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# Genome-Wide Identification and Characterization of Tea SGR Family Members Reveal Their Potential Roles in Chlorophyll Degradation and Stress Tolerance

**Hengze Ren [1,†], Yating Yu [1,†], Chao Huang [1], Danying Li [1], Jiale Ni [1], Wuyun Lv [1], Kang Wei [2], Liyuan Wang [2,*] and Yuchun Wang [1,*]**

[1] College of Tea Science and Tea Culture, Zhejiang A&F University, Hangzhou 311300, China; renhengze@zafu.edu.cn (H.R.); yuyating@stu.zafu.edu.cn (Y.Y.); huangchao@tricaas.com (C.H.); 17326081030@163.com (D.L.); jailene77@163.com (J.N.); lvwuyun_blue@163.com (W.L.)

[2] Key Laboratory of Biology, Genetics and Breeding of Special Economic Animals and Plants, Ministry of Agriculture and Rural Affairs, National Center for Tea Improvement, Tea Research Institute, Chinese Academy of Agricultural Sciences (TRICAAS), Hangzhou 310008, China; weikang@tricaas.com

* Correspondence: wangly@tricaas.com (L.W.); ycwang0201@126.com (Y.W.)

† These authors contributed equally to this work.

**Abstract:** Photosynthesis plays vital roles in plant growth and development. Stay-green (SGR) proteins are responsible for chlorophyll degradation and photosynthetic metabolism. To identify SGR family members and determine their potential functions in tea plants, we identified and cloned three *SGR* genes. Phylogenetic analysis revealed that the tea SGR homologs were classified into the SGR subfamily (named CsSGR1 and CsSGR2) and the SGRL subfamily (named CsSGRL). *Cis*-element analysis indicated that the promoters of *CsSGR1*, *CsSGR2* and *CsSGRL* contained light-, phytohormone- and stress-related elements. Subcellular localization confirmed that CsSGR1 was localized in the chloroplast, while CsSGR2 and CsSGRL were localized in the chloroplast, membrane and nucleus. The RT-qPCR results showed that the three genes in the matures of albino tea cultivars were expressed higher than in the green tea cultivar. However, only *CsSGR2*-overexpressing tobacco leaves exhibited a yellowish phenotype and significantly lower Fv/Fm values. *CsSGR1* and *CsSGR2* exhibited similar expression patterns in different tissues after infection with the pathogen *Colletotrichum camelliae*, which was opposite to the pattern observed for *CsSGRL*. In addition, *CsSGR1* was significantly induced in response to cold stress, SA, JA and ABA in *C. camelliae*. These findings identified valuable candidate genes for elucidating the mechanism of leaf albinism, stress response and phytohormone signaling in tea plants.

**Keywords:** *Camellia sinensis*; CsSGR; chlorophyll; stress; phytohormone



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## 1. Introduction

Chlorophylls (Chls), which mainly include Chl *a* and Chl *b*, are a class of tetrapyrrole molecules and indispensable for light energy absorption and electron transfer during photosynthesis [1]. Changes in leaf or fruit color, shifting from green to yellow or red, often coincide with the depletion of Chl [2,3]. Notably, excess Chl and its derivatives are potential cellular phytotoxins and must be rapidly degraded [4,5]. The *STAY-GREEN* (*SGR*) gene, crucial for Mg-dechelatase, plays a pivotal role in extracting central Mg from Chl *a* [6]. SGR1 and SGRL can interact with light-harvesting complex II (LHCII) and chlorophyll catabolic enzymes (CCEs) to form the SGR1/SGRL-CCE-LHCII complex, which accelerates Chl degradation and mitigates the damage caused by catabolites to cell membranes [2,5,7].

Armstead et al. were the first to identify the gene governing Mendel's green cotyledon trait in *Arabidopsis thaliana*, *Pisum sativum* and *Festuca pratensis* [8], which is now called *STAY-GREEN 1* (*SGR1*) or *NONYELLOEING1* (*NYE1*). Subsequently, *SGR* homologs were widely

identified in other plants, such as *Oryza sativa* [9], *Solanum lycopersicum* [10], *Capsicum annuum* [10], *Medicago truncatula* [11], *Glycine max* [12], *Cucumis melo* L. [13] and *Citrus sinensis* [14]. The SGR family is categorized into two subfamilies: the SGR and SGR-like (SGRL) subfamilies. All SGR homologs are localized in chloroplasts and contain chloroplast transit peptides, conserved SGR domains and variable C-terminal regions [2,15]. The distinguishing feature between SGR and SGRL lies in the presence of a cysteine-rich motif (CRM, C-X3C-X-C2-F-P-X5-P), essential for Mg dechelatase activity and significant in Chl degradation, which is found in the C-terminus of most SGR proteins but absent in SGRL proteins [13,15].

SGR homologs primarily participate in regulating Chl degradation, carotenoid metabolism, fruit ripening and leaf senescence [2,14,16]. Within the *Arabidopsis* genome, three *SGR* members exist as the following: *SGR1/NYE1*, *SGR2* and *SGRL*. *SGR1/NYE1* modulates pheophorbide *a* oxygenase activity to facilitate Chl degradation during plant senescence [17]. This process is regulated by the NAC016 transcription factor, which can bind to the promoter of *SGR1* [7]. In contrast, the *SGR2*-overexpressing line remains green, and the *sgr2-1* mutant displays early leaf yellowing [5]. However, Wu et al. [18] reported that *SGR2* promotes Chl degradation. SGRL contributes to Chl breakdown or turnover in pre-senescent leaves [5]. In the melon, the *CmSGR1*- and *CmSGR2*-overexpressing lines exhibited fruit ripening and leaf yellowing, while the suppression of *CmSGR1* and *CmSGR2* delayed the degradation of Chl [13]. In the tomato, the inhibition of *SlSGR1* in transgenic fruits elevated the accumulation of lycopene and β-carotene [19]. In citrus, *SGRa* plays a positive role in Chl degradation, and *SGRa* and *SGRb* play negative roles in carotenoid biosynthesis [14]. In addition, studies also showed that *SGR* genes play roles in plant resistance. A candidate *SGR* gene for anthracnose was identified in a Gy14 cucumber [20]. Amino acid substitution at position 108 of the SGR protein is accountable for disease resistance in the Gy14 cucumber [21]. In *Medicago truncatula*, the *sgr* mutant showed enhanced defense responses to Asian soybean rust [22].

