKM of *CsGA20ox4.3*, and the branch angle and GA_{20} content were both negatively correlated with the FPKM of *CsGA2ox5.3*. Secondly, GA_1 content was positively correlated with the FPKM of *CsGA20ox4.3* and was negatively correlated with the FPKM value of *CsGA2ox5.3*. Thirdly, the FPKM of *CsGA20ox.un3* was highly positively correlated with the contents of GA_1 and GA_{20} (Figure 5B).

The FPKM values of *CsGA20ox4.3*, *CsGA20ox4.4*, and *CsGA3ox15* in the draped cv. DT were significantly higher than those in both upright cvs. UT1 and UT2, whereas the FPKM value of *CsGA20ox.un3* was significantly higher in DT than in UT1 but

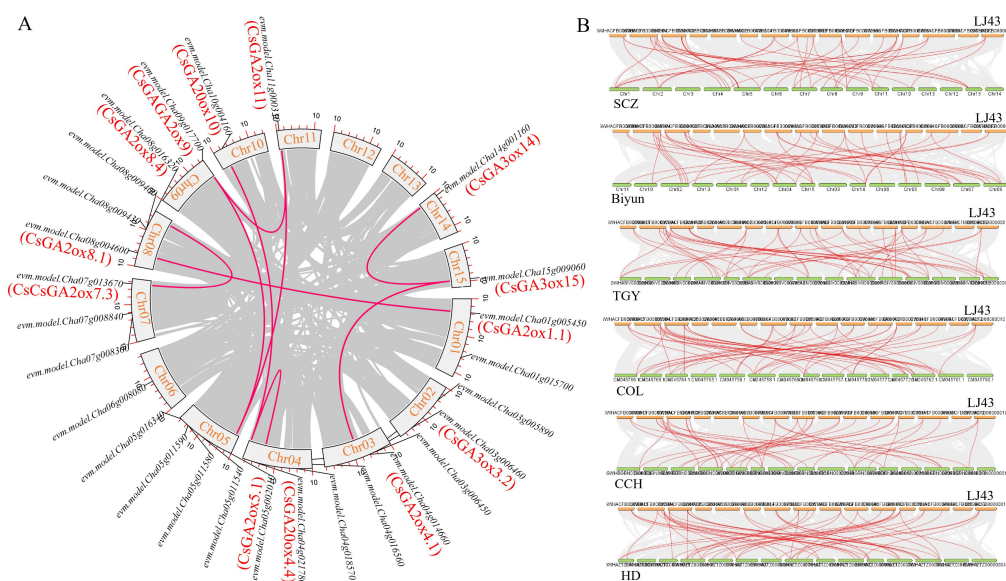


FIGURE 4

Gene duplication and synteny analyses of *CsGAox* genes. (A) Synteny analysis of *CsGAox* genes within the LJ43 genome. Gray lines in the background indicate collinear blocks, whereas red lines connect duplicated *CsGAox* gene pairs. (B) Synteny analysis of *CsGAox* genes between LJ43 and six other *Camellia* genomes (SCZ, BY, TGY, COL, CCH, and HD). Gray lines in the background indicate collinear blocks, whereas red lines connect syntenic *CsGAox* gene pairs. Abbreviations: *CsGAox*, *Camellia sinensis* gibberellin oxidase; LJ43, Longjing 43; SCZ, Shuchazao; BY, Biyun; TGY, Tieguanyin; COL, *Camellia oleifera*; CCH, *Camellia chekiangoleosa*; HD, Huangdan; Chr, chromosome.

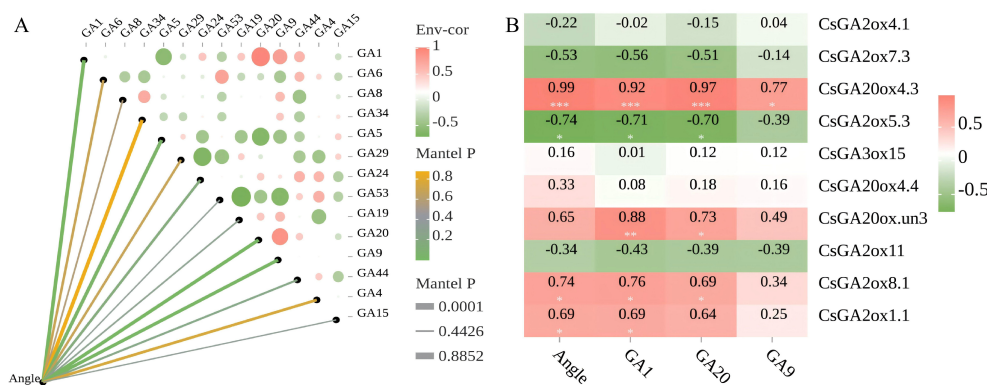


FIGURE 5

Correlation analysis among branch angle, gibberellin (GA) contents, and *CsGAox* gene expression in three tea cultivars with different branch angles: UT1 (CT2009-0025, upright tea 1), UT2 (CT2013-0114, upright tea 2), and DT (CT2009-0160, draped tea). (A) Correlation network heat map between branch angle and GA contents. (B) Correlation heat map showing the associations of branch angle and GA contents with *CsGAox* gene expression. The heat maps were generated using OmicShare Tools. Abbreviations: *CsGAox*, *Camellia sinensis* gibberellin oxidase; Env-cor, correlation coefficient among variables; Mantel *R*, correlation coefficient of the Mantel test; Mantel *P*, *P* value of the Mantel test; *, **, and *** indicate significant correlations at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

