Transcriptome and phytochemical analyses provide new insights into long non-coding RNAs and characteristic secondary metabolites of oolong tea (*Camellia sinensis*) in solar-withering

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Abstract

Background Oolong tea, a semi-fermented tea, was deeply loved by consumers. Among tea processing, withering is the first indispensable process for improving the unique flavor. However, the roles of long non-coding RNAs (lncRNAs) and characteristic secondary metabolites in withering of oolong tea remain unknown. **Results** Using phytochemical analyses, the total flavonoid, total catechins, EGC, CG, GCG, ECG, and EGCG were all present at significantly lower levels in solar-withered leaves (SW) than in fresh leaves (FL) and indoor-withered leaves (IW). However, terpenoid, JA, and MeJA were present at a higher level in SW than FL and IW. By analyzing the transcriptome data, we obtained a total of 32,036 lncRNAs. On the basis of Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, flavonoid metabolic pathway, terpenoid metabolic pathway, and JA/MeJA biosynthesis and signal transduction pathway were the representative pathways in this study. And a total of 63 differentially expressed-lncRNAs (DE-lncRNAs) and 23 target genes were identified in the 3 pathways. Analysis of the expression profiles of DE-lncRNAs and their target genes in SW compared with IW, we found that 4 up-regulated genes (FLS, CCR, CAD, and HCT), 7 up-regulated lncRNAs, 4 down-regulated genes (4CL, CHI, F3H, and F3'H), and 3 down-regulated lncRNAs in flavonoid metabolism; 9 up-regulated genes (DXS, CMK, HDS, HDR, AACT, MVK, PMK, GGPPS, and TPS), 3 up-regulated lncRNAs, 6 down-regulated lncRNAs in terpenoid metabolism; 6 up-regulated genes (LOX, AOS, AOC, OPR, ACX, and MFP2), 4 up-regulated lncRNAs, and 3 down-regulated lncRNAs in JA/MeJA biosynthesis and signal transduction pathway. **Conclusions** These results suggested that the expression of DE-lncRNAs and their targets involved in the 3 pathways maybe related to the low content of total flavonoid, total catechins, EGC, CG, GCG, ECG, and EGCG, and high content of terpenoid in SW. Moreover, solar light, high content of JA and MeJA, and the endogenous target mimics (eTMs) regulatory mechanism in SW were also crucial factors in increasing the terpenoid. These findings provide new insights into the view of "Kàn qīng shài qīng" that the solar-withering is more contribute to the high quality flavor of oolong tea compared to the indoor-withering.

Background

Long non-coding RNAs (lncRNAs) are defined as a class of RNA transcripts, which are greater than 200 nt in length and have no obvious open reading frame (ORF) [1, 2]. Besides, it usually has structural features similar to mRNA, such as 5'-cap structures and poly (A) tails. Due to the low expression level of lncRNAs, previous studies have suggested that lncRNAs are noise produced by RNA polymerase II during transcriptional process and do not have any biological function [3, 4]. With the development of lncRNA research, it has found that most of the transcriptional regions are transcribed into the lncRNA, and it may have abundant functions [5, 6]. Till now, it has been reported that lncRNAs play significant roles in transcriptional level [7], post-transcriptional level [8], and epigenetic levels [9] through diverse regulatory mechanisms, including chromatin modification, transcriptional activation, transcriptional interference, and splicing regulation. In plants, lncRNAs have been found to regulate the several metabolism, including sugar metabolism, organic acid metabolism, amino acid metabolism. Moreover, some lncRNAs with miRNA binding sites can be used as endogenous target mimics (eTMs) to bind with miRNAs and reduce

their inhibition on their target genes [10]. In *Arabidopsis thaliana*, lncRNAs *IPS1*, as an eTM of ath-miR399, can condense the cleavage of *PHO2* gene mediated by ath-miR399 [11]. Similarly, it has been reported that several lncRNAs function by interacting with miRNAs in other plants [12-15]. Furthermore, nta-eTMX27 in *Nicotiana tabacum* [16], osa-eTM160 in *Oryza sativa* [17], eTM (lncRNA23468) in *Solanum lycopersicum* [18], eTM (LTCNS-00042843) in *Dimocarpus longan* [12] have been shown to act as the eTMs to modulate targets by decoying relevant miRNAs. With the innovation of high-throughput sequencing and bioinformatics tools, a large number of lncRNAs have been found in several plants, not only in model plants, including *A. thaliana* [19, 20], *Oryza sativa* [21], and *Brachypodium distachyon* [22], but also in horticultural plants, including *Solanum lycopersicum* [23], *Cucumis sativus* [14], *Musa itinerans* [24], *D. longan* [12], and *Citrus sinensis* [25]. Moreover, lncRNAs in plants have been found to be involved in regulating the secondary metabolisms and stress responses [16, 24, 26, 27]. However, few investigators focus on the identification and analysis of lncRNAs in tea plant based on genome-wide levels. And no study has been published on explore the roles of lncRNAs involved in secondary metabolisms in the tea processing.

Tea plant(*Camellia sinensis*) is an important economic crop which originated from China. Oolong tea, a semi-fermented type of tea, was produced mainly in Fujian, Guangdong and Taiwan provinces of China, and was deeply loved by consumers, because of its attractive unique floral and fruity aroma and mellow taste. Its postharvest processing is always accompanied by a series of biochemical reactions and may regulate the accumulation of various secondary metabolites, so as to promote and improve the formation of flavor quality and product added value of oolong tea.