The tea plant (*Camellia sinensis* (L). O. Kuntze) stands as an economically vital perennial woody species, revered for its leaves being processed into tea for drinking. Albino leaf occurrences in tea plants often stem from Chl deficiency [23]. Typically, plant leaf albinism is viewed as an aberrant phenotype leading to yield reduction and heightened vulnerability to pathogens and pests [23]. However, albino leaves often confer tea with a relatively high amino acid content and good flavor [24]. To date, several studies have revealed that *SGR* genes play important roles in tea leaf albinism. Transcriptional profiling revealed that the expression of the *SGR* gene was greater in the chlorina shoots than in green shoots of tea plants [23,25,26]. The CsSGR-CssHSP-CsLHCII complex potentially regulates the albino phenotype of tea leaves [27]. Nevertheless, the comprehensive genomic identification of *SGR* genes and their prospective functionalities await further elucidation.

Therefore, to identify *SGR* gene members and investigate their potential roles in tea plants, the genome-wide identification and cloning of *CsSGRs* was conducted in this study. The evolutionary relationships, protein characteristics, subcellular localizations, expression patterns and functions of CsSGRs in Chl degradation were analyzed. Our study identified *CsSGR* genes in tea plants and provides valuable insights into their potential roles. The results will lay the foundation for further functional elucidation of CsSGR members, which will be helpful for breeding elite tea cultivars with albino or other desirable phenotypes.

## 2. Materials and Methods

### *2.1. Plant Materials and Treatments*

Tissues from various parts of tea plants, including the buds, leaves from different positions (1st, 2nd, 3rd and 4th leaves) and stems, were gathered for RT-qPCR analysis from 5-year-old '*Longjing 43*' (LJ43) tea plants cultivated at the Tea Research Institute of the Chinese Academy of Agricultural Science in Hangzhou, China. Given the association of *SGR* genes with Chl degradation during leaf senescence, mature leaves from a green tea cultivar

('*Longjing43*'), light-sensitive albino tea cultivars ('*Zhonghuang 1*' and '*Huangjinya*') and a temperature-sensitive albino tea cultivar ('*Baiye 1*') were collected for RT-qPCR analysis.

For biotic stress induction, 5-year-old 'LJ43' tea plants, grown in a greenhouse for a month, were subjected to acupuncture and inoculated with a conidial suspension of *C. camelliae*, as previously described [28]. Samples were gathered at 0, 6, 12, 24, 48 and 72 h post-treatment, while a mock group was inoculated with sterile water. For cold treatment, the 'LJ43' tea plants were exposed to either 4 °C or 22 °C. Young leaves were collected at 6, 12, 24 and 48 h post-treatment. Regarding jasmonic acid (JA), salicylic acid (SA) and abascisic acid (ABA) treatments, detached branches of 'LJ43' were sprayed with 150 μg $mL^{-1}$ JA, 1 mM SA and 100 μM ABA [29]. One bud and one leaf were collected at 1, 3, 6, 12 and 24 h post-treatment, with samples sprayed with sterile water serving as controls. Each experiment utilized three independent biological replicates.

### 2.2. Identification and Bioinformatics Analysis of SGR Genes in Tea Plants

All sequences pertaining to tea plants were sourced from the Tea Plant Information Archive (TPIA, http://tpia.teaplants.cn/index.html, accessed on 4 November 2023). SGR protein sequences from *Arabidopsis thaliana* and *Solanum lycopersicum* [2] were queried via the TPIA online tool with BLASTp (e-value $\leq 1 \times 10^{-10}$) against the '*Longjing 43*', '*Shuchazao*' and '*Tieguanyin*' databases to pinpoint tea plant *CsSGR* genes. Furthermore, the Pfam database (http://pfam.xfam.org, accessed on 4 November 2023) and the Conserved Domain Database (CDD, https://www.ncbi.nlm.nih.gov/cdd, accessed on 4 November 2023) were utilized to verify that the identified genes harbored a stay-green domain. Additionally, the conserved motifs within CsSGR proteins were determined using the MEME database (https://meme-suite.org/meme/, accessed on 4 November 2023). A phylogenetic tree was constructed using MEGA version 11 software employing the neighbor-joining method with 1000 bootstrap trials. Multiple sequence alignment of SGR proteins was conducted using DNAMAN. Lastly, the PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/, accessed on 7 November 2023) was employed to analyze *cis*-acting regulatory elements within the promoters.

### 2.3. Cloning and RT-qPCR of CsSGR1, CsSGR2 and CsSGRL

All samples were promptly frozen in liquid nitrogen, followed by storage at −80 °C for subsequent analyses. Total RNA extraction from tea samples was performed using a FastPure Plant Total RNA Isolation Kit (Polysaccharides & Polyphenolics-Rich) (Nanjing Vazyme Biotech Co., Ltd., Nanjing, China) according to the manufacturer's instructions. Subsequently, 1 μg of total RNA was utilized for first-strand cDNA synthesis by employing the HiScript II 1st Strand cDNA Synthesis Kit (with gDNA wiper) (Nanjing Vazyme Biotech Co., Ltd., Nanjing, China). The cDNA from 'LJ43' leaves served as a template for amplifying the CDS of *CsSGR1*, *CsSGR2* and *CsSGRL* using Phanta® Max ultra-fidelity DNA polymerase (Nanjing Vazyme Biotech Co., Ltd., Nanjing, China). RT-qPCR was performed following established protocols [30,31], with the *CsPTB* reference gene serving as the internal control [32]. The primer sequences utilized for gene cloning and RT-qPCR are detailed in Supplementary Table S1.

### 2.4. Subcellular Localization of CsSGR1, CsSGR2 and CsSGR

To elucidate the subcellular localization of CsSGR1, CsSGR2 and CsSGRL, their ORFs lacking stop codons were integrated into the pCAMBIA2300-35S::GFP vector, resulting in the 35S::CsSGR1-GFP, 35S::CsSGR2-GFP and 35S::CsSGRL-GFP vectors. These recombinant vector plasmids were subsequently introduced into the *Agrobacterium* strain GV3101 and transiently expressed in *Nicotiana benthamiana* leaves. After 48 h, fluorescence signals were assessed utilizing a laser scanning confocal microscope (LSM 980; Zeiss, Oberkochen, Germany). The primers employed for constructing recombinant vectors are detailed in Supplementary Table S1.

### 2.5. Measurement of Fv/Fm

The subcellular localization vectors were used for transient overexpression analysis. The entire leaves of *Nicotiana benthamiana* were inoculated with the pCAMBIA2300, 35S::CsSGR1-GFP, 35S::CsSGR2-GFP and 35S::CsSGRL-GFP vectors via the *Agrobacterium tumefaciens*-mediated method. After 48 h, the inoculated plants were subjected to a 20 min dark period, following which the Fv/Fm of the transformed leaves was measured using a FluorCam 7 (Photon Systems Instruments, Banbury, UK) [33]. The PAM mode was used for Fv/Fm analysis. For Fo measurement, the duration was set as 5.04 s. For Fm measurement, the pulse duration and intensity were set as 0.8 s and 20%, respectively. Each treatment was conducted with nine independent biological replicates.