not UT2. Conversely, the FPKM values of *CsGA2ox4.1* and *CsGA2ox5.3* were significantly lower in DT than in both upright cultivars, whereas *CsGA2ox7.3* was significantly lower in DT than in UT1 but not UT2. Furthermore, the expression patterns of these seven *CsGAox* genes were further validated by qRT-PCR (Figure 6). The results showed that the relative expression of these seven *CsGAox* genes all showed trends similar to their FPKM values. Thus, *CsGA2ox4.3*, *CsGA2ox4.4*, *CsGA2ox.un3*, *CsGA3ox15*, *CsGA2ox4.1*, *CsGA2ox5.3*, and *CsGA2ox7.3* were identified as candidate *CsGAox* genes associated with branch angle variation in tea plants.

3.9 Exogenous GA₃ treatment increased branch angle and altered *CsGAox* gene expression in tea lateral buds

Our recent report suggested that exogenous spraying of 0.5 and 5 mg/L GA₃ both significantly increased the branch angle of tea plants. To further examine the responses of the seven candidate *CsGAox* genes to exogenous GA₃ treatment, their relative expression levels in lateral buds were determined by qRT-PCR. Firstly, 0.5 and 5 mg/L GA₃ up-regulated the expression of two GA biosynthetic genes, *CsGA2ox4.3* and *CsGA2ox.un3*. Secondly, 0.5 mg/L

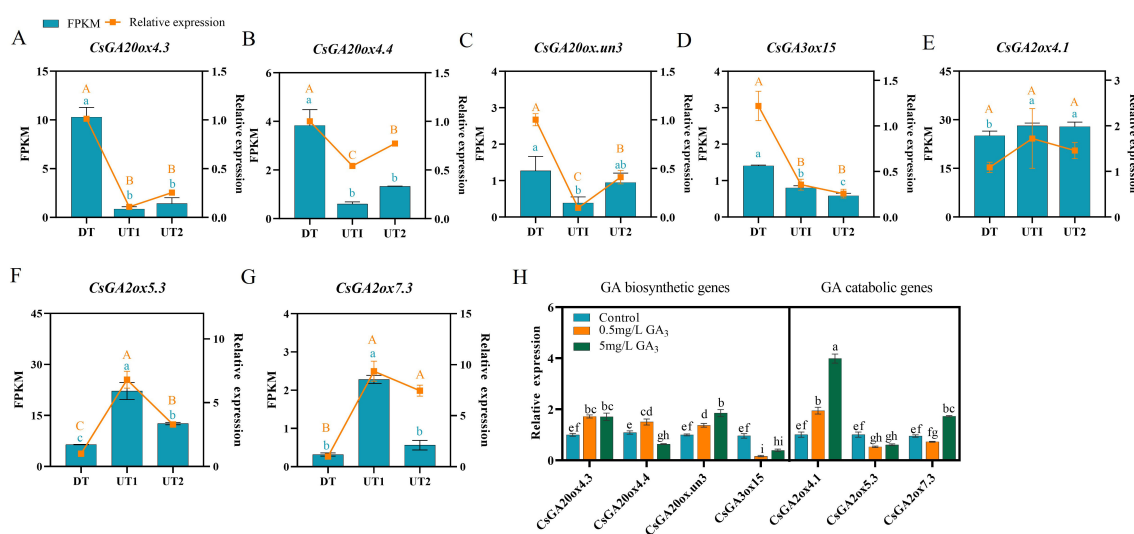


FIGURE 6

Expression patterns of *CsGAox* genes associated with branch angle in tea plants. (A–G) Fragments per kilobase of transcript per million mapped reads (FPKM) values and relative expression levels of seven *CsGAox* genes in the lateral buds of three tea cultivars with different branch angles: DT (CT2009-0160, draped tea), UT1 (CT2009-0025, upright tea 1), and UT2 (CT2013-0114, upright tea 2). The draped cultivar DT was used as the control. FPKM values are shown on the left y-axis, and relative expression levels determined by quantitative real-time PCR (qRT-PCR) are shown on the right y-axis. Different lowercase and uppercase letters indicate significant differences in FPKM values and relative expression levels, respectively, among the three cultivars ($P < 0.05$). (H) Relative expression levels of the seven *CsGAox* genes in the lateral buds of Zhongcha 108 (ZC108) under 0, 0.5, and 5 mg/L gibberellin A₃ (GA₃) treatments. Different lowercase letters indicate significant differences among all gene–treatment combinations ($P < 0.05$). Data are presented as means $\pm$ standard error (SE) of three independent biological replicates.