It is well known that the tea flavor is greatly influenced by the manufacturing process [28-31]. Among these processings, withering is the first indispensable process for improving flavors in the postharvest processing of oolong tea, black tea and white tea, which ties closely to the subsequent tea processing and was important to form the unique aroma and taste. In the traditional oolong tea processing, there is a tea proverb about withering "Kàn qīng shài qīng", which means adjusting the solar-withering degree according to the condition of tea leaves. And it also showed that solar-withering is an important processing of oolong tea production. Moreover, solar-withering has positive regulation effect on floral and fruity aromas in tea, and can obviously improve aroma quality index of oolong tea [32, 33]. During the solar-withering processing, the fresh tea leaves gradually shrink and soften due to spontaneous and slow dehydration, and the tea flavor compounds undergo biochemical changes, which may be a factor that benefits the unique mellow and thick taste of oolong tea. In sensory quality of oolong tea, the taste and aroma are the most important factors. The characteristic compounds associated with tea quality include an extensive array of secondary metabolites. Among them, the flavonoids is one of the representative secondary metabolites in tea plant, including flavanols, phenolic acids, anthocyanin, and other substances [34], which is also the most important ingredient in tea that has bitterness and astringent. Volatile is another important factor to evaluate the flavor quality of oolong tea. According to their metabolic pathways, the major volatile compounds in tea can be divided into 4 different classes, including fatty acid derivatives, terpenoids, phenylpropanoids/benzenoids, and carotenoids derived compounds [35, 36]. Among them, volatile sesquiterpenoid and monoterpenoid in terpenoid have strong

sweet and floral aromas, which are the main terpenoid metabolites [35]. Additionally, it has been reported that the transcription level and content of tea flavor compounds changed significantly during tea withering processing [37]. Furthermore, some studies reveal that jasmonic acid (JA) and methyl jasmonate (MeJA), which acts as an effective elicitor, could improve the plant aroma by promoting volatiles [38-41]. In the past decades, many efforts have been made to uncover the aroma formation in the tea processing based on the analysis of transcript level and metabolite content [30, 42-45]. Nevertheless, the regulatory mechanism of the lncRNAs involved in tea flavor compounds formation in withering processing is still unclear, especially the important roles of lncRNAs in the improvement of oolong tea quality in solar-withering.

With the release of the tea genome [46, 47], it is possible to accurately identify lncRNAs in tea plants. To investigate the mechanism of lncRNAs affecting the tea flavor-related metabolisms in the withering processing of oolong tea, we systematically identified lncRNAs in FL, IW, and SW through transcriptome sequencing. On the basis of these identified lncRNAs, we analyzed the target genes regulated by these lncRNAs and explored the function of lncRNAs involved in tea flavor-related metabolisms. Then, these lncRNAs, which can be involved in the regulation of miRNAs and mRNAs as eTMs, were also predicted and identified. Additionally, the contents of tea flavor-related metabolites, JA, and MeJA were analyzed. And the role of JA/MeJA biosynthesis and signal transduction in the withering processing of oolong tea was also explored. This is the first time that the lncRNA involved in tea processing has been reported at the genome-wide level. And these analyses provide important insights into the transcriptional changes and regulatory relationships in lncRNAs and their targets in the withering processing of oolong tea. Moreover, the important effect of solar-withering on the flavor quality of oolong tea was uncovered. And the relationship between tea flavor metabolites and the expression patterns of lncRNAs and their target genes were also unveiled in this study.

Methods

Plant materials, sample preparation and sensory analysis

The eight-year-old tea plants (*C. sinensis* cv. Tieguanyin) was cultivated at Fujian Agriculture and Forestry University, Fuzhou, Fujian Province, China (E 119°14', N 26°05'). The original plant was identified by associate professor Yuqiong Guo of the Fujian Agriculture and Forestry University and a voucher specimen was deposited in the Fujian Agriculture and Forestry University. The fresh shoot and first to third leaves were uniform picked from each tea plant. Then, the fresh plucked tea leaves are equally divided into two parts, each part weighing 2 kg. The first part of fresh leaves was subject to solar-withering under sunlight (temperature 25 ± 2 °C, relative humidity 60 ± 5 %, illumination intensity 40000 ± 1000 lx, and leaf layered thickness 1 cm) for 45 min. Another part of tea leaves were evenly layered in the indoor condition (illumination intensity 100 ± 5 lx) for 45 min. And the other parameters in the indoor-withering processing were all consistent with that in the solar-withering processing. The fresh leaves (FL), indoor-withered leaves (IW), and solar-withered leaves (SW) were collected for analyses in this study. Then all samples were frozen in liquid nitrogen immediately and stored at -80 °C for further experiments.

Three independent biological replicates were collected. Each replicate was collected from more than 10 randomly selected tea plants.

To initial study the taste and aroma of FL, IW, and SW, the three sample after 24 h of freeze-drying were all submitted to sensory evaluation. Then the samples were evaluated in accordance with previous method [48].

Determination of total flavonoids, catechins, volatiles, JA, and MeJA contents

The total flavonoids content of three tea samples were extracted and detected using the aluminum chloride colorimetric method [49]. The weight (0.1 g) of tea samples was diluted with methanol until 100 mg/mL. The 2.0 mL diluted extract was added to extraction solution (0.1 mL of 10% aluminum chloride and 0.1 mL of 0.1 mmol/L potassium acetate solution) and incubated at room temperature for 30 min. Then, the absorbance of the extracts was read at 415 nm using a ultraviolet (UV)-spectrophotometer. And the contents of total flavonoids were calculated and quantified according to the previous method [49]. Moreover, the catechins were extracted and determined from the samples according to previous method [50]. Catechins were analyzed using a Waters 2695 high performance liquid chromatography (HPLC) system equipped with a 2489 UV-visible detector. The detection wavelength was set to 278 nm and column temperature were maintained at 25 °C. Authentic standards of catechin (C), gallocatechin (GC), epicatechin (EC), epigallocatechin (EGC), catechin gallate (CG), gallocatechin gallate (GCG), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) were purchased from Solarbio (Beijing, China). Each sample was detected in triplicate.