### 2.6. Statistical Analysis

The data in this study are depicted as the means ± SEMs of at least three biological independent replicates. Statistical analyses were conducted using SPSS version 27 software, employing ANOVA followed by the LSD test or *t*-test. Statistical significance is denoted by asterisks (* $p < 0.05$ and ** $p < 0.01$). Figures were generated using GraphPad Prism 8 and Adobe Photoshop 23.0.

## 3. Results

### 3.1. Identification, Cloning and Evolutionary Analysis of CsSGRs in Tea Plants

In this study, a total of six, putative, full-length genes encoding stay-green proteins were identified via BLASTp searches of AtSGR and SlSGR protein sequences against the tea plant genome and verified via CDD and MEME analyses. Three of the six CsSGRs were classified into the SGR subfamily, and TEA010303 and GWHPASIV042313 shared 100% similarity in protein sequences. The other three CsSGRs were classified into the SGRL subfamily, and GWHTACFB016019 and GWHTASIV012516 shared 94.12% similarity in protein sequences (Supplementary Figure S1; Supplementary Table S2). According to these reference sequences, multiple specific primers were designed and RT-PCR was performed using cDNA from the '*Longjing 43*' tea cultivar as a template to clone *CsSGR* genes. Finally, three *CsSGR* genes were obtained and their protein sequences were used to construct a phylogenetic tree to explore the evolutionary relationships of CsSGR homologs. The two CsSGR proteins that were most closely related to VvSGR1 were classified into the SGR subfamily and named CsSGR1 and CsSGR2. One protein that shared the highest homology with SlSGRL and NtSGRL was divided into the SGRL subfamily and named CsSGRL (Figure 1A).

### 3.2. Sequence and Conserved Element Analysis of CsSGR1, CsSGR2 and CsSGRL in Tea Plants

To explore the structural features and functional differentiation of CsSGR1, CsSGR2 and CsSGRL, we analyzed their sequences and conserved elements. CsSGR1, CsSGR2 and CsSGRL consisted of 216, 286 and 260 amino acid residues, respectively (Figure 2). All three members contained a chloroplast transmit peptide, an SGR domain and a variable C-terminal region (Figure 1B, Figure 2). Conserved motif analysis indicated that CsSGR2 possesses a cysteine-rich motif (CRM) in the C-terminus (Figure 1C–motif 9, Figure 2), which is crucial for Mg dechelatase-mediated Chl degradation [2,15], but this motif was absent in CsSGR1 or CsSGRL. These results suggest that CsSGR2 is more likely to participate in Chl degradation.

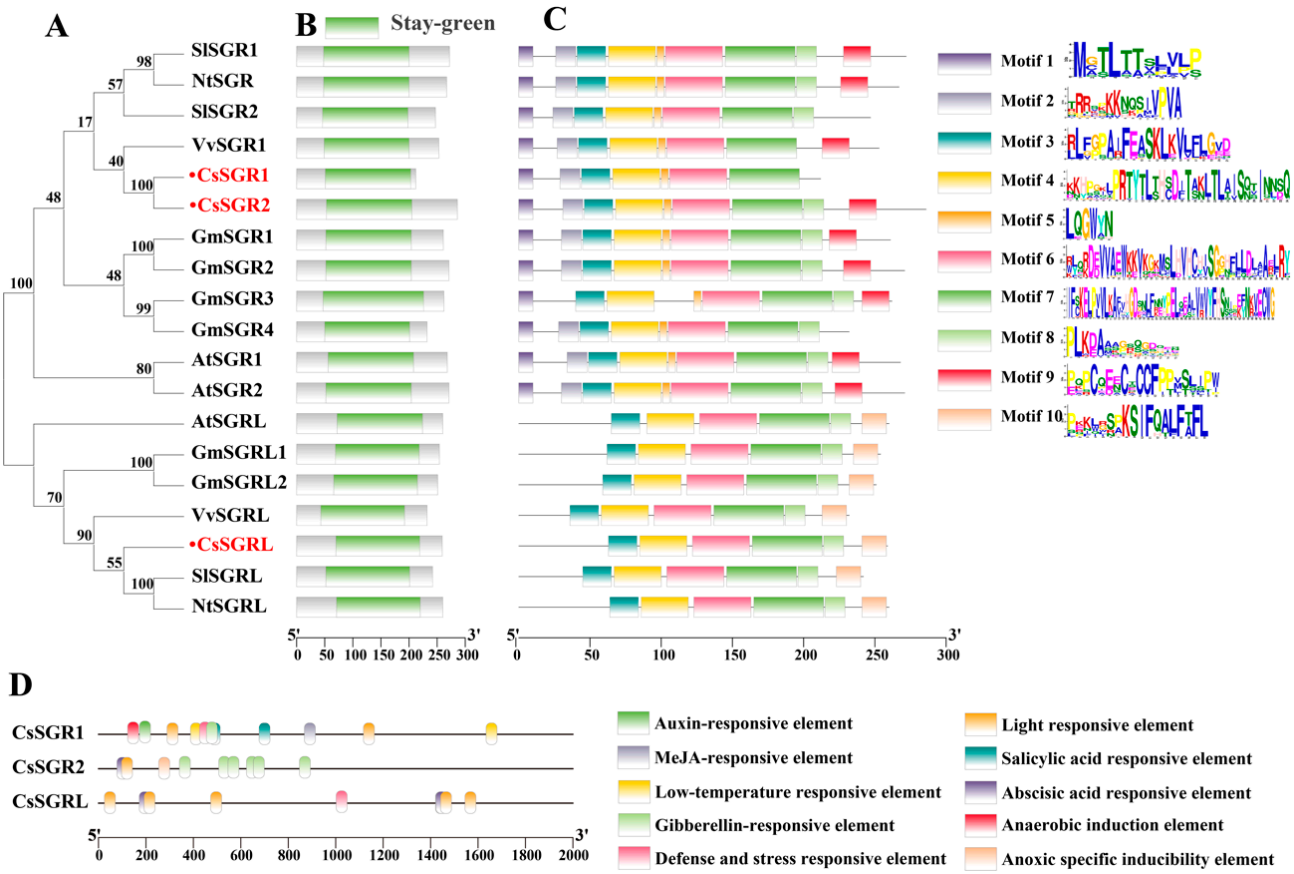


**Figure 1.** Phylogenetic tree and structural characteristics of the SGRs. (**A**) Phylogenetic analysis of SGR proteins. The analyzed sequences were the following: AtSGR1 (NP_001328989.1), AtSGR2 (NP_192928.2) and AtSGRL (NP_564489.1) of *Arabidopsis thaliana*; NtSGR (ABY19382.1) of *Nicotiana tabacum*; NtSGRL (XP_009602525.1) of *Nicotiana tomentosiformis*; VvSGR1 (CBI34771.3) and VvSGRL (CBI18940.3) of *Vitis vinifera*; GmSGR1 (NP_001238357.1), GmSGR2 (NP_001236690.1), GmSGR3 (XP_003550881.2), GmSGR4 (XP_003549871.1), GmSGRL1 (XP_003523416.1) and GmSGRL2 (NP_001351655.1) of *Glycine max*; and CsSGR1, CsSGR2 and CsSGRL of *Camellia sinensis*. (**B**) Conserved domains of the SGR proteins. (**C**) Conserved motifs of the SGR proteins. (**D**) *Cis*-acting regulatory elements of the *CsSGR1*, *CsSGR2* and *CsSGRL* promoters.