GA₃ treatment upregulated the expression of *CsGA20ox4.4*, whereas 5 mg/L GA₃ downregulated its expression, indicating a concentration-dependent transcriptional response to exogenous GA₃. Thirdly, 0.5 and 5 mg/L GA₃ both down-regulated the expression of *CsGA3ox15*. Fourthly, 0.5 and 5 mg/L GA₃ both up-regulated the expression of *CsGA20ox4.1*. Thus, *CsGA20ox4.1* showed a positive transcriptional response to exogenous GA₃ treatment. Fifthly, both 0.5 and 5 mg/L GA₃ treatments downregulated the expression of the GA-catabolic gene *CsGA20ox5.3*. This expression response, together with the increased branch angle following GA₃ treatment, further supports an association of *CsGA20ox5.3* with GA-responsive branch angle formation. Sixthly, 0.5 mg/L GA₃ treatment decreased *CsGA20ox7.3* expression, whereas 5 mg/L GA₃ treatment increased its expression, indicating a concentration-dependent transcriptional response to exogenous GA₃. In summary, *CsGA20ox4.3*, *CsGA20ox5.3* and *CsGA20ox.un1* were identified as candidate *CsGAox* genes associated with GA-responsive branch angle formation in tea plants.

3.10 Association of *CsGAox* gene expression with internode elongation in tea plants cvs. LJ43 and ZC102

During the growth of tea new shoots from the 2- to 5-leaf stage, the FPKM values of *CsGA20ox4.3* and *CsGA3ox3.3* in the internode of new shoots were both significantly positively correlated with the internode length. The FPKM value of *CsGA20ox1.1* was significantly negatively correlated with internode length (Supplementary Table 11). Furthermore, the FPKM value of *CsGA20ox.un1* was significantly negatively correlated with GA₃ contents at the 2-leaf stage; the FPKM value of *CsGA20ox4.3* was significantly positively correlated with internode length as well as the contents of GA₃ and GA₇ at the 5-leaf stage. Subsequently, the expression patterns of *CsGAox* genes were further validated by qRT-PCR.

Transcriptome results showed that with internode development, the FPKM value of *CsGA20ox4.3* showed a trend of gradual increase in two cultivars. Furthermore, the FPKM value of *CsGA20ox4.3* in LJ43 was significantly higher than that in ZC102 at the 2-, 3-, and 5-leaf stages. Similarly, with internode development, the FPKM value of *CsGA3ox3.3* showed a trend of gradual increase in the two cultivars. With internode development, the FPKM of *CsGA20ox1.1* showed a decreasing trend in the two cultivars; the FPKM values of *CsGA20ox4.3* and *CsGA3ox3.3* both gradually increased with internode development in the two cultivars; the FPKM of *CsGA20ox.un1* showed a gradually decreasing trend in ZC102. On the other hand, the FPKM values in the two cultivars at the same time point were compared. The results showed that the FPKM of *CsGA20ox4.3* in LJ43 was higher than that in ZC102 at all three time points, whereas the FPKM of *CsGA20ox1.1* in LJ43 was significantly lower than that in ZC102 at each stage. The results of qRT-PCR showed trends similar to the FPKM values of *CsGAox* genes (Figure 7). In addition, the relative expression of *CsGA20ox4.4* increased with internode development in both cultivars; its FPKM value increased progressively in ZC102, whereas in LJ43 it increased from the 2- to 3-leaf stage and then decreased at the 5-leaf stage. The FPKM value and relative expression of *CsGA20ox5.2* showed a trend of gradual decrease in the two cultivars, while the FPKM value and relative expression of *CsGA20ox5.4* showed a trend of gradual decrease in LJ43. In conclusion, *CsGA20ox4.3*, *CsGA3ox3.3*, *CsGA20ox1.1*, and *CsGA20ox.un1* were identified as candidate genes associated with internode elongation in LJ43 and ZC102.

3.11 Association of *CsGAox* gene expression with internode elongation in tea plants cvs. LJ43, QC1 and FY

A previous report suggested that the internode length of tea cv. LJ43, QC1, and FY increased successively (Mi et al., 2025). The