To detect volatiles released from FL, IW, and SW, volatiles were extracted and determined following the previous method [51]. A Clarus SQ 8 gas chromatograph-mass spectrometer (GC-MS; PerkinElmer, New York, NY, USA) and a Turbmatix Headspace System (PerkinElmer) was used to detected the volatile compounds. Each sample was detected in triplicate. The GC-MS analysis was performed based on the TurboMass 6.1 software (PerkinElmer). And separated compounds were identified according to their retention index and the National Institute of Standards and Technology (NIST) Mass Spectral Library. And ethyl decanoate was used as an internal standard. The contents of volatiles in these three tea sample were calculated and quantified following the previous criteria [51].

JA were extracted and determined from three tea samples according to the previous method [38]. The each extracted sample (10 µL) subjected to a Waters 2695 HPLC system equipped with a 2489 UV-visible detector, and eluted JA were detected at 230 nm. In order to determine MeJA, equal weights (0.3 g) of tea leaves collected from three tea samples were immersed in 80% ethyl alcohol solution for 24 h. After centrifugation at 12,000 g for 10 min, the supernatant was filtered through a 0.22 µm organic membrane. Then each sample (10 µL) also subjected to the HPLC system equipped with the UV-visible detector. The detection wavelength was set to 210 nm and column temperature were also maintained at 30 °C. Each sample was detected in triplicate. Authentic standards of JA and MeJA were purchased from Solarbio (Beijing, China) and added to the extracted mixture as internal standards. The contents of JA and MeJA

were quantified by calculating the area of each individual peak against the peak area of the authentic standard.

Total RNA extraction, library construction and high-throughput sequencing

For high-throughput sequencing, total RNA was extracted separately from FL, SW, and IW using a Trizol Reagent Kit (TransGen Biotech, Beijing, China). And the RNA integrity checked by gel electrophoresis and micro-ultraviolet spectrophotometry (Nanodrop). After RNA integrity checked, ribosomal RNA (rRNA) was depleted using the Ribo-Zero rRNA Removal Kit (Illumina, San Diego, CA, USA). Then, strand-specific cDNA was synthesized to construct these nine libraries (each sample with three biological replicates) using the TruSeq Stranded Kit (Illumina, San Diego, CA, USA), DNA polymerase I, and Ribonuclease H (RNaseH). Each library was sequenced pair end on the Illumina HiSeq X Ten platform (BGI; Shenzhen, China).

Transcriptome assembly and lncRNAs identification

After removing rRNA, low quality reads, adapter sequences, and contaminating reads, the clean reads were obtained. Then, all clean reads were aligned to the tea reference genome [47] using the hierarchical indexing for spliced alignment of transcripts (Hisat) software. And transcriptome of each sample was assembled independently using StringTie software [52, 53]. The expression level of transcript was calculated and normalized using FPKM value [54].

In order to check the assembled quality, transcripts that overlapped with known genes in tea reference genome were discarded. Then, transcripts with short sequence length (length ≤ 200 bp) and low FPKM (FPKM < 0.5) were also removed. The candidate lncRNAs selected for further investigation were those that satisfied all the criteria in Coding Potential Calculator (CPC, score < 0) [55], txCdsPredict (score < 500) [56], Coding-Non-Coding Index (CNCI, score < 0) [57], and the transcript cannot be aligned to Pfam database [58].

Analysis of differentially expressed lncRNAs and their targets

Differentially expressed lncRNAs (DE-lncRNAs) and differentially expressed genes (DEGs) was detected by DEGseq software [59]. And $|\text{Fold change}| \geq 2$ and false discovery rate (FDR) ≤ 0.001 were defined as the threshold to identified the DE-lncRNAs and DEGs in these samples. Pearson correlation coefficient (PCC) and Spearman correlation coefficient (SCC), these two correlation coefficients were used to identify the potential target protein-coding genes of lncRNAs ($|\text{PCC value}| \geq 0.6$ and $|\text{SCC value}| \geq 0.6$). Moreover, the regulation of lncRNAs on its target genes can be divided into *cis*- and *trans*-acting based on the coordinates of lncRNAs and its target genes [60, 61]. When lncRNAs located 100 kb upstream and downstream of their target genes, it was defined as *cis*-regulation. If lncRNAs exceeded this range, *trans*-regulation identification was performed according to the binding energy (value ≤ 30) of lncRNAs to mRNAs. To further investigate the potential functions of DE-lncRNAs and their related target genes, all target genes were searched against non-redundant protein (NR), Swiss-Prot, gene ontology (GO), and

kyoto encyclopedia of genes and genomes (KEGG) database by using Blast [62] ($E\text{-value} < 1.0E^{-5}$). In KEGG enrichment analysis, $FDR \leq 0.01$ were defined as the threshold to identified the significantly enrichment pathway. On the basis of FPKM value, the expression levels of DE-lncRNAs and DEGs were visualized by TBtools software [63].

Prediction of the eTMs and targets of miRNAs

To explore the relationships between lncRNAs and miRNAs, lncRNAs as eTMs of miRNAs in tea withering processing were identified by TAPIR software, and the criteria details were referred to Bonnet et al [64]. Furthermore, some lncRNAs and genes may serve as miRNA targets, which were directly regulated by miRNAs. The lncRNAs and genes as targets of miRNAs were predicted by Plant Small RNA Target Analysis Server (psRNATarget) software, respectively. The lncRNAs and genes with the expected value greater than 5 will be filtered, and the remaining lncRNAs and genes will be identified as potential targets of miRNAs. The miRNAs dataset in FL, IW, and SW were obtained through small RNA sequencing.