### *3.3. Cis Element Analysis of CsSGR Promoters*

The *cis*-acting regulatory elements were analyzed using 2000 bp promoter sequences upstream of the coding region to explore the potential functions of CsSGR1, CsSGR2 and CsSGRL. The results showed that light- and phytohormone-responsive elements were present in *CsSGR1* (light-, auxin-, salicylic acid-, MeJA- and gibberellin-responsive elements), *CsSGR2* (light-, abscisic acid- and gibberellin-responsive elements) and *CsSGRL* (light-, MeJA- and abscisic acid-responsive elements). In addition, stress-related responsive elements such as low-temperature, defense and anaerobic elements were present in the *CsSGR1* promoter, while anoxic-specific inducibility elements were present in the *CsSGR2* promoter, and defense elements were present in the *CsSGRL* promoter (Figure 1D; Supplementary Table S3). These results suggest that in addition to being involved in photosynthetic processes, *CsSGR1*, *CsSGR2* and *CsSGRL* might be involved in stress response processes and participate in phytohormone signaling pathways.

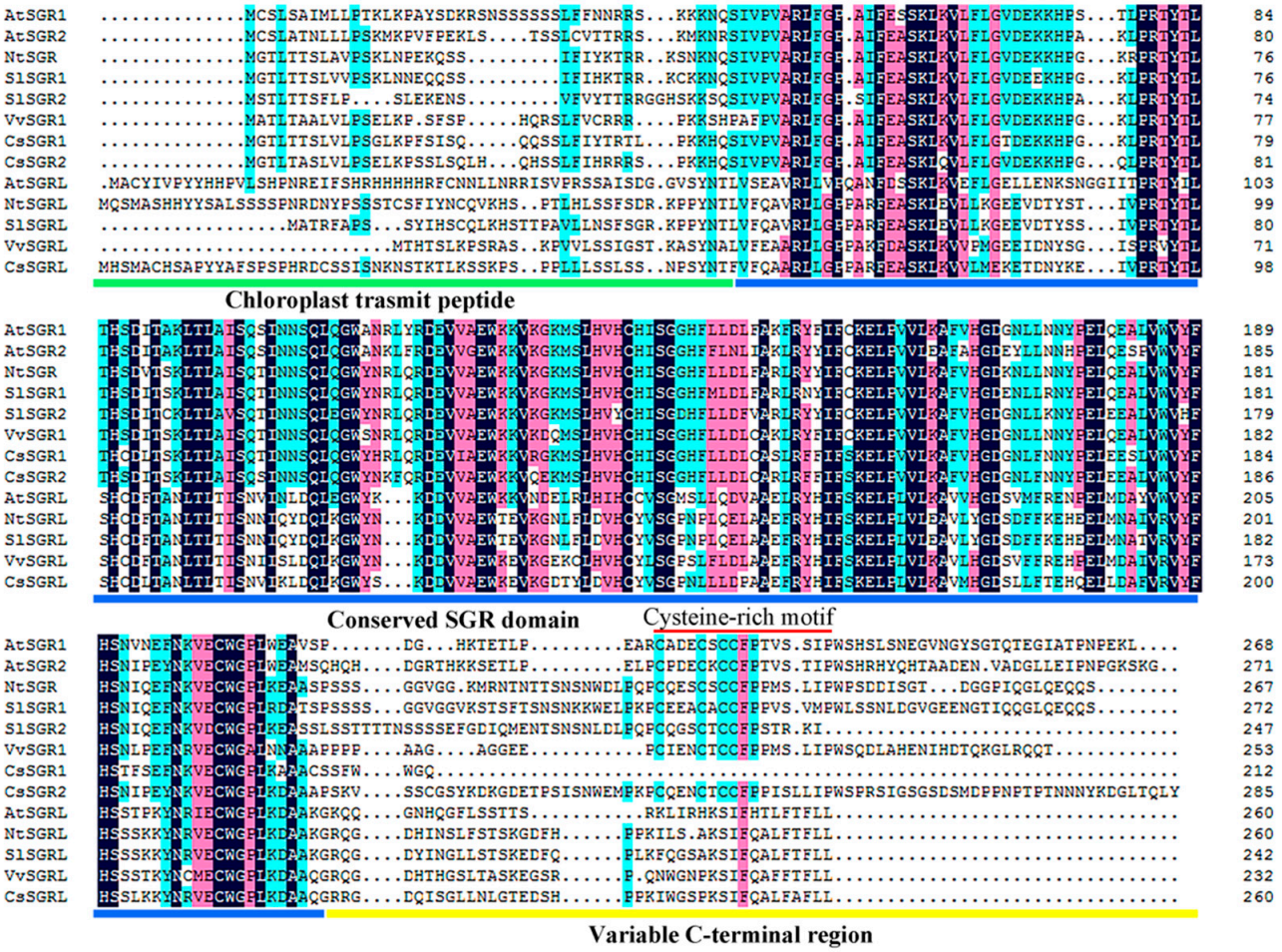


**Figure 2.** Multiple sequence alignment of SGR proteins. The chloroplast transmit peptide (green), conserved SGR domain (blue) and variable C-terminal region (yellow) are shown using three rectangles. The cysteine-rich motif is marked using a red line. Dark blue, pink and light blue shading represent 100%, 75% and 50% sequence identities, respectively.

### *3.4. Subcellular Localization of CsSGR1, CsSGR2 and CsSGRL*

To investigate the subcellular localizations and potential functions of CsSGR1, CsSGR2 and CsSGRL, their coding sequences without stop codons were inserted into the pCAMBIA2300-35S::GFP vector. The resulting recombinant plasmids, namely 35S::CsSGR1-GFP, 35S::CsSGR2-GFP and 35S::CsSGRL-GFP, were transiently expressed in H2B-RFP transgenic *N. benthamiana* leaves via *Agrobacterium tumefaciens*-mediated transformation [34]. The GFP fluorescence signal of the vector control (35S::GFP) was observed throughout the cell. CsSGR1, CsSGR2 and CsSGRL were localized in the chloroplast. In addition, CsSGR2 and CsSGRL were also detected in the membrane and nucleus (Figure 3). These results further imply the potential functions of CsSGR1, CsSGR2 and CsSGRL in Chl degradation or leaf photosynthesis.