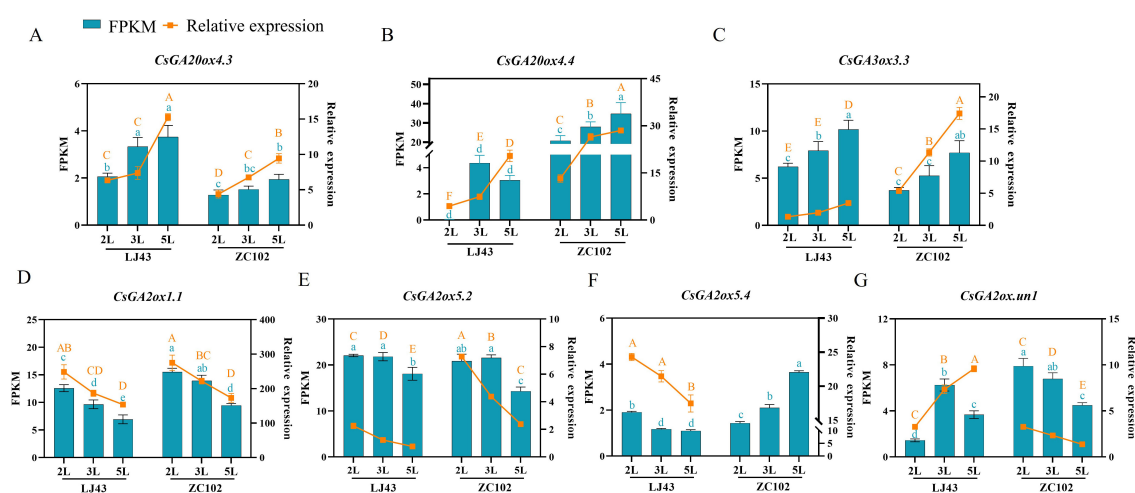


FIGURE 7

Expression patterns of *CsGAox* genes in the internodes of two tea cultivars with different internode lengths, Longjing 43 (LJ43) and Zhongcha 102 (ZC102), at the 2-, 3-, and 5-leaf stages. (A–G) Fragments per kilobase of transcript per million mapped reads (FPKM) values and relative expression levels of seven *CsGAox* genes. FPKM values are shown on the left y-axis, and relative expression levels determined by quantitative real-time PCR (qRT-PCR) are shown on the right y-axis. Different lowercase and uppercase letters indicate significant differences in FPKM values and relative expression levels, respectively, among the six cultivar–developmental stage combinations ($P < 0.05$). Data are presented as means $\pm$ standard error (SE) of three independent biological replicates. 2L, 3L, and 5L indicate the 2-, 3-, and 5-leaf stages, respectively.

transcriptome data were downloaded and analyzed. The results of correlation analysis suggested that the FPKM values of *CsGA2ox* genes, including *CsGA2ox1.1*, *CsGA2ox5.2*, and *CsGA2ox5.3*, were significantly negatively correlated with internode length, while the FPKM value of *CsGA2ox10* was significantly positively correlated with internode length (Figure 8A; Supplementary Table 11). The FPKM values of these four *CsGAox* genes were displayed in Figure 8B. It can be seen that, firstly, there were no significant differences in the FPKM values of *CsGA2ox1.1* and *CsGA2ox5.3* between LJ43 and QC1, and their FPKM values in LJ43 and QC1 were significantly higher than those in the internode of FY. Secondly, the FPKM values of *CsGA2ox5.2* decreased successively in the internode of LJ43, QC1, and FY. Thirdly, different from the above three GA degrading genes, the FPKM values of the GA biosynthetic gene *CsGA2ox10* increased successively in the internode of LJ43, QC1, and FY. Thus, *CsGA2ox1.1*, *CsGA2ox5.2*, *CsGA2ox5.3*, and *CsGA2ox10* were identified as candidate genes associated with internode length variation among the three tea cultivars. Based on the two internode transcriptome datasets, *CsGA2ox4.3*, *CsGA3ox3.3*, *CsGA2ox1.1*, *CsGA2ox5.2*, and *CsGA2ox.un1* were prioritized as candidate genes potentially associated with internode elongation in tea plants.

4 Discussion

GAs play crucial roles in regulating the growth and development of higher plants. *GAox* genes are crucial GA metabolic genes and play key roles in regulating GA balance in plants (Davière et al., 2014; He et al., 2025; Nagai et al., 2020; Zhu et al., 2025). A total of 33 unique *CsGAox* proteins were obtained from the LJ43 genome in this study.

Firstly, the proposed consensus sequence NXYPXCXXP of 2-ODDs may be involved in the binding of a common co-substrate, 2-oxoglutaric acid. It was highly conserved in all of the *CsGA2ox* and

CsGA3ox proteins, and only a partial sequence NXYPXC was highly conserved in *CsGA2ox* of tea cv. YK10 (Pan et al., 2017). Compared with YK10, only the partial sequence NXYPXC was highly conserved in all *CsGAox* members of LJ43 here. Secondly, the sequence LPWKET may be related to GA substrate binding and was conserved in *GA2ox* proteins of tea cv. YK10, pumpkin and *Arabidopsis*. However, it was not found in the *GA2ox* proteins of LJ43. The MWSEGXT sequence may be conserved in plant *GA3ox* proteins (Pan et al., 2017); however, it was not found in the *CsGA3ox* proteins of LJ43. Thirdly, previous studies suggested that the D and H residues could bind to the active-site Fe^{2+} , and the H, R, and S residues could interact with the 5-carboxylate of 2-oxoglutarate (Li Y. et al., 2021). In this study, consistent with *CsGAox* proteins in YK10 (Pan et al., 2017), three H, three R, and four S residues were conserved in all putative *CsGAox* proteins of LJ43, whereas four D residues were identified, with the second D residue present only in *CsGA2ox7.1*, *CsGA2ox.un1*, and *CsGA2ox5.4* (Figure 2).

