Response to the Cold Stress Signaling of the Tea Plant (Camellia sinensis) Elicited by Chitosan Oligosaccharide

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Abstract: Cold stress caused by a low temperature is a significant threat to tea production. The application of chitosan oligosaccharide (COS) can alleviate the effect of low temperature stress on tea plants. However, how COS affects the cold stress signaling in tea plants is still unclear. In this study, we investigated the level of physiological indicators in tea leaves treated with COS, and then the molecular response to the cold stress of tea leaves treated with COS was analyzed by transcriptomics with RNA-Sequencing (RNA-Seq). The results show that the activity of superoxide dismutase (SOD) activity, peroxidase (POD) activity, content of chlorophyll and soluble sugar in tea leaves in COS-treated tea plant were significantly increased and that photosynthesis and carbon metabolism were enriched. Besides, our results suggest that COS may impact to the cold stress signaling via enhancing the photosynthesis and carbon process. Our research provides valuable information for the mechanisms of COS application in tea plants under cold stress.

Keywords: tea plant; cold stress; chitosan oligosaccharide; physiological response; transcriptome

1. Introduction

The tea plant (Camellia sinensis (L.) O. Kuntze) is one of the most important commercial beverage crops in the world and an important revenue source in tea-producing countries [1]. The tea production in over 50 countries has reached over 5.95 million tons on 4.1 million hectares around the world [2]. Among them, the cultivar ‘Anji Baicha’ is a special green-revertible albino mutant widely cultivated in China, especially in Zhejiang, Hubei and Guizhou provinces, which exhibits periodic albinism during the development of young shoots [3,4]. It is rare and represent precious tea germplasm because of its special flavor, and also has high levels of total amino acids and low levels of polyphenols, which differs from conventional tea [3–8]. In addition, it has a higher commercial value than green tea [4].

The tea plant can grow in different agroclimates and adapted to optimal temperature of 18 to 30 °C and pH ranging from 4.5 to 5.5, but the thermophilic nature of tea plants confines their growth to temperate area [9–11]. Furthermore, tea plants that are exposed to a low temperature, such as a sudden frost in fall or early spring, may be at risk of cold stress [12]. Cold environment can adversely affect tea plants on their growth, development, and spatial distribution with decreasing yield and quality, which is one of the factors restricting the healthy development of the tea industry [13–15]. So, it is significant to explore the ways to improve the cold resistance of tea plants. Some studies have reported that the cold resistance of tea plant can be effectively improved by cultivating cold-resistant tea plant
varieties (e.g., Fudingdabai, Shuchazao), cold acclimation of tea plant and the application of exogenous substances [16–19].

Chitosan oligosaccharide (COS) prepared from chitosan, is an environmentally friendly plant growth regulator and stress tolerance inducer [20–24]. Chitosan is a linear polysaccharide composed of β-1,4-glucosamines. The hydrolysis of the glycosidic chitosan chains yields oligosaccharides, including the water-soluble oligochitosan [21,22]. Chitosan and COS have a rich history of being researched for applications in agriculture, primarily for plant defense and yield increase [23,24]. As a natural biocontroller and elicitor of defense responses, COS can boost the innate ability of plants to defend themselves by stimulating secondary metabolite synthesis, and increasing the chlorophyll content and photosynthetic ability [20,21], enrich the soluble sugar in plant [25], and enhancing the activities of antioxidant enzymes [25–27]. COS stimulated the signaling pathways involved in disease resistance in rice [28], and its role in tobacco mosaic virus (TMV) resistance in Arabidopsis has been investigated [29]. And studies have shown that COS enhances carbon metabolism, nitrogen metabolism, photosynthesis, and defense against abiotic stress in plants [30]. As reported, COS was able to mitigate the effects of abiotic stresses in plant, including salt, cold and drought [25–27,31,32]. The mechanism of COS in increasing abiotic stress tolerances was summarized as: enhancing the activities of antioxidant enzymes [25], photosynthesis, and stimulate secondary metabolite synthesis [31]. For example, COS has been applied to wheat seedlings for improved chilling tolerance by enhancing antioxidant activities of superoxide dismutase (SOD) and peroxidase (POD) and increasing content of chlorophyll.

These physiological responses of plants elicited by COS are closely related to the regulation of plant gene expression. Transcriptome sequencing has been widely applied to tea plant, which is has the advantage of highly accurate, highly efficient and sensitive profiling in recent years [33]. RNA sequencing (RNA-Seq) technology for measuring transcriptomes of organisms can analyze genes related to abiotic and biotic stress responses, growth, development and metabolites [34–37], to improve our understanding of the molecular mechanism of the tea plant [13–16,38], and RNA-Seq will also be a valuable tool to reveal the role of exogenous substances in tea plant cold resistance.