Relative expression analyses of the selected lncRNAs, mRNAs, and miRNAs

Total RNA of FL, SW, and IW was reverse transcribed into first-strand cDNA for mRNAs and lncRNAs qRT-PCR using a Transcript First-Strand cDNA Synthesis SuperMix (TransGen Biotech, Beijing, China). In addition, first-strand cDNA of miRNA was synthesized using a Transcript miRNA First-strand cDNA synthesis SuperMix (TransGen Biotech, Beijing, China). The mRNAs, miRNAs and lncRNAs qRT-PCR was performed by the LightCycler 480 platform (Roche Applied Sciences, Basel, Switzerland) with Transtart Tip Green qPCR SuperMix (TransGen Biotech, Beijing, China), respectively. The setting of qRT-PCR procedure and reaction system were both referred to the method of Chen et al [12]. The *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* and *β -actin* genes were used as reference genes for mRNAs and lncRNAs normalization. And *5.8S ribosomal RNA (5.8S rRNA)* and *U6 small nuclear RNA (U6 snRNA)* were used to normalize miRNAs. The relative expression levels was calculated using $2^{-\Delta\Delta CT}$ method, and all primers used for qRT-PCR are designed using the automatic primer designer tool at Tea Plant Information Archive (Additional file 1: Table S1) [65]. All qRT-PCR analyses were performed in 3 biological replications, respectively.

Statistical analyses

All data are expressed as the means $\pm$ standard deviations (SD). Group differences was performed by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. And significant differences among various groups are represented by different letters. Lowercase letter means significant difference ($P < 0.05$); uppercase letter means extremely significant difference ($P < 0.01$). The data were analyzed by SPSS 25 software.

Results

Analysis of total flavonoids, catechins, volatiles, JA, and MeJA contents in fresh leaves, indoor-withered leaves, and solar-withered leaves

The phenotypes of tea leaves of FL, IW, and SW were recorded (Figure 1). The tea leaves of FL were glossy, straight, and green color. Compared with FL, the leaves of IW showed slight curling and deformation, while the leaves of SW showed obvious curling and the color of leaves became dull green. After sensory evaluation, we found that the three samples also have differences in taste and aroma, the leaves of FL and IW both have strong bitter and astringent tastes, and strong grass aroma, while the leaves of SW have weak astringent and mellow tastes, light grass flavor, and slightly floral and fruity aromas.

To explore the changes of characteristic tea compounds in FL, IW, and SW, we determined the contents of total flavonoids, catechins, and volatiles in these samples. The total flavonoids contents were 57.02, 53.28, and 47.98 mg/g in FL, IW, and SW, respectively (Figure 2). And the total flavonoids content in SW was significantly lower than that in FL and IW. Moreover, the total catechins were also present at significantly lower content in SW than in FL and IW. Further analysis of individual catechins found that EGCG were the most abundant catechins in all three sample. The content of EGCG showed both significantly higher in FL (61.37 mg/g) and IW (57.55 mg/g) than SW (55.35 mg/g). Similarly, other four individual catechins, including, EGC, CG, GCG, and ECG, showed significantly higher content in FL and IW than in SW. However, the contents of C, GC, and EC have no significant difference in FL vs. SW, FL vs. IW, IW vs. SW. In conclusion, total flavonoids, total catechins, and five individual catechins, especially galloylated catechins (CG, GCG, ECG, and EGCG), were both present a significantly lower content in SW compared to FL and IW.

The contents of top 15 volatiles in FL, IW, and SW detected by GC-MS, including dimethyl sulfide, 1-penten-3-one, 2-ethylfuran, (Z)-3-hexenal, (E)-2-hexenal, (E)-3-hepten-1-ol, 5-methyl-2-hexene, (E)-3-hexen-1-ol acetate, β -ocimene, limonene, γ -terpinene, α -farnesene, methyl salicylate, β -myrcene, and β -ionone (Table 1). Analysis showed that (Z)-3-hexenal and (E)-2-hexenal were mostly volatile components in FL. After withering, the contents of (Z)-3-hexenal and (E)-2-hexenal in IW and SW are both significantly lower in FL. Further analysis found that the (Z)-3-hexenal contents in SW is 12.70%, significantly lower than that in IW (15.41%). Meanwhile, (E)-2-hexenal contents in SW (12.20%) is significantly lower than that in IW (16.92%). Terpenoid volatiles, including β -ocimene, limonene, γ -terpinene, α -farnesene, and β -myrcene, were significantly increased in IW and SW than that in FL. Similarly, methyl salicylate and β -ionone had high contents in IW and SW than in FL. Further comparing the contents of terpenoid volatiles in IW and SW, we found that all five terpenoid volatiles were present at significantly higher contents in SW than in IW. Therefore, the contents of (Z)-3-hexenal, (E)-2-hexenal, and five terpenoid compounds in oolong tea may be significantly affected by the withering processing, especially solar-withering, it can trigger a significant increase of these five terpenoid compounds and decrease in two types of hexenal.

Phytohormones, especially JA and MeJA, have been reported to act as signal substances to promote the synthesis of related volatiles [66, 67]. In order to understand the accumulation of JA and MeJA in

withering processing, we also determined the content of JA and MeJA in fresh leaves and withered leaves. The JA contents in FL, IW, and SW were 0.92, 1.81, and 3.14 $\mu\text{g/g}$, respectively. Statistical analyses showed that JA content was present at a significantly higher level in SW (3.14 $\mu\text{g/g}$) than FL (0.92 $\mu\text{g/g}$) and IW (1.81 $\mu\text{g/g}$). Similarly, The MeJA content also showed significantly higher in SW (0.45 $\mu\text{g/g}$) than that in FL (0.08 $\mu\text{g/g}$) and IW (0.21 $\mu\text{g/g}$). Combined with volatiles contents in FL, IW, and SW, the difference in jasmonates contents among these three samples may be related to the accumulation of volatiles.