The *cis*-acting elements play vital roles in transcription regulation (Hu et al., 2021). In general, MeJA- and light-responsive elements were ubiquitous and relatively abundant in the promoters of *CsGAox* genes in LJ43, suggesting potential responsiveness of these genes to MeJA- and light-related signals. The presence of MYB-binding sites also suggests that *CsGAox* genes may be responsive to MYB-mediated transcriptional regulation under light-related conditions.

4.1 Collinearity and duplication of *CsGAox* genes within LJ43 and among *Camellia* genomes

CsGA3ox3.2 and *CsGA3ox3.3* (located on Chr03), *CsGA2ox5.2*, *CsGA2ox5.3*, and *CsGA2ox5.4* (Chr05), *CsGA3ox8.2* and *CsGA3ox8.3* (on Chr08) were all very close to each other, respectively, and whether they might have similar biological functions needs to be confirmed. It is worth noting that *CsGA3ox3.2* and *CsGA3ox3.3* formed a pair of

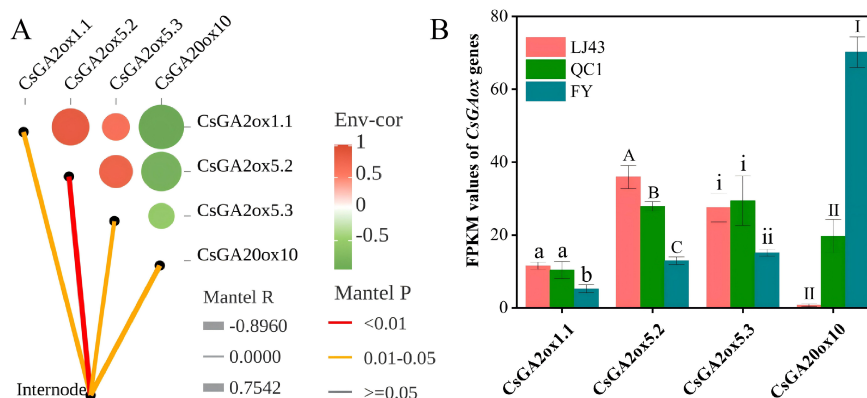


FIGURE 8

Association between internode length and *CsGAox* gene expression in three tea cultivars, Longjing 43 (LJ43), Qiancha 1 (QC1), and Feiyun (FY). (A) Correlation analysis between internode length and the fragments per kilobase of transcript per million mapped reads (FPKM) values of *CsGAox* genes. Circle color and size indicate the direction and strength of correlations among *CsGAox* genes, whereas line color and width indicate Mantel P and Mantel R, respectively. Env-cor denotes the correlation coefficient among variables. (B) FPKM values of four *CsGAox* genes in the internodes of LJ43, QC1, and FY. Different letters indicate significant differences among the three cultivars for each gene ($P < 0.05$, Duncan's multiple range test). Data are presented as means $\pm$ standard error (SE) of three independent biological replicates.

tandemly duplicated genes located within a distance of 666 bp (Supplementary Table 3). The results of collinearity analysis within LJ43 suggested that *CsGA20ox4.1* and *CsGA20ox4.4* belong to different subfamilies, although they formed a gene pair. Except for this gene pair, the two genes within each of the other gene pairs belonged to the same subfamily. The nine identified *CsGAox* gene pairs in LJ43 were all associated with WGD/SD events, indicating that WGD or SD may be a major force driving the expansion of the *CsGAox* gene family and that functional divergence may have occurred following duplication. The analysis of *CsGAox* duplication events revealed numerous collinear regions between LJ43 and the other seven *Camellia* genomes. The results showed that, except for YK10, the WGD/SD, dispersed, tandem, and proximal duplications may play important roles in the expansion of the *CsGAox* gene family in seven *Camellia* genomes, including LJ43, SCZ, BY, TGY, COL, CCH, and HD. However, the *GAox* genes in YK10 were classified only as dispersed duplications.

The *Ka/Ks* ratios of gene pairs can be used to explore the evolutionary constraints and the trend of gene divergence after duplication (Sun et al., 2023). Here, except for BY and YK10, the *Ka/Ks* ratios of the analyzed gene pairs were all less than one in the other six genomes, suggesting that these duplicated gene pairs have predominantly evolved under purifying selection. Many *CsGAox* genes in LJ43 shared more than one orthologous gene pair with the other *Camellia* genomes. It can be speculated that these genes may have contributed to the expansion of the *CsGAox* family during *Camellia* evolution and that these orthologous relationships may have arisen before the divergence of the analyzed lineages.