Though many investigators provided valuable information to cold stress in tea plant, the action mode of COS eliciting responses to cold stress of tea plant is unclear. Therefore, in this report, we studied the effect of exogenous COS on the molecular mechanism of tea plant under low temperature stress. Herein, the physiological parameters of tea plants with and without COS-treatment were compared. The molecular response to cold resistance within tea plant was analyzed by RNA-Seq technology. This research improves the understanding of the cold resistance mechanism of COS-treated tea plant and provides important guidance for COS application under low temperature stress.

2. Materials and Methods

2.1. Plant Materials and Cold Treatments

Two-year-old albino tea cultivar (Camellia sinensis (L.) O. Kuntze cv. ‘Anji Baicha’) were used in the experiment from AnShun County, Guizhou Province, China. Additionally, the tea plants were transplanted into the plastic pot. Plants were grown in a growth chamber at the experimental of Guizhou University, Guizhou Province, China (16 h day/8 h night at 25 °C/20 °C and relative humidity of 70%). After a month, tea plants were treated with 10 mL of following elicitors by surface spraying with sterile distilled water (control, CK), or with 1.25 mL/L COS solution (COS comes from Hainan Zhengye Zhongnong High-tech Co., Ltd., Haikou, Hainan Province, China). After 24 h, the two groups of tea plants were separately maintained in a chamber at −4 and −8 °C at cold treatment for 24 h, with one group maintained under normal room temperature conditions. Three independent biological repeats were collected for each treatment. Fresh leaves from the stable stage (re-greening stage) of chlorophyll development of Anji Baicha were harvested at 24 h and frozen immediately in liquid nitrogen and stored at −80 °C for further study.
2.2. Physiological Response Assay

Physiological indexes of tea leaves (containing 1st, 2nd, 3rd leaf and old leaves), involving the activities of SOD and POD, and content of chlorophyll and soluble sugar, were determined. Additionally, the assay kits used included the SOD assay kit, the POD assay kit, the chlorophyll assay kit, the soluble sugar assay kit (Solarbio, Cat. No. BC0175, BC0095, BC0995, BC0035, respectively, Beijing, China). All assays were performed according to the manufacturer’s instructions.

2.3. cDNA Library Construction and Sequencing

We selected tea leaves from control and COS treatment on −4 °C for RNA-Seq analysis. Total RNA was extracted from tea leaves using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer’s instruction. Poly (A) + mRNA was purified with oligo (dT) beads. The mRNA was randomly cut into short fragments using Fragmentation Buffer, which were used as a template for the short fragment mRNA, first-strand cDNA was synthesized with 6 bp random primers, and then the Buffer, dNTPs and DNA polymerase I were added to synthesize the second-strand cDNA. RNA Integrity was confirmed using 1.5% agarose gel. RNA quality was checked by a NanoDrop™ OneC spectrophotometer (Thermo Fisher Scientific, New York, NY, USA). RNA qualified was measured by Qubit™ RNA BR Assay Kit in Qubit® 2.0 (Life Technologies, Carlsbad, CA, USA). The cDNA library construction and Illumina sequencing of the samples were performed using a 150 bp paired-end Illumina Nova-seq 6000 (Illumina, San Diego, CA, USA) by Seqhealth Technology Co., Ltd. (Wuhan, China).

2.4. RNA-Seq Data Analysis

The raw reads were first filtered to obtain the clean reads by removing the adaptor sequences, unknown sequences “N” and low-quality reads using Trimmomatic (version 0.36). After filtering, the clean reads were mapped to the reference genome of Camellia sinensis using STATR software (version 2.5.3a).

2.5. Identification of Differentially Expressed Genes

The expression levels of each gene were calculated and normalized by the corresponding Reads Per Kilobase of transcript per Million mapped reads (RPKM). The RPKM method can eliminate the influence of gene length and sequencing amount differences on gene expression. FeatureCounts (version 1.5.1) was used to count the read numbers mapped to each gene [39]. Additionally, differentially expressed genes (DEGs) were identified with the edge R package (version 3.12.1) [40]. The resulting p-values were adjusted using Benjamini and Hochberg’s method for controlling the false discovery rate (FDR). Genes with p-value < 0.05 and a logarithm two-fold change |log₂FC| > 1 were defined as DEGs.