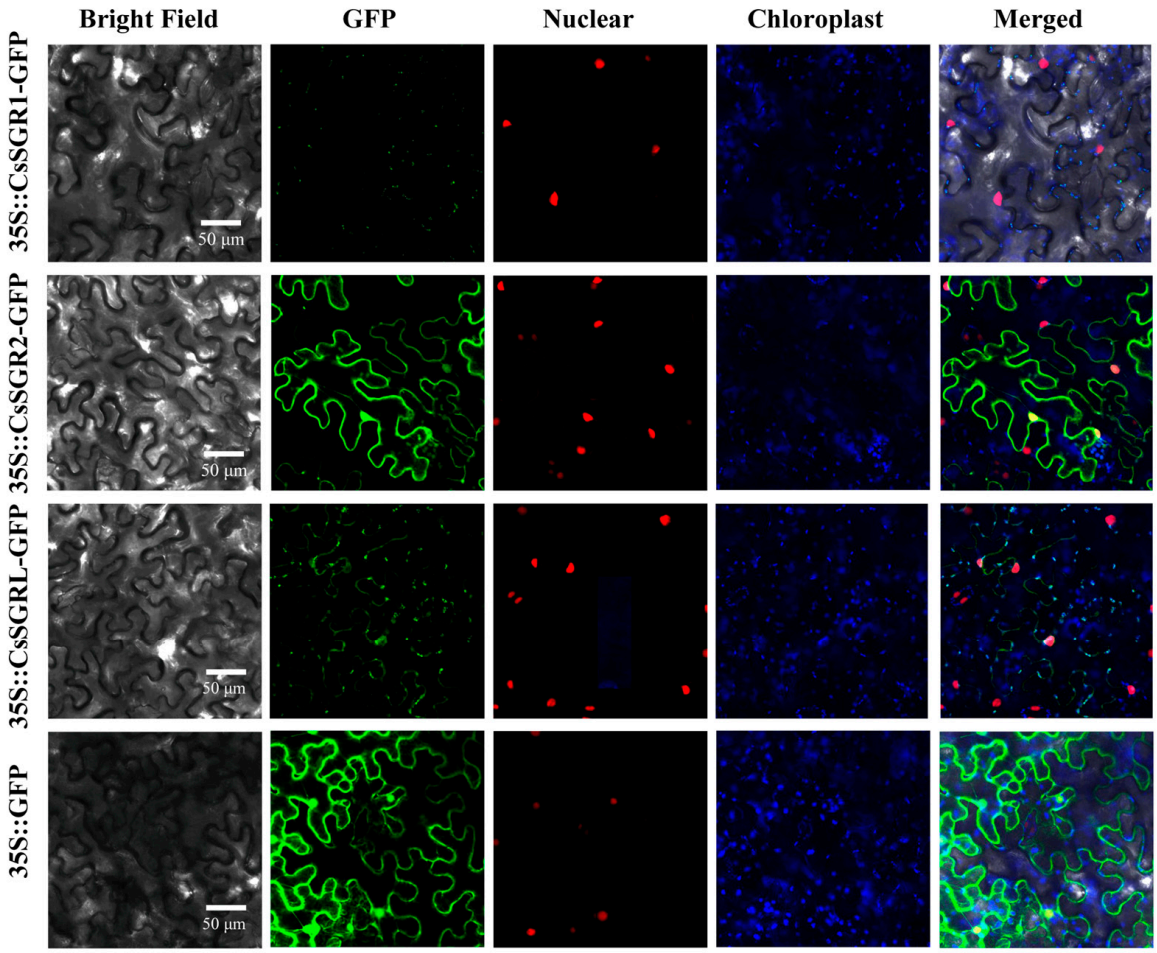


**Figure 3.** Subcellular localization of the *CsSGR1*, *CsSGR2* and *CsSGRL* proteins in H2B-REP transgenic *Nicotiana benthamiana* leaves. The 35S::GFP served as a positive control. Nuclei are depicted in red, while chloroplasts are represented in blue. Scale bar = 50 μm.

### *3.5. Expression Patterns of CsSGR1, CsSGR2 and CsSGRL in Different Tea Cultivars and Tissues*

The *SGR* genes play crucial role in Chl degradation, closely linked to the leaf albinism in tea plants. Consequently, the expression levels of *CsSGR1*, *CsSGR2* and *CsSGRL* were examined in the mature leaves of the green tea cultivar (‘*Longjing43*’) and light-sensitive albino tea cultivars (‘*Zhonghuang 1*’ and ‘*Huangjinya*’), as well as the temperature-sensitive albino tea cultivar (‘*Baiye 1*’). The results showed that compared with those in ‘*Longjing 43*’, the expression patterns of *CsSGR1*, *CsSGR2* and *CsSGRL* were similar and were greatest in the mature leaves of ‘*Baiye 1*’, followed by ‘*Huangjinya*’ and ‘*Zhonghuang 1*’ mature leaves (Figure 4A,B), suggesting that these genes may play important roles in the albinism of tea leaves.

Furthermore, the expression patterns of *CsSGR1*, *CsSGR2* and *CsSGRL* were examined across various tissues of tea plants. The RT-qPCR results revealed that the expression patterns of *CsSGR1* and *CsSGR2* were similar, contrasting with that of *CsSGRL*. *CsSGR1* and *CsSGR2* were highly expressed in the stem (S), followed by the first leaf (L1), the fourth leaf (L4), the second leaf (L2) and the third leaf (L3), while those of *CsSGR2* were S > L1 > L4 > L3 > L2. *CsSGRL* was highly expressed in the tender tissues, including the L1 and L2, followed by the L3, bud, L4 and stem (Figure 4C). These findings suggest that the three genes may fulfill distinct roles in the development of various tissues.