4.2 Associations of *CsGAox* genes with tea plant architecture

In tea production, the branch angle and internode length of newly formed shoots are two key traits affecting the integrity rate of tea bud leaves during mechanical-picking (Luo et al., 2023; Zhu et al., 2025; Mi et al., 2025). The bioactive GAs play important roles in regulating internode elongation and branch angle of plants (Davière et al., 2014; He et al., 2025; Nagai et al., 2020; Zhu et al., 2025). *GA20ox*, *GA3ox*, and *GA2ox* genes encode key enzymes involved in the later stages of GA biosynthesis and catabolism. Identifying candidate *CsGAox* genes associated with branch angle and internode length may provide a basis for future functional studies and genetic improvement of tea plant architecture.

Here, the results of correlation analysis between GA contents and the *CsGAox* FPKM values suggested that, firstly, GA_9 and GA_{20} are both the direct products of *GA20ox* and the substrates of *GA3ox* and *GA2ox* enzymes. The branch angle as well as the contents of GA_9 and GA_{20} were all highly positively correlated with the FPKM value of *CsGA20ox4.3*, and the branch angle and GA_{20} content were both negatively correlated with the *CsGA20ox5.3* FPKM value. Secondly, as a direct product of the *GA3ox* enzyme, an indirect product of *GA20ox*, and also a substrate of *GA2ox* enzymes, GA_1 content was positively correlated and negatively correlated with the FPKM value of *CsGA20ox4.3* and *CsGA20ox5.3*, respectively. Thirdly, the FPKM value of *CsGA20ox.un3* was highly positively correlated with the contents of GA_1 and GA_{20} . Based on these correlations,

CsGA20ox4.3, *CsGA20ox5.3*, and *CsGA20ox.un3* were prioritized as candidate genes associated with branch angle in tea plants.

Exogenous application of 0.5 and 5 mg/L GA_3 significantly increased branch angle and was accompanied by the upregulation of *CsGA20ox4.3* and *CsGA20ox.un3* and the downregulation of *CsGA20ox5.3*, whereas *CsGA20ox7.3* showed a concentration-dependent transcriptional response. These coordinated phenotypic and transcriptional responses further support an association of these *CsGAox* genes with GA-responsive branch angle formation. Previous studies reported that bioactive GAs could negatively regulate *GA20ox* and *GA3ox* expression and positively regulate *GA2ox* expression through feedback mechanisms (Pan et al., 2017; He et al., 2019). Consistent with previously reported feedback responses in GA metabolism, 5 mg/L GA_3 treatment decreased the transcript abundance of *CsGA20ox4.4*, while both 0.5 and 5 mg/L GA_3 treatments decreased *CsGA3ox15* expression in tea lateral buds. In contrast, 0.5 and 5 mg/L GA_3 treatments increased *CsGA20ox4.1* expression in tea lateral buds. Previous studies suggested that the expression of *GA20ox* and *GA3ox* genes plays important regulatory roles in plant stem and internode elongation. For example, the maize *ZmGA3ox* mutant *dwarf1* exhibited a dwarf phenotype (Song et al., 2011), and miRNA-mediated suppression of *ZmGA20ox3* and *ZmGA20ox5* reduced GA levels and produced a short-statured maize ideotype (Paciorek et al., 2022). The rice *OsGA3ox2* mutant *d18* exhibited a severe dwarf phenotype, while the *OsGA20ox2* mutant *sd1* showed a semi-dwarf phenotype due to decreased active GA contents in the elongating stem (He et al., 2025). In rice, deepwater conditions promoted internode elongation by increasing *GA20ox2* expression and activity in the internode (Davière et al., 2014; Nagai et al., 2020). Overexpression of *ZmGA20ox1* or *PsGA3ox1* increased the length of maize stems and pea internodes, respectively (Paciorek et al., 2022; Ren et al., 2023). On the other hand, upregulation or overexpression of *GA2ox* in various plants reduces endogenous bioactive GA levels, resulting in plant dwarfing and internode shortening (Luo et al., 2023; He et al., 2025; Mi et al., 2025; Cao et al., 2024). Consistent with these findings in other plant species, the integrated FPKM and qRT-PCR analyses identified *CsGA20ox4.3*, *CsGA3ox3.3*, *CsGA2ox1.1*, *CsGA2ox5.2*, and *CsGA2ox.un1* as candidate genes associated with internode elongation in tea plants.

Several limitations of the present study should be acknowledged. The prioritization of the candidate *CsGAox* genes in this study was mainly based on comparative genomic analysis, expression profiling, correlation analysis, endogenous GA contents, and responses to exogenous GA_3 treatment, and their direct biological functions have not yet been experimentally validated. Further studies using overexpression, gene silencing or gene editing, together with enzyme activity assays, are required to clarify their specific roles in tea plant architecture.