2.6. Gene Ontology and KEGG Pathway Analysis

Gene ontology (GO) analysis and Kyoto encyclopedia of genes and genomes (KEGG) enrichment analysis of DEGs were both implemented by KEGG orthology based annotation system (KOBAS) software (version 2.1.1) with p-value < 0.05 to judge statistically significant enrichment [41].

2.7. Quantitative RT-PCR (qRT-PCR) Analysis

To verify the RNA-Seq analysis, we randomly selected five unigenes and used qRT-PCR to confirm their participation in the high-temperature reaction. RT-qPCR was conducted on ABI ViiA™ 7 Real-Time PCR System (Applied Biosystems, Foster, CA, USA) using GoTaq® qPCR Master Mix (Promega, Madison, WI, USA). The PCR amplifications were consisted of 95 °C for 3 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s, and then 72 °C for 30 s. Gene expression was normalized using the glyceraldehyde-3-phosphate dehydrogenase (GADPH) as an internal reference gene, and the relative changes of gene expression were calculated using the 2−ΔΔCt method. The list of primers is presented in Table S1.
2.8. Statistical Analysis

Data were expressed as the mean ± standard error, and the data were subjected to one-way analysis of variance (ANOVA) (p < 0.05) followed by a significant difference test (LSD) using SPSS statistics v17.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Physiological Parameter Response to a Low Temperature

To analyze the effects of COS on tea plant growth, we measured the change in activity of SOD, and POD enzymes and content of chlorophyll, soluble sugar in COS-treated tea plant and their respond to low temperature stress, with sterile distilled water served as control. As shown in Figure 1, under a low temperature, the tea plant responds to cold stress with all the physiological parameters changed and COS-enhanced freeze protection. As in the control group, a low temperature caused increases in those physiological parameters. As shown in Figure 1A, the enzyme activity of SOD was significantly increased by 24.04% at −4 °C and 32.68% at −8 °C. Similarly, the enzyme activity of POD was significantly increased by 38.05% at −4 °C and 8.81% at −8 °C. Cold stress significantly reduced the chlorophyll content by 20.18% and 21.96% at −4 and −8 °C, respectively (Figure 1C). Moreover, soluble sugar content was significantly increased by 29.87% at −4 °C and 28.16% at −8 °C, respectively (Figure 1D). The results show that cold stress consistently increased SOD and POD activity, and soluble sugar content, when the temperature was switched from 25 °C to −4 °C or −8 °C, but POD activity was highest at −4 °C.

When exogenous COS was used, it consistently enhanced SOD and POD activities, and the soluble sugar content and chlorophyll content in the tea plant. For example, COS improved SOD activity by 11.75% at 25 °C, 25.93% at −4 °C and 9.21% at −8 °C, respectively, as compared with the control. Similarly, POD activity was enhanced by 19.91%, 19.23% and 30.09% on 25 °C, −4 °C and −8 °C, respectively.

![Figure 1](image_url)

**Figure 1.** Effect of chitosan oligosaccharide (COS) on physiological parameters of tea leaves. (A) Superoxide dismutase (SOD) activity; (B) peroxidase (POD); (C) chlorophyll content; (D) soluble sugar content. The data represent the means ± SD of three replicates samples. Different letters indicate significant differences at p < 0.05.

For all the tested parameters, the effects of COS were more pronounced under cold stress. When tea plants were treated with COS combined with cold stress, SOD enhanced by 56.21% and 44.91% at −4 and −8 °C, respectively. Similarly, POD increased 37.26% and 18.04%. The content of soluble sugar also increased by 45.22% and 40.25% at −4 and −8 °C, respectively. Chlorophyll content was decreased by 13.47% and 14.99%, respectively. The results show that COS treatment consistently increased...
chlorophyll content, but three parameters of SOD, POD and soluble sugar were highest at −4 °C of cold stress combined with COS.

3.2. Transcriptome Sequencing and Assembly

To understand the response of the tea plant to cold stress and the effect of COS on the molecular level, we compared the transcriptomes between COS treatment and the control group at −4 °C by RNA-Seq. Replicate samples of the control group (ConT3_1/2/3) and COS-treatment group (TreT3_1/2/3) were included in this study. We obtained 5.59–6.60 million raw reads in control and 5.79–6.77 million raw reads in the COS-treatment group. After filtering and removing low-quality reads, the clean reads were limited 5.26–6.21 million and 5.45–6.34 million, respectively. Of these clean reads, the GC content was 46.46–47.21% and the Q30 values were over 98.45%. The ratio of total mapped reads between the control and COS-treatment groups was 94.69–94.90% and 94.85–95.20% for *Camellia sinensis* according to the Genome Database. Unique mapped reads were 91.48–92.10% in the control group and 88.02–90.66% in the COS-treatment group (Table 1).