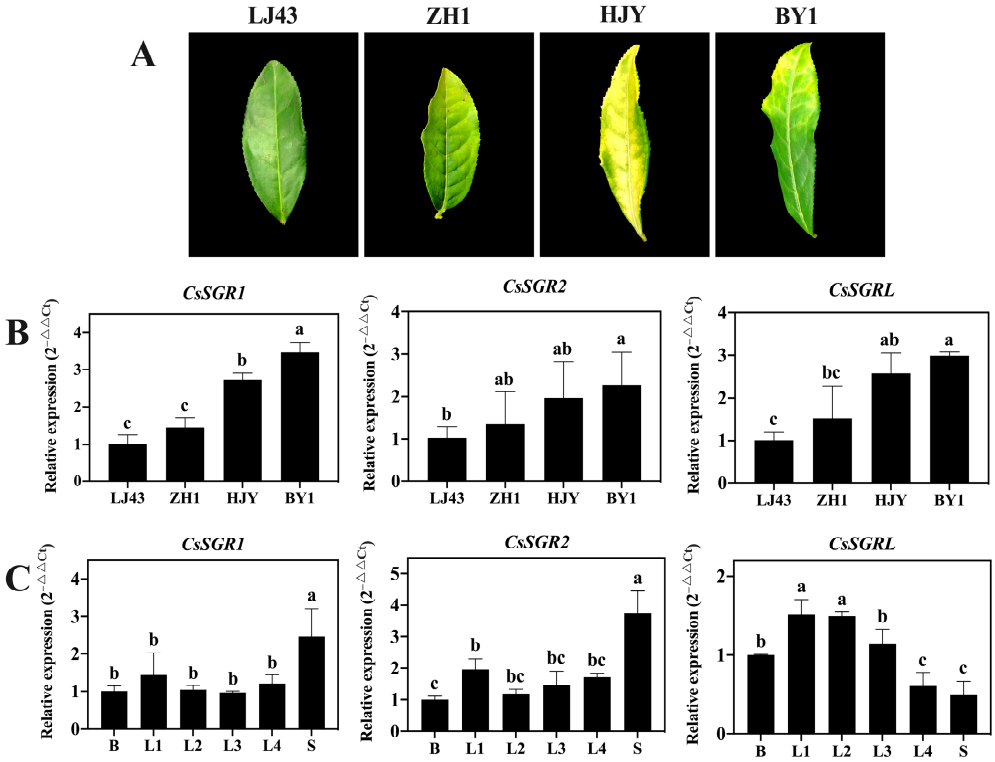


**Figure 4.** Expression profiles of *CsSGR1*, *CsSGR2* and *CsSGRL* in different tea cultivars and tissues. (**A**) The phenotypes of '*Longjing 43*' (LJ43), '*Zhonghuang 1*' (ZH1), '*Huangjinya*' (HJY) and '*Baiye 1*' (BY1) mature leaves. (**B**) Expression levels of *CsSGR1*, *CsSGR2* and *CsSGRL* in 'LJ43', 'ZH1', 'HJY' and 'BY1'. (**C**) Expression levels of *CsSGR1*, *CsSGR2* and *CsSGRL* in different tea tissues. Buds (B), first leaves (L1), second leaves (L2), third leaves (L3), fourth leaves (L4) and stems (S). Different lowercase letters above columns indicate significant differences at the $p < 0.05$ level using the LSD test. Three independent biological replicates were performed for each treatment.

### *3.6. Expression Patterns of CsSGR1, CsSGR2 and CsSGRL under Various Stresses and Phytohormone Treatments*

Stress-related and phytohormone-responsive elements were detected in the promoters of *CsSGR1*, *CsSGR2* and *CsSGRL*. To determine the potential functions of the three genes, RT-qPCR analyses were performed on tea plants subjected to biotic stress (*C. camelliae*), abiotic stress (low temperature) and phytohormones (SA, JA and ABA) treatment (Figure 5). *CsSGR1* and *CsSGR2* were significantly induced by *C. camelliae* infection and by JA and ABA treatment. In addition, *CsSGR1* was also significantly induced at 48 h under 4 °C treatment, while the expression of *CsSGR2* was repressed at 6, 12 and 24 h and increased at 48 h under 4 °C treatment. The expression of the *CsSGRL* gene significantly increased at 6 and 12 h but decreased at 24, 48 and 72 h after treatment with *C. camelliae*. There were no significant changes in the expression of *CsSGRL* under low-temperature or phytohormone treatments. These results indicate that *CsSGR1* and *CsSGR2* might play roles in the response to biotic and abiotic stresses and that phytohormones may be involved in these processes.