5 Conclusions

Overall, this study provides a comparative genomic framework for the *CsGAox* family across multiple *Camellia* genomes and

identifies several candidate *CsGAox* genes associated with branch angle and internode elongation. These genes represent candidates for future functional studies and further evaluation in tea plant architecture breeding, particularly for improving the suitability of tea plants for mechanical harvesting.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/[Supplementary Material](#).

Author contributions

HS: Investigation, Writing – original draft. LZ: Conceptualization, Writing – original draft. HJL: Investigation, Writing – review & editing. SG: Formal analysis, Software, Writing – review & editing. HYL: Investigation, Writing – review & editing. YL: Funding acquisition, Writing – review & editing. PW: Formal analysis, Validation, Data curation, Writing – review & editing. JPZ: Formal analysis, Validation, Data curation, Writing – review & editing. AM: Writing – review & editing. XL: Funding acquisition, Writing – review & editing. JMZ: Conceptualization, Writing – review & editing. SZ: Conceptualization, Supervision, Funding acquisition, Project administration, Writing – review & editing.

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References

- Cao, C., Guo, S., Deng, P., Yang, S., Xu, J., Hu, T., et al. (2024). The BEL1-like homeodomain protein OsBLH4 regulates rice plant height, grain number, and heading date by repressing the expression of *OsGA2ox1*. *Plant J.* 119, 1369–1385. doi: 10.1111/tpj.16857
- Davière, J. M., Wild, M., Regnault, T., Baumberger, N., Eisler, H., Genschik, P., et al. (2014). Class I TCP-DELLA interactions in inflorescence shoot apex determine plant height. *Curr. Biol.* 24, 1923–1928. doi: 10.1016/j.cub.2014.07.012
- Dutt, M., Mahmoud, L. M., Nehela, Y., Grosser, J. W., and Killiny, N. (2022). The *Citrus sinensis* TILLER ANGLE CONTROL 1 (*CsTAC1*) gene regulates tree architecture in sweet oranges by modulating the endogenous hormone content. *Plant Sci.* 323, 111401. doi: 10.1016/j.plantsci.2022.111401
- Fukazawa, J., Mori, M., Watanabe, S., Miyamoto, C., Ito, T., and Takahashi, Y. (2017). DELLA-GAF1 Complex is a main component in gibberellin feedback regulation of GA20 Oxidase 2. *Plant Physiol.* 175, 1395–1406. doi: 10.1104/pp.17.00282
- He, H., Liang, G., Lu, S., Wang, P., Liu, T., Ma, Z., et al. (2019). Genome-wide identification and expression analysis of *GA2ox*, *GA3ox*, and *GA20ox* are related to gibberellin oxidase genes in grape (*Vitis vinifera* L.). *Genes* 10, 680. doi: 10.3390/genes10090680
- He, Q., Wu, H., Zeng, L., Yin, C., Wang, L., Tan, Y., et al. (2025). OsKANAD11 and OsYABBY5 regulate rice plant height by targeting *GIBBERELLIN 2-OXIDASE6*. *Plant Cell* 37, koae276. doi: 10.1093/plcell/koae276
- Honi, U., Amin, M. R., Kabir, S. M. T., Bashar, K. K., Moniruzzaman, M., Jahan, R., et al. (2020). Genome-wide identification, characterization and expression profiling of gibberellin metabolism genes in jute. *BMC Plant Biol.* 20, 306. doi: 10.1186/s12870-020-02512-2
- Hu, L., Wang, P., Hao, Z., Lu, Y., Xue, G., Cao, Z., et al. (2021). Gibberellin oxidase gene family in *L. chinense*: genome-wide identification and gene expression analysis. *Int. J. Mol. Sci.* 22, 7167. doi: 10.3390/ijms22137167
- Jiao, S., Liu, Z., Kang, Y., Zhang, R., Wang, Y., Zhang, J., et al. (2024). Identification of the *GAox* gene family in potato (*Solanum tuberosum* L.) and its expression analysis in response to drought stress. *Chem. Biol. Technol. Agric.* 11, 52. doi: 10.1111/tpj.16857