### Table 1. Statistical analyses and mapping results of RNA sequencing reads.

<table>
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<tr>
<th>Sample</th>
<th>ConT3_1</th>
<th>ConT3_2</th>
<th>ConT3_3</th>
<th>TreT3_1</th>
<th>TreT3_2</th>
<th>TreT3_3</th>
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</thead>
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<tr>
<td>Raw reads</td>
<td>55,965,032</td>
<td>56,476,808</td>
<td>66,044,722</td>
<td>57,864,054</td>
<td>67,743,104</td>
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<td>Clean reads</td>
<td>52,619,470</td>
<td>53,061,678</td>
<td>62,155,286</td>
<td>54,555,936</td>
<td>63,422,124</td>
<td>61,416,118</td>
</tr>
<tr>
<td>Q30 (%)</td>
<td>98.45</td>
<td>98.45</td>
<td>98.70</td>
<td>98.65</td>
<td>98.55</td>
<td>98.45</td>
</tr>
<tr>
<td>GC content (%)</td>
<td>46.60</td>
<td>46.46</td>
<td>46.63</td>
<td>46.82</td>
<td>46.83</td>
<td>47.21</td>
</tr>
<tr>
<td>Total reads</td>
<td>44,163,580</td>
<td>43,980,650</td>
<td>52,344,630</td>
<td>45,455,332</td>
<td>52,920,720</td>
<td>51,188,834</td>
</tr>
<tr>
<td>Total mapped</td>
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<td>41,644,005</td>
<td>49,676,907</td>
<td>43,274,546</td>
<td>50,292,573</td>
<td>48,351,418</td>
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<tr>
<td>Unique mapped</td>
<td>38,522,223</td>
<td>38,095,351</td>
<td>45,663,412</td>
<td>39,232,023</td>
<td>45,576,183</td>
<td>42,734,470</td>
</tr>
</tbody>
</table>

3.3. Differentially Expressed Genes Analysis

In order to verify the correlation of gene expression level between samples, we demonstrated that the biological repeatability between samples was great through spearman correlation analysis based on the RPKM of different samples. Genes with *p*-value < 0.05 and ∣log2(Foldchange)∣ > 1 were defined as differentially expressed genes between control and COS. There were identified 4503 differentially expressed genes (DEGs) between the control and COS, including 1605 up-regulated and 2898 down-regulated genes in the leaves of tea plant (Figure 2 and Table S2).

![Volcano plot of differentially expressed genes (DEGs) showed up-regulated and down-regulated between control and COS under −4 °C treatment. The red dots represent up-regulated genes, the blue dots represent down-regulated genes, and the gray dots represent no significant difference. The horizontal coordinates indicate the change in multiple expression, the longitudinal coordinates indicate the magnitude of differences.](image-url)
3.4. Gene Ontology (GO) Annotation

The differentially expressed mRNAs were analyzed by GO enrichment, as shown in Figure 3 and Table S3. The differentially expressed genes were mostly enriched in biological process (Figure 3). In the biological process categorization, functional enrichment mainly focuses on metabolic processes and nutrient synthesis processes, such as “single-organism biosynthetic process” (GO: 0044711), “metabolic process” (GO: 0008152), “carbohydrate metabolic process” (GO: 0005975) and “carbohydrate derivative biosynthetic process” (GO: 1901137). The molecular function category includes the expression of transmembrane transporters and catalytic enzyme-related genes, such as “catalytic activity” (GO: 0003824), “transporter activity” (GO: 0005215), “transmembrane transporter activity” (GO: 0022857), and “ion transmembrane transporter activity” (GO: 0015075). Besides, “serine-type endopeptidase activity” (GO: 0004252) was mostly enriched in the molecular function category.