Sequencing and assembly of transcriptome data

To investigate the regulation mechanism of lncRNAs in withering processing of oolong tea, the FL, IW and SW were sequenced from three independent biological replicates using Illumina HiSeq X Ten platform. After removing rRNA, low quality reads, adapter sequences, and contaminating reads, the average of clean data were obtained per sample is 12.66 Gb (Additional file 2: Table S2). Then, all clean data were aligned to tea reference genome. A total of 82,077 transcripts were detected, including 32,036 lncRNAs and 50,041 mRNAs. Further analysis of the length distribution and exon number of lncRNAs and mRNAs, it was found that most lncRNAs (72.36%) were shorter than 1000 bp in length and only 3.94% were longer than 3000 bp. In contrast, the proportion of mRNAs in the range of 0~1000 bp was 40.46%, and the proportion of mRNAs exceeding 3000 bp was 10.07%. Moreover, 68.22% of lncRNAs contain only one exon, while most mRNAs (85.98%) contain more than two exons. This result is consistent with the findings in maize [3], kiwifruit [68] and longan [12], the lncRNAs contains fewer exons than annotated mRNAs and the majority of the lncRNAs in plants are single exon transcripts.

On the basis of transcriptome dataset, the quantitative expression analysis revealed that 28,677 lncRNAs were expressed. And 26,740 expressed lncRNAs were identified in FL, 26,508 expressed lncRNAs were identified in IW, and 26,479 expressed lncRNAs were identified in SW. Further analysis of lncRNAs expressions revealed that the expression levels of majority lncRNAs in FL, IW, and SW were both at a low level ($\text{FPKM} \leq 1$), while the number of highly expressed ($\text{FPKM} \geq 10$) lncRNAs did not exceed 5% of the total number of lncRNAs (Figure 3).

Identification of DE-lncRNAs and their target genes in tea withering processing

To identify DE-lncRNAs in tea withering processing, we compared FL vs. IW, FL vs. SW, and IW vs. SW, respectively. The expression profiling through FPKM values showed that a total of 12,487 DE-lncRNAs were found in FL, IW, and SW. And 9,847 DE-lncRNAs were identified in FL vs. IW, among which 4,243 were up-regulated and 5,604 were down-regulated; 6,506 DE-lncRNAs were identified in FL vs. SW, among which 2,659 were up-regulated and 3,847 were down-regulated; and 4,964 DE-lncRNAs were identified in IW vs. SW, among which 2,443 were up-regulated and 2,521 were down-regulated.

Furthermore, the function of lncRNAs are mainly achieved by acting on target genes by *cis*- or *trans*-regulation. Thus target genes prediction was performed on these DE-lncRNAs. The results showed that 2,892 DE-lncRNAs were identified to contain target genes, including 2,180 *cis*-regulated target genes and

1,096 *trans*-regulated target genes. Among these, 1,694 DE-lncRNAs only targeted one coding gene and only 14 DE-lncRNAs had more than 6 target genes. Further analysis of target genes revealed that 1,904 target genes were regulated by only one DE-lncRNA, while 15 target genes were corresponded to more than 6 DE-lncRNAs.

Functional annotation of target genes of DE-lncRNAs based on GO and KEGG

To determine the specific function of target gene, all target genes were aligned to the GO databases by using the blast program. The GO classification annotation of DE target genes, showing 3,543 DE target genes in FL vs. IW were annotated to three major categories (Figure 4a), namely biological process, cellular component, and molecular function. The top 3 subgroups in biological process were cellular process, metabolic process, and biological regulation. In cellular component, most DE target genes were annotated into membrane, membrane part, and cell subgroups. In the classification of molecular function, most of the DE target genes were classified into three subgroups, binding, catalytic activity, and transporter activity.

In FL vs. SW, 2,085 target genes were also enriched in these 3 major categories (Figure 4b). At the same time, the top 3 subgroups of the cellular process, metabolic process, and biological regulation in FL vs. SW were consistent with the results in FL vs. IW.

In IW vs. SW, only 890 target genes were annotated to cellular process, metabolic process, and biological regulation categories (Figure 4c). In cellular process, the most enriched 3 subgroups were cellular process, metabolic process, and response to stimulus. And the results of the top 3 enriched subgroups in the remaining two categories were also consistent with the enrichment results in FL vs. IW and FL vs. SW.

Then we performed the KEGG annotation analysis on these target genes of DE-lncRNAs. In the FL vs. IW, 3,543 DE target genes were enriched in 129 pathways (Figure 5a). The top 20 enriched pathways were focused on plant-pathogen interaction, sesquiterpenoid and triterpenoid biosynthesis, alanine, aspartate and glutamate metabolism, amino sugar and nucleotide sugar metabolism, brassinosteroid biosynthesis, plant hormone signal transduction, pentose phosphate pathway, monoterpene biosynthesis, terpenoid backbone biosynthesis, stilbenoid, diarylheptanoid and gingerol biosynthesis, glucosinolate biosynthesis, photosynthesis, DNA replication, flavonoid biosynthesis, diterpenoid biosynthesis, metabolic pathways, glycosphingolipid biosynthesis, fructose and mannose metabolism, folate biosynthesis, and lysine biosynthesis.