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Supplementary material

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- Lange, T., Krämer, C., and Lange, M. J. P. (2020). The class III gibberellin 2-oxidases AtGA2ox9 and AtGA2ox10 contribute to cold stress tolerance and fertility. *Plant Physiol.* 184, 478–486. doi: 10.1104/pp.20.00594
- Li, W., Xiang, F., Su, Y., Luo, Z., Luo, W., Zhou, L., et al. (2021). Gibberellin increases the bud yield and theanine accumulation in *Camellia sinensis* (L.) Kuntze. *Molecules* 26, 3290. doi: 10.3390/molecules26113290
- Li, Y., Shan, X., Jiang, Z., Zhao, L., and Jin, F. (2021). Genome-wide identification and expression analysis of the GA2ox gene family in maize (*Zea mays* L.) under various abiotic stress conditions. *Plant Physiol. Biochem.* 166, 621–633. doi: 10.1016/j.plaphy.2021.06.043
- Liu, Y., Chen, S., Pal, S., Yu, J., Zhou, Y., Tran, L.-S., et al. (2024). The hormonal, metabolic, and environmental regulation of plant shoot branching. *New Crops* 1, 100028. doi: 10.1016/j.ncrops.2024.100028
- Luo, Y., Yu, Q., Xie, Y., Xu, C., Cheng, L., Shi, Q., et al. (2023). Internode length is correlated with GA₃ content and is crucial to the harvesting performance of tea-picking machines. *Plants* 12, 2508. doi: 10.3390/plants12132508
- Mi, X., Qiao, D., Yang, C., Chen, J., Liang, S., and Guo, Y. (2025). Transcriptome sequencing revealed the regulation of stem internode length associated with mechanical harvesting in three tea varieties. *Front. Plant Sci.* 16, 1626915. doi: 10.3389/fpls.2025.1626915
- Nagai, K., Mori, Y., Ishikawa, S., Furuta, T., Gamuyao, R., Niimi, Y., et al. (2020). Antagonistic regulation of the gibberellin acid response during stem growth in rice. *Nature* 584, 109–114. doi: 10.1038/s41586-020-2501-8
- Paciorek, T., Chiapelli, B. J., Wang, J. Y., Paciorek, M., Yang, H., Sant, A., et al. (2022). Targeted suppression of gibberellin biosynthetic genes *ZmGA20ox3* and *ZmGA20ox5* produces a short stature maize ideotype. *Plant Biotechnol. J.* 20, 1140–1153. doi: 10.1111/pbi.13797
- Pan, C., Tian, K., Ban, Q., Wang, L., Sun, Q., He, Y., et al. (2017). Genome-wide analysis of the biosynthesis and deactivation of gibberellin-dioxygenases gene family in *Camellia sinensis* (L.) O. Kuntze. *Genes* 8, 235. doi: 10.3390/genes8090235
- Plackett, A. R. G., Powers, S. J., Fernandez-Garcia, N., Urbanova, T., Takebayashi, Y., Seo, M., et al. (2012). Analysis of the developmental roles of the *Arabidopsis* gibberellin 20-oxidases demonstrates that GA20ox1, -2, and -3 are the dominant paralogs. *Plant Cell* 24, 941–960. doi: 10.1105/tpc.111.095109
- Ren, M., Wang, Q., Zhang, F., Wang, Y., Wang, Y., Li, W., et al. (2024). Genome-wide identification of the GAox gene family and functional characterization of *PbGA3ox4* during stone cell formation in Chinese white pear. *Sci. Hortic.* 330, 113063. doi: 10.1016/j.scienta.2024.113063
- Ren, Z., Liu, Y., Li, L., Wang, X., Zhou, Y., Zhang, M., et al. (2023). Deciphering transcriptional mechanisms of maize internodal elongation by regulatory network analysis. *J. Exp. Bot.* 74, 4503–4519. doi: 10.1093/jxb/erad178
- Song, J., Guo, B., Song, F., Peng, H., Yao, Y., Zhang, Y., et al. (2011). Genome-wide identification of gibberellins metabolic enzyme genes and expression profiling analysis during seed germination in Maize. *Gene* 482, 34–42. doi: 10.1016/j.gene.2011.05.008
- Sun, H., Pang, B., Yan, J., Wan, T., Wang, L., Chen, C., et al. (2018). Comprehensive analysis of cucumber gibberellin oxidase family genes and functional characterization of *CsGA20ox1* in root development in *Arabidopsis*. *Int. J. Mol. Sci.* 19, 3135. doi: 10.7717/peerj.16279
- Sun, J., Zhang, X., Fu, C., Ahmad, N., Zhao, C., Hou, L., et al. (2023). Genome-wide identification and expression analysis of GA20ox and GA3ox genes during pod development in peanut. *PeerJ* 11, e16279. doi: 10.1007/s13562-015-0346-2
- Yan, Q., Li, J., Lu, L. Y., Gao, L. J., Lai, D. W., Yao, N., et al. (2021). Integrated analyses of phenotype, phytohormone, and transcriptome to elucidate the mechanism governing internode elongation in two contrasting elephant grass (*Cenchrus purpureus*) cultivars. *Ind. Crops Prod.* 170, 113693. doi: 10.1016/j.indcrop.2021.113693
- Zhang, L., Shen, H., Zhu, S., Mao, Y., Li, Y., Liu, H., et al. (2026). Transcriptome and phytohormone analysis reveal the regulatory mechanism of internode elongation in new shoots of tea plants (*Camellia sinensis*). *J. Plant Growth Regul.* 45 (4), 2744–2756. doi: 10.1007/s00344-025-12027-5
- Zhu, J., Li, X., Huang, J., Wang, L., Zheng, Q., Li, H., et al. (2025). Transcriptomics and plant hormone analysis reveal the mechanism of branching angle formation in tea plants (*Camellia sinensis*). *Int. J. Mol. Sci.* 26, 604. doi: 10.3390/ijms26020604