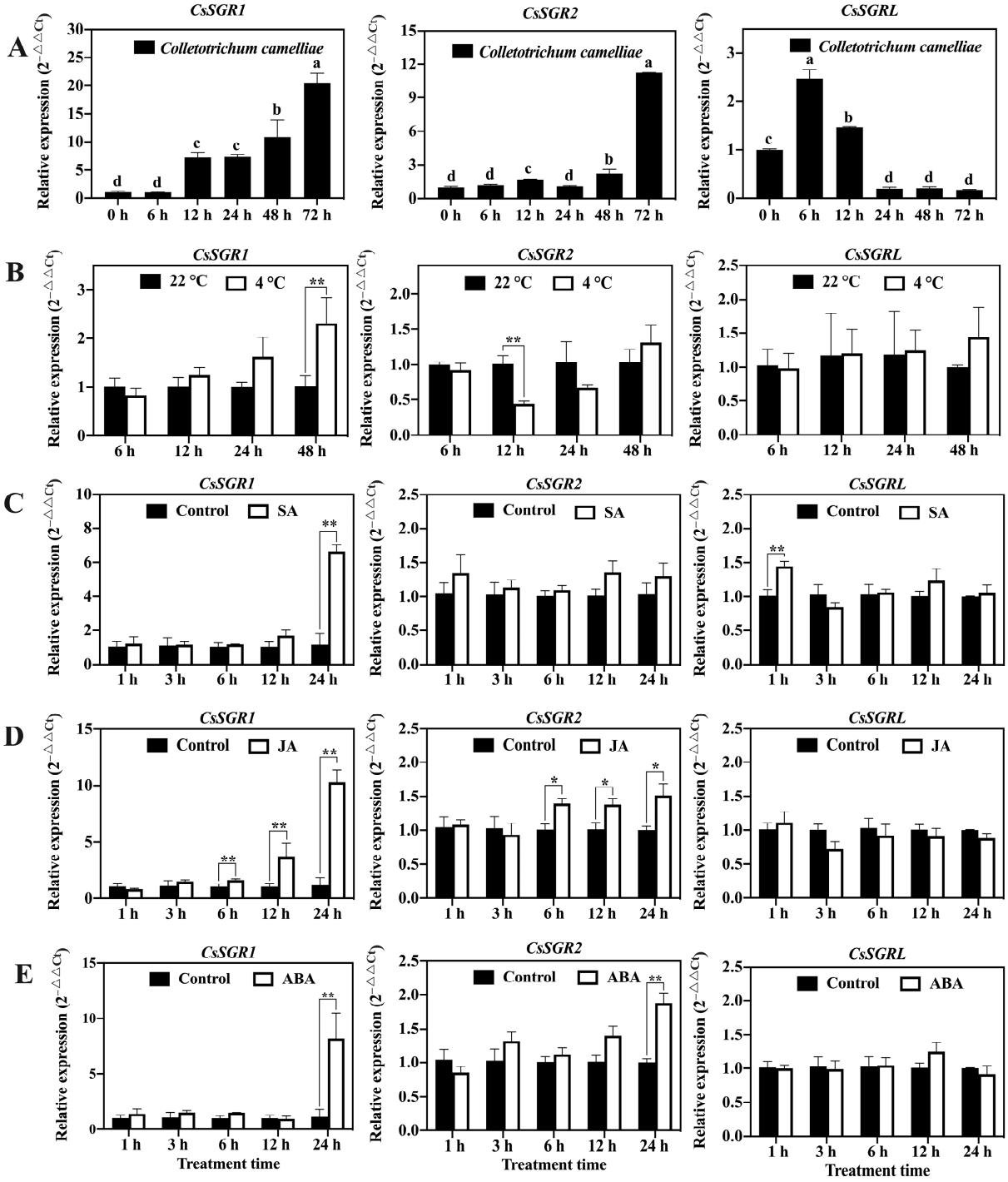


**Figure 5.** Expression profiles of *CsSGR1*, *CsSGR2* and *CsSGRL* in response to biotic and abiotic stress and phytohormones. (**A**,**B**) Expression levels in tea leaves after treatment with *C. camelliae* and cold (4 °C) stress. (**C**–**E**) Expression levels in tea leaves in response to exogenous 150 μg $mL^{-1}$ JA, 1 mM SA and 100 μM ABA treatment. The data are shown as the means ± SEs (n = 3). Different lowercase letters above columns indicate significant differences at the $p < 0.05$ level using the LSD test. * and ** denote significant differences at the $p < 0.05$ and $p < 0.01$ levels using the *t*-test, respectively. Three independent biological replicates were performed for each treatment.

### *3.7. Functions of CsSGR1, CsSGR2 and CsSGRL in Chlorophyll Degradation*

To determine the functions of *CsSGR1*, *CsSGR2* and *CsSGRL* in Chl degradation, transient overexpression of *CsSGR1*, *CsSGR2* and *CsSGRL* was performed in tobacco leaves. Compared with those of a wild type, pCAMBIA2300, 35S::*CsSGR1* and 35S::*CsSGRL* leaves,

35S::*CsSGR2* tobacco leaves exhibited a yellowish phenotype and significantly lower Fv/Fm values (Figure 6). Notably, compared with *CsSGR1* and *CsSGRL*, *CsSGR2* plays important roles in Chl degradation.

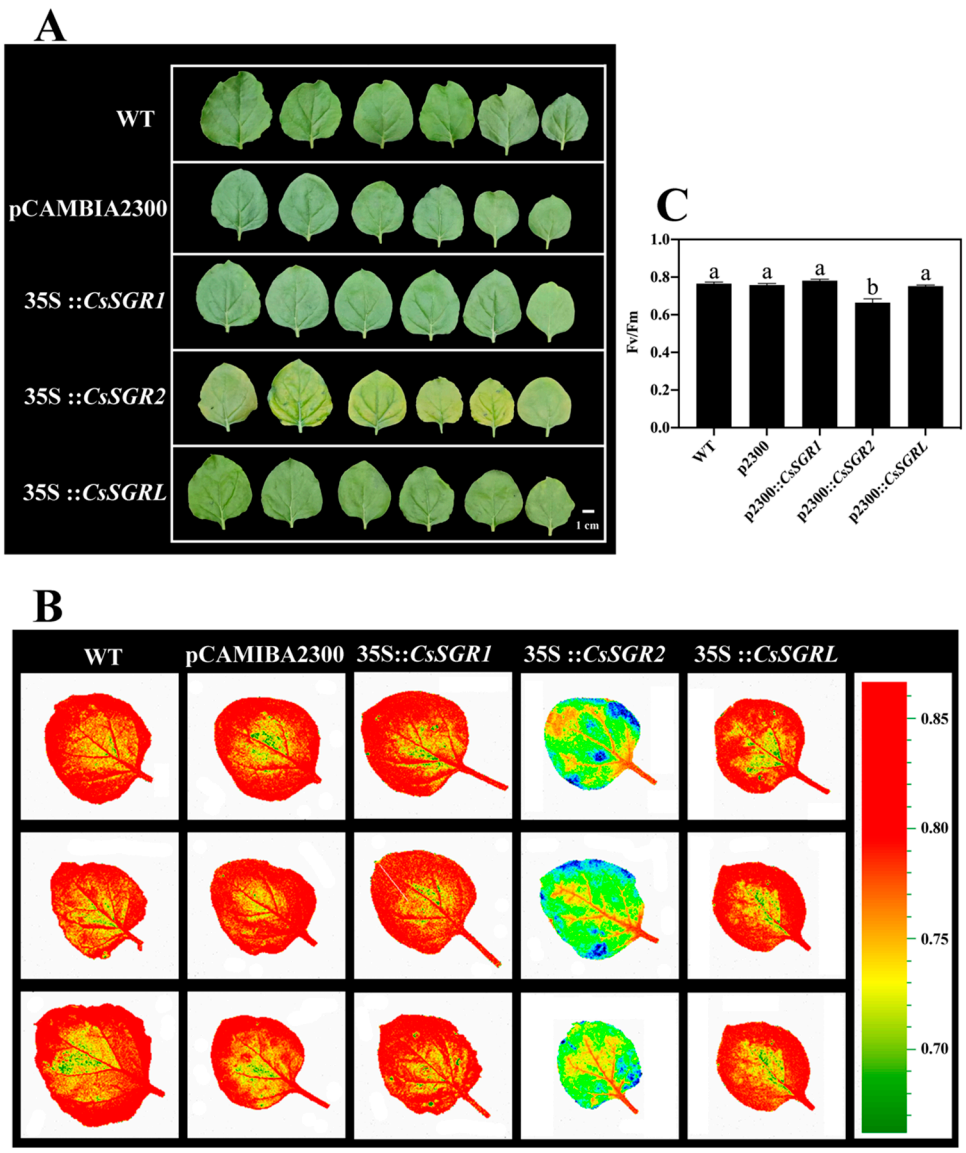


**Figure 6.** Transient overexpression of *CsSGR1*, *CsSGR2* and *CsSGRL* in tobacco leaves. (**A**) Phenotypes of tobacco leaves. (**B**) Chlorophyll fluorescence images. (**C**) Fv/Fm values in tobacco leaves. Different lowercase letters above columns indicate significant differences at the $p < 0.05$ level using the LSD test. Nine independent biological replicates were performed for each treatment.