In FL vs. IW and FL vs. SW, 7 pathways in the top 20 enriched pathways were consistent (Figure 5a and 5b). These 7 pathways included plant-pathogen interaction, plant hormone signal transduction, stilbenoid, diarylheptanoid and gingerol biosynthesis, flavonoid biosynthesis, sesquiterpenoid and triterpenoid biosynthesis, glucosinolate biosynthesis, and monoterpene biosynthesis. Considering the effects of flavonoids on the tea taste and terpenoids on the tea aroma, we paid more attention to the flavonoids and terpenoids metabolic pathways. Plant hormone signal transduction pathway is also involved, in which linoleic acid metabolism is related to the biosynthesis of JA, and MeJA, while

jasmonate in plants hormone signal transduction is beneficial to induce the formation of terpenoid volatiles.

Then, further analysis of transcriptome differences between IW and SW (Figure 5c), 13 of the top 20 enrichment pathways were consistent with FL vs. IW and FL vs. SW. The terpenoids metabolism, including sesquiterpenoid and triterpenoid biosynthesis, monoterpene biosynthesis, and diterpenoid biosynthesis, were found to be a crucial metabolic pathway in our study. In addition, the pathways related to flavonoid metabolism and plant hormone signal transduction were also worthy of further study.

Analysis of DEGs and DE-lncRNAs involved in flavonoid metabolic pathways in tea withering

On the basis of KEGG analysis, flavonoid metabolic pathway was one of the representative pathways in our study. A total of 124 differentially expressed transcripts (DE-transcripts) were assigned to flavonoid metabolic pathways. In Additional file 3: Table S3, there were 14 genes, including *PAL* (*phenylalanine ammonia-lyase*), *C4H* (*cinnamate 4-hydroxylase*), *4CL* (*4-coumarate CoA ligase*), *CHS* (*chalcone synthase*), *CHI* (*chalcone isomerase*), *F3H* (*flavanone 3-hydroxylase*), *F3'H* (*flavonoid 3'-hydroxylase*), *FLS* (*flavonol synthase*), *DFR* (*dihydroflavonol 4-reductase*), *ANS* (*anthocyanidin synthase*), *ANR* (*anthocyanidin reductase*), *CCR* (*cinnamoyl-CoA reductase*), *CAD* (*cinnamyl alcohol dehydrogenase*), and *HCT* (*hydroxycinnamoyltransferase*) genes, were found to be differentially expressed in FL vs. IW, FL vs. SW, and IW vs. SW. On the basis of these identified DEGs, the proposed flavonoid metabolic pathway was constructed (Figure 6).

The expression trends of the 124 transcripts involved in flavonoid metabolic pathway of transcriptome data were compared based on FPKM value (Figure 6). In short, all transcripts of 6 genes such as *C4H*, *4CL*, *F3H*, *F3'H*, *FLS*, and *HCT*, showed the higher expression in FL and IW than SW; most transcripts of 6 genes, including *PAL*, *CHS*, *CHI*, *ANS*, *ANR*, and *DFR* genes, also showed the higher expression in FL and IW than SW. Moreover, 9 transcripts belonging to the *CCR* gene had the highest expression in SW. And more than half of the transcripts belong to the *CAD* gene, which also showed the higher expression in SW compared to FL and IW. In addition, 83 transcripts in the flavonoid metabolic pathway had the higher expression levels in IW compared to SW. These results implied that the flavonoid metabolism in SW might had a weak metabolic flux than that in IW. Therefore, solar-withering could inhibit the accumulation of flavonoids in the withered leaves.

To decipher the regulation mechanism of lncRNAs in flavonoid metabolism, DE-lncRNAs and their target genes related to flavonoid metabolism were analyzed. Among them, 31 DE-lncRNAs and 8 differentially expressed target genes involved in flavonoid metabolic pathway were identified from FL, IW, and SW transcriptome dataset (Additional file 4: Table S4). These identified target genes included *4CL*, *CHI*, *F3H*, *F3'H*, *FLS*, *CCR*, *CAD*, and *HCT* genes. In addition, lncRNAs do not have a one-to-one correspondence to the regulation of their target genes. We defined a lncRNA to regulate a mRNA as a pair of regulatory relationship. And a total of 43 pairs of regulatory relationships were identified from these DE-lncRNAs and their target genes (Figure 7). Moreover, the number of lncRNAs that regulated *HCT* and *CAD* genes was

the highest, and these two genes were each regulated by 10 lncRNAs, while *4CL*, *CHI*, *F3H* and *F3'H* genes were regulated by only one lncRNA, respectively.

To further study the expression pattern of lncRNAs related to flavonoid metabolism, the expression patterns of these 31 DE-lncRNAs were analyzed in FL, IW, and SW based on FPKM values (Figure 8). The expression levels of lncRNAs in FL and IW was similar. Among the regulatory relations, there was a positive regulatory relationships between lncRNAs and their target genes in the 36 pairs of regulatory relationships, while other 7 pairs of regulatory relationships were negatively regulated. Besides, we found that some target genes were also regulated by multiple lncRNAs, and some lncRNAs can simultaneously regulate multiple target genes. Thus, it indicated that lncRNAs may be involved in the regulation of flavonoids by affecting the expression level of genes related to flavonoids metabolism through complex regulatory mechanisms.