![Figure 3. Gene ontology (GO) classification analysis based on DEGs induced by COS under −4 °C treatment. The horizontal coordinates indicate GO terms, the longitudinal coordinates indicate rich factor, rich factor represents the ratio between the number of different genes enriched in the term and the background genes in GO term.](image)

3.5. Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Annotation

The KEGG enrichment scatter plot is a graphical representation of the statistical analyses that visualizes the pathway enrichment (Figure 4). The degree of KEGG enrichment was measured in terms of richness factor, p-value, and the number of genes in the pathway. The important enriched pathways with high generation, low p-value and large numbers of genes are shown in the Figure 4 and Table S4. As shown in Figure 4, these enriched pathways, including “photosynthesis” (ko00195), “carbon fixation in photosynthetic organisms” (ko00710), “photosynthesis–antenna proteins” (ko00196), “ribosome” (ko03010), “carbon metabolism” (ko01200).

Compared with the control group, 71 genes were significantly induced to up-regulated by COS treatment, including PSII, PSI, cytochrome b6/f complex, photosynthetic electron transport and F-type ATPase (Table S5). In the carbon metabolism pathway, a total of 77 genes were differentially expressed, including 52 up-regulated and 25 down-regulated (Table S6). A total of 43 genes were assigned to
the plant hormone signal transduction pathway, including 16 genes that were up-regulated in auxin, abscisic acid, ethylene, salicylic acid (Table S7). These results suggest that the addition of COS at a low temperature have a complex effect on biological process and metabolism of the tea plant.

**Figure 4.** Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis based on DEGs induced by COS under −4 °C treatment. The significance of enrichment is shown on the horizontal coordinates (represented by $-\log_{10}(p$-value), the greater the value, the more significant the enrichment), and the KEGG pathway is shown on the longitudinal coordinates. The size of the dots indicates the number of different genes contained in the KEGG pathway, and the color of the dots indicates the degree of rich factor enrichment.

3.6. qRT-PCR Validation of Differentially Expressed Transcripts from RNA-Seq

Five transcripts were randomly selected for qRT-PCR analysis, which used to confirm validity and accuracy the RNA-Seq data. The results show that the trend of qRT-PCR is consistent with the results of RNA-Seq in Figure S1.

4. Discussion

Cold stress affects photosynthetic activities and metabolic functions in plants, which further affected growth, development, and metabolism. It has a negative effect on the yield and quality of tea. Anji Baicha is a temperature-sensitive albino tea cultivar. When the environment temperature is below 20 °C in early spring, the white shoots phenomenon will appear. After about two weeks, the plant gradually turns as green, as does those of common tea cultivars [4–6]. The change of leaf color was mainly due to chloroplast development in the albescent stage, the etioplast–chloroplast transition was blocked, and the accumulation of chlorophyll was inhibited under low temperature [4–8,37]. In this study, we chose Anji Baicha in the stable stage of chlorophyll development as a research object,
the results revealed that COS could enhance antioxidant activity, increase accumulation of sugar content and chlorophyll content in tea plant. It is confirmed that COS could play an important role in improving stress tolerance of Anji Baicha.

Cold stress can cause excessive production of reactive oxygen species (ROS), disrupt the normal physiological and metabolic balance of plants, lead to the increase of membrane lipid peroxidation and damage to vital biomolecules [42,43]. Plants have evolved complex mechanisms to combat against the damage induced by ROS, including improve the antioxidant enzymes [44,45]. In this study, under cold stress, the tea plant natively reacted to protect themselves by increasing the activity of SOD and POD enzyme, and the application of COS provided external assistance plant. Chlorophyll content in COS-treated tea plant was higher than in control, which indicated that COS application mitigated the cold-induced decline in chlorophyll content. Soluble sugar can maintain the osmotic balance, and the soluble sugar in COS treated tea plant was higher than that without COS treatment, suggesting that COS can stabilize cell membrane and enhance cold resistance of plant. Those results indicated that the utilization of COS can positively affect these physiological parameters in tea plants, and beneficially regulate the natural defense system and improve growth and developmental processes of tea plants under cold stress. Moreover, this was also demonstrated in wheat seedlings where the application of COS could enhance the activities of antioxidant enzymes and the content of chlorophyll and alleviate the damage of abiotic stress in wheat [25–27,46]. In wheat, COS could enhance the activities of antioxidant enzymes and the content of chlorophyll, alleviate plant the damage of abiotic stress [25–27,46]. These differentially expressed genes indicate that the application of COS has complex effects on metabolism and signaling pathways of tea plants at low temperature. From RNA sequencing, we found that COS significantly altered the level of gene expression involved in photosynthesis and carbon metabolism under cold stress.