## 4. Discussion

SGR homologs play important roles in Chl degradation during leaf senescence and fruit ripening. In recent years, several studies have revealed that *SGR* genes are involved in leaf albinism in tea plants via transcriptomic profiling. However, comprehensive studies on *SGR* genes in *C. sinensis* are lacking. In our study, we screened and cloned three *SGR* members in tea plants. Phylogenetic analysis indicated that these genes could be divided into the SGR and SGRL subfamilies, which were named CsSGR1, CsSGR2 and CsSGRL, respectively. All members were found to localize in chloroplasts and featured a chloroplast transit peptide, an SGR domain and a variable C-terminal region, consistent with observations in other plant species [2,15]. Notably, the cys-rich motif (CRM, C-X3-C-X-C2-F-P-X5-P), crucial for Mg-dechelatase activity, is commonly present in most SGR proteins

but absent in SGRL proteins [2]. Our MEME and multiple sequence alignment results revealed that the CRM motif was present in the CsSGR2 protein but not in the CsSGR1 and CsSGRL proteins. The nonexistence of the CRM motif was also found in the CmSGR3 and CmSGR4 proteins of *Cucumis melo* L. [13] and the GmaSGR4 protein of *Glycine max* [2]. Due to the Chl degradation function of CRM, CsSGR2 may have a greater ability to mediate Chl degradation than *CsSGR1* and *CsSGRL*. The 35S::*CsSGR2* tobacco leaves exhibited a yellowish phenotype and significantly lower Fv/Fm values in our study, further suggesting that the CRM motif is important for Chl degradation. Notably, although the CRM motif is absent in SGR or SGRL proteins, these proteins may be recruited or activated by maturity, stress or phytohormones to mediate Chl degradation [35]; however, this mechanism needs to be further studied in tea plants. In addition, *SGR* and *SGRL* may function at different stages of plant development in *Arabidopsis*, *SGR1* functions in Chl degradation during natural senescence and the expression level of *SGR1* mainly increases with tissue aging, and *SGRL* may play a vital role in Chl turnover under pre-senescence conditions with a high expression level in green leaves [2]. The expression patterns of *CsSGR* and *CsSGRL* in tea plants are similar to those in *Arabidopsis*, indicating that *CsSGR* and *CsSGRL* may play different roles in Chl degradation at different stages of tea senescence.

Traditionally, SGR proteins are responsible for the extraction of central Mg from Chl *a*, which results in Chl degradation. CRM, which participates in inter- or intramolecular crosslinking or redox regulation, is responsible for Chl degradation [15]. However, the presence or absence of CRM results in different functions in different species. Early termination of *SGR* led to a lack of CRM and resulted in a stay-green trait in pak choi [36,37]. In citrus, the overexpression of truncated $SGRa^{stop}$ resulted in no obvious Chl degradation [14]. Interestingly, we found that the length of CsSGR1 (216 amino acid residues) in tea plants was shorter than that of most other SGR proteins, and the CRM motif was also absent in CsSGR1. It may be that the truncated version of SGR possesses different functions compared with traditional SGR homologs. Although our study revealed significantly higher expression of *CsSGR1* in '*Huangjinya*' and '*Baiye 1*' leaves compared to '*Longjing 43*' leaves, the 35S::*CsSGR1* tobacco leaves displayed a phenotype similar to that of the wild-type plants. These findings suggest that *CsSGR1* may not have a decisive role in Chl degradation, and the absence of CRM deficiency might contribute to this observation.

In addition to functioning in promoting Chl degradation by SGR homologs, recent studies have shown that they participate in biotic and abiotic stress resistance via the Chl degradation pathway. The central Mg of Chl *a* is extracted by Mg-dechelatase encoded by *SGR1* to form pheophytin *a* [6], which is subsequently catalyzed to pheophorbide *a* (Pheide *a*) and red Chl catabolite (RCC) [35]. Pheide *a* and RCC, which can potentially be phototoxic, have the ability to induce cell death or generate ROS (especially $H_2O_2$) which function as signaling molecules to mediate defense responses against pathogenic infection [21,35,38]. In addition, SGR can interact with chlorophyll's catabolic enzymes (CCEs) and light-harvesting complex II (LHCII) to form the SGR-CCE-LHCII complex, contributing to the detoxification of phototoxic catabolites [2]. In *Cucumis sativus* and *Medicago truncatula*, the mutation or silencing of *SGR* is responsible for anthracnose resistance by regulating the level of ROS and the expression of defense-related genes [20–22]. *LpSGR*-mediated Chl *a* catabolism is involved in ryegrass heat stress tolerance [39]. In addition, phytohormones such as ABA are involved in SGR-mediated Chl breakdown and other processes [2,30]. *Cis*-element analysis of the promoters revealed that phytohormones and stress-related responsive elements were present in the promoters of *CsSGR1*, *CsSGR2* and *CsSGRL*. Our RT-qPCR results indicated that the expression level of *CsSGR1* was significantly increased in response to treatment with *C. camelliae*, low temperature, SA, JA and ABA. The abundance of *CsSGR2* increased under treatment with *C. camelliae*, JA and ABA. However, the expression of *CsSGRL* significantly decreased in response to *C. camelliae*, which was opposite to what was observed for *CsSGR1* and *CsSGR2*. These findings suggest that *CsSGR* genes, especially *CsSGR1*, may play crucial roles in stress-related processes and phytohormone signaling pathways, which needs to be further investigated. Our results also found that the *CsSGR*

genes were expressed higher in the mature leaves of albino tea cultivars than those of the green tea cultivar. However, the resistance of the tea plant to stress is determined by many factors. Defects in chloroplast development and lower contents of secondary metabolites such as catechins and anthocyanins often present in albino tea cultivars may affect their stress tolerance [23,40]. Thus, the exact resistance function of *CsSGRs* in different tea cultivars remains to be clarified.

## 5. Conclusions

*CsSGR1*, *CsSGR2* and *CsSGRL* were identified and cloned from tea plants. The promoter of the three genes contained light-, phytohormone- and stress-related elements. CsSGR1 was localized in the chloroplast, while CsSGR2 and CsSGRL were localized in the chloroplast, membrane and nucleus. RT-qPCR showed that the expression patterns of *CsSGR1* and *CsSGR2* were increased with tissue aging, which was opposite to *CsSGRL*. The expression levels of the three genes were greater in the albino tea cultivars than in the green tea cultivar. However, only *CsSGR2*-overexpressing tobacco leaves exhibited a yellowish phenotype and significantly lower Fv/Fm values. In addition, *CsSGR1* was significantly induced in response to *C. camelliae*, cold stress, SA, JA and ABA treatments. This study provides candidate genes for the further functional elucidation of the mechanism of *CsSGRs* in the leaf albinism, stress response and phytohormone signaling of tea plants.

**Supplementary Materials:** The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy14040769/s1, Figure S1: Phylogenetic analysis of the putative tea CsSGRs with SGRs of other species.; Table S1: Primers used for the cloning of CsSGR1, CsSGR2 and CsSGRL; Table S2: The six candidate CsSGR homologs obtained from the tea genome via bioinformatic analysis; Table S3: The numbers of cis-elements in the promoters of CsSGR1, *CsSGR2* and *CsSGRL*.

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**Data Availability Statement:** The cloned sequences of *CsSGR1* (PP215819), *CsSGR2* (PP215820) and *CsSGRL* (PP215821) were deposited in the National Center for Biotechnology Information (NCBI) database.

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