To confirm the lncRNAs and their target genes expressions in the transcriptome dataset, the expression patterns of 16 genes and 10 lncRNAs were analyzed by qRT-PCR in FL, IW, and SW (Figure 9). These 16 genes included *PAL* (CSA016076.1), *C4H* (CSA022101.1), *4CL* (CSA015903.1), *CHS* (CSA024718.1), *CHI* (MTCONS_00060938), *F3H* (CSA004930.1), *F3'H* (MTCONS_00044513), *FLS* (MTCONS_00031810), *DFR* (MTCONS_00092979), *ANS* (CSA000829.1), *ANR* (MTCONS_00086693), *CCR* (CSA010774.1), *CAD* (CSA020376.1, CSA019604.1), and *HCT* (CSA021003.1, CSA008809.1) genes. And the 10 lncRNAs involved LTCONS_00054003 (targeting *4CL* gene), LTCONS_00060939 (targeting *4CHI* gene), LTCONS_00056216 (targeting *F3H* gene), LTCONS_00044497 (targeting *F3'H* gene), LTCONS_00031811 (targeting *FLS* gene), LTCONS_00001863 (targeting *CCR* gene), LTCONS_00000233 (targeting *CAD* gene), LTCONS_00090121 (targeting *CAD* gene), LTCONS_00030131 (targeting *HCT* gene), and LTCONS_00101116 (targeting *HCT* gene). The results showed that the expression patterns of the detected lncRNAs and their target genes were consistent with the transcriptome dataset, demonstrating that lncRNAs participated in the regulation of flavonoid metabolism by affecting the expression levels of related genes. And it may be an important factor in obvious differences in the content of flavonoid metabolite between IW and SW.

Analysis of DEGs and DE-lncRNAs involved in the terpenoid metabolic pathway, and JA/MeJA biosynthesis and signal transduction pathway in tea withering

To further understand the expression of DEGs involved in terpenoid metabolic pathways in FL, IW, and SW, we focused on the 59 DE-transcripts annotated to this pathway (Additional file 5: Table S5). Except for *DXR* (*1-deoxy-D-xylulose 5-phosphate reductoisomerase*), *MCT* (*2-C-methyl-D-erythritol 4-phosphate cytidyltransferase*), *CMK* (*4-diphosphocytidyl-2-C-methyl-D-erythritol kinase*), *GPPS* (*geranyl diphosphate synthase*), *PMK* (*phosphomevalonate kinase*), *MDC* (*Mevalonate pyrophosphate decarboxylase*), and *FPS* (*farnesyl diphosphate synthase*) genes, the other 11 gene contained more than 2 transcripts, including *DXS* (*1-deoxy-D-xylulose 5-phosphate synthase*), *MDS* (*2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase*), *HDS* (*4-hydroxy-3-methylbut-2-enyl-diphosphate synthase*), *HDR* (*4-hydroxy-3-methylbut-2-enyl-diphosphate reductase*), *IDI* (*isopentenyl diphosphate isomerase*), *GGPPS*

(*geranylgeranyl diphosphate synthase*), *AACT* (*acetyl-CoA C-acetyltransferase*), *HMGS* (*hydroxymethylglutaryl-CoA synthase*), *HMGR* (*hydroxymethylglutaryl-CoA reductase*), *MVK* (*mevalonate kinase*), and *TPS* (*terpene synthase*) genes.

In addition, we further analyzed the JA/MeJA biosynthesis and signal transduction pathway. In this pathway, a total of 48 DE-transcripts were assigned to 7 genes, including *LOX* (*lipoxygenase*), *AOS* (*allene oxide synthase*), *AOC* (*allene oxide cyclase*), *OPR* (*oxophytodienoic acid reductase*), *ACX* (*acyl-CoA oxidase*), *MFP2* (*enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase*), and *JAR* (*jasmonic acid-amino synthetase*) (Additional file 6: Table S6). All the 7 genes contained more than 2 transcripts, respectively. Among these, the *ACX* gene had the largest number of transcripts. On the basis of these identified DEGs, a proposed terpenoids metabolic pathway was constructed (Figure 10).

Comparisons of the transcript levels (FPKM values) of these DEGs revealed that 17 DEGs showed higher transcript levels in IW and SW than that in FL (Figure 10), including *DXS* (7 transcripts), *DXR* (1 transcript), *MCT* (1 transcript), *CMK* (1 transcript), *HDS* (3 transcripts), *HDR* (2 transcripts), *IDI* (1 transcript), *GGPPS* (3 transcripts), *GPPS* (1 transcript), *AACT* (3 transcripts), *HMGS* (3 transcripts), *HMGR* (8 transcripts), *MVK* (2 transcripts), *PMK* (1 transcript), *MDC* (1 transcript), *FPS* (1 transcript), and *TPS* (10 transcripts). Moreover, *MDS* genes showed complex expression trends in different transcripts.

On the basis of FPKM values, we also compared the expression trends of the 48 transcripts involved in JA/MeJA biosynthesis and signal transduction pathway between fresh leaves and withered leaves (Figure 10). In the expression patterns of these genes, most transcripts showed higher transcript levels in SW than that in IW. Among these, all transcripts corresponding to *LOX*, *AOS*, *AOC*, *OPR*, *MFP2*, and *JAR* genes showed highest transcript levels in SW. And 17 transcripts corresponding to *ACX* genes also showed higher expression levels in SW than that in IW. It suggested that the high transcript level of these 6 JA/MeJA biosynthesis-related genes in SW may affect the accumulation of JA and MeJA.

In short, high expression levels of most transcripts corresponding to the 17 genes involved in terpenoids metabolic pathway may be positively correlated with the content of terpenoids. Also, the high expression levels of 7 genes involved in JA/MeJA biosynthesis and signal transduction pathway may promote the accumulation of jasmonate. And jasmonate is also beneficial for the synthesis of terpenoid volatiles [66]. Thus, these results suggested that the transcript levels of these DEGs may be related to the terpenoids contents in FL, IW, and SW.