The up-regulated differentially expressed genes could be important for the pathology and biological processes of response to cold stress. Chlorophyll content is an important parameter frequently used to indicate chloroplast development, and which is sensitive to abiotic stresses [47]. COS can increase chlorophyll content under cold stress, which is consistent with the observations from RNA-Seq. Compared with the control group, COS treatment may increase the photosynthesis of plants by significantly up-regulating photosystem I (PSI), photosystem II (PSII)-related genes (Table S5). In the PSII core complex, PsbR is an important link, which can stabilize the assembly of the oxygen-evolving complex protein PsbP [48]. In the present study, PsbR was up-regulated, which was consistent with the action of chitosan heptamer response in wheat seedling [49]. Besides, Chlorophyll a/b-binding protein can participate in light uptake, transfer energy to the reaction centers of the photosystem I and photosystem II, and regulate the excitation energy distribution to maintain the structure of the thylakoid membrane [50], and all of 23 chlorophyll a/b-binding protein genes were also up-regulated, which can imply the recovery of photosynthesis activities by COS treatment under cold stress [51]. These results indicate that COS may enhance photosynthesis via the upregulation of related proteins to improve the cold resistance of tea plant.

In the carbon metabolism pathway, genes encoding ribulose bisphosphate carboxylase small subunit (rbcS), phosphoglycerate kinase, glyceraldehyde-3-phosphate dehydrogenase, triosephosphate isomerase were up-regulated significantly (Table S6). RbcS is one of the subunits of Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo), and the activity of rbcS decreased to inhibit photosynthesis under cold stress [52]. This result was consistent with previous research demonstrating the application of COS to regulate the photosynthetic mechanism and carbon metabolism and thereby the plant growth [53].

During plant development, the response of plants to endogenous and environmental signals is mediated by several hormones, which are involved in almost every aspect of plant growth. For example, plants respond very quickly to auxin, including cell growth and the activation of multiple auxin-responsive genes [53]. Indole-3-acetic acid (GH3) and the ethylene receptor (ETR) were up-regulated genes in the plant hormone signal transduction pathway (Table S7). GH3 is an
important response gene of auxin-responsive protein (IAA), which can encode a class of IAA-amido synthetases responsible for balancing endogenous free IAA content and plays an important role in IAA-regulated plant growth and development [54,55]. The ETR responds to ethylene and abscisic acid (ABA) signaling. ETR is the most important ethylene receptor protein in plants, and the lack of ETR will hinder the transduction of ethylene signal cascade reaction, resulting in the insensitivity to ethylene in plant [56–58].

The application COS can improve antioxidant enzyme activities, and the content of chlorophyll and soluble sugar. Besides, compared with the control group, the addition of COS significantly changed the photosynthesis pathway and carbon metabolism of tea plants under low temperature stress, which may contribute to COS’ ability to improve the cold tolerance of tea plants. These results may represent that COS participates in the specific regulatory mechanism related to cold adaptation in the cold resistance of Anji Baicha. As for the comparison of cold resistance between Anji Baicha and other tea plants (e.g., Xiaoxueya, Fudingdabai), we are further carrying out relevant experimental verification.

5. Conclusions

In summary, low temperature will impact the key physiological and developmental processes that determine the yield of tea. This study indicates that the utilization of COS can positively affect these physiological parameters in tea plants by improving antioxidant enzyme activities, and the content of chlorophyll and soluble sugar. Hence, COS can beneficially regulate the natural defense system and improve the growth and developmental processes of tea plants under cold stress. With transcriptome sequencing and differentially expressed genes analysis, we identified 1605 up-regulated and 2898 down-regulated genes in COS compared to the control, and photosynthesis and the carbon metabolism pathway of enrichment may play a role in the COS-improved cold resistance of a tea plant. The results may provide the foundation for further research on the regulation mechanism of COS on plant cold tolerance.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/6/915/s1, Table S1: Primer sequences used for qRT-PCR. Table S2: The list of different expression genes. Table S3: GO enrichment list of different expression genes. Table S4: KEGG pathway enrichment list of different expression genes. Table S5: Differentially expressed genes in photosynthesis related pathway. Table S6: Differentially expressed genes in carbon metabolism pathway. Table S7: Differentially expressed genes in plant hormone signal transduction pathway. Figure S1. Verification of relative expression levels of DEGs in transcriptome date by qRT-PCR between control and COS.

Author Contributions: Y.L. conducted the experiments; Y.L., L.O. and D.J. designed and performed the experiments; Y.L., Q.Z., T.L. and R.L. analyzed the data; X.L. and L.J. conceived and supervised the project. All authors have read and agreed to the published version of the manuscript.

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