To further analysis the regulation mechanism of lncRNAs in terpenoids metabolic pathway, and JA/MeJA biosynthesis and signal transduction pathway, we also predicted the DE-lncRNAs and their target genes involved in these 2 pathways. We found a total of 10 DE-lncRNAs owning 14 *cis*-target genes and other 4 DE-lncRNAs owning 5 *trans*-target genes in terpenoids metabolic pathway (Additional file 7: Table S7). Further analysis revealed that *GGPPS* and *TPS* genes were each regulated by 4 lncRNAs, while 4 genes (*DXS*, *CMK*, *AACT*, and *PMK*) only targeted by one lncRNA, respectively (Figure 11).

In JA/MeJA biosynthesis and signal transduction pathway, 18 DE-lncRNAs and 18 differentially expressed target genes were identified from FL, IW, and SW (Additional file 8: Table S8). Of which, a total of 6 genes involved in JA/MeJA biosynthesis and signal transduction pathway were regulated by lncRNAs, including *LOX*, *AOS*, *AOC*, *OPR*, *ACX*, and *MFP2* genes. In addition, we also identified the specific lncRNA-mRNA regulatory relationship. In brief, a total of 22 pairs of lncRNA-mRNA regulatory relationships were identified from these DE-lncRNAs and their target genes involved in JA/MeJA biosynthesis and signal transduction pathway (Figure 11).

To analyze the relationship between expression patterns of DE-lncRNAs and their target genes, we compared their expression in FL, IW, and SW. And the expression patterns of these 32 DE-lncRNAs were analyzed in FL, IW, and SW based on FPKM values (Figure 12). The overall expression levels of these DE-lncRNAs were more similar in IW and SW, while the expression levels of FL differed significantly from those of IW and SW. Among the 41 lncRNA-mRNA regulatory pairs, 25 lncRNA-mRNA pairs showed the same expression trend of lncRNA and potential target gene involved in terpenoids metabolic pathway and JA/MeJA biosynthesis and signal transduction pathway, while other 16 lncRNA-mRNA pairs showed an opposite expression trend between lncRNA and their target genes. Based on the regulatory relationship between lncRNA and mRNA, we found that there are 8 lncRNAs target more than one gene. Meanwhile 24 lncRNAs have only one target gene. This result implied most of the lncRNAs involved in these two pathways own a single regulatory function by regulating the expression level of target genes. However, a few lncRNAs that target several genes may have multiple functions.

To validate the accuracy of the expression pattern of lncRNA and its target genes, we conducted qRT-PCR analyses in terpenoids metabolic pathway to determine the transcript levels of *DXS* (MTCONS_00098554), *DXR* (CSA031231.1), *MCT* (MTCONS_00095823), *CMK* (MTCONS_00012675), *MDS* (MTCONS_00024440), *HDS* (MTCONS_00002131), *HDR* (CSA027533.1), *IDI* (MTCONS_00054647), *GGPPS* (CSA009630.1), *GPPS* (CSA036178.1), *AACT* (CSA004241.1), *HMGS* (CSA033887.1), *HMGR* (MTCONS_00002297), *MVK* (CSA017167.1), *PMK* (MTCONS_00101060), *MDC* (MTCONS_00071582), *FPS* (CSA030638.1), and *TPS* (MTCONS_00043145). In JA/MeJA biosynthesis and signal transduction pathways, the transcript levels of *LOX* (CSA036050.1), *AOS* (CSA023743.1), *AOC* (CSA017471.1), *OPR* (CSA027386.1), *ACX* (MTCONS_00079947 and MTCONS_00087178), *MFP2* (CSA008532.1), and *JAR* (CSA018958.1) were also analyzed by qRT-PCR. Furthermore, we also conducted qRT-PCR analyses to detect the expression levels of these lncRNAs, including LTCONS_00093140 (targeting *DXS* gene), LTCONS_00012676 (targeting *CMK* gene), LTCONS_00002173 (targeting *HDS* gene), LTCONS_00078708 (targeting *HDR* gene), LTCONS_00039845 (targeting *GGPPS* gene), LTCONS_00025739 (targeting *AACT* gene), LTCONS_00091745 (targeting *MVK* gene), LTCONS_00092790 (targeting *PMK* gene), LTCONS_00043160 (targeting *TPS* gene), LTCONS_00040667 (targeting *LOX* gene), LTCONS_00087608 (targeting *AOS* gene), LTCONS_00035664 (targeting *AOC* gene), LTCONS_00032547 (targeting *OPR* gene), LTCONS_00064473 (targeting *ACX* gene), LTCONS_00087182 (targeting *ACX* gene), and LTCONS_00061187 (targeting *MFP2* gene), involved in terpenoids metabolic pathway and JA/MeJA biosynthesis and signal transduction pathways. The expression trends of selected genes and lncRNAs were similar between the qRT-PCR and transcriptome dataset analyses (Figure 13). Together, these results

confirmed that there are significant differences in the transcription levels of terpenoid metabolism-related lncRNAs and target genes in FL vs. IW, FL vs. SW, and IW vs. SW.

Relationships between the lncRNAs and miRNAs involved in flavonoid metabolism, terpenoid metabolism, and JA/MeJA biosynthesis and signal transduction

To analyze relationships between lncRNAs and miRNAs involved in flavonoid metabolism, terpenoid metabolism, and JA/MeJA biosynthesis and signal transduction, the psRNATarget program was used to predict the potential regulatory relationships between lncRNAs and miRNAs. In total, 41 lncRNAs involved in these three metabolic pathways were predicted to be targets of 76 miRNAs in 35 families. And 133 lncRNA-miRNA interaction pairs were identified (Additional file 9: Table S9). Among them, 28 lncRNAs could be targeted by multiple miRNAs. However, 13 lncRNAs are regulated by only one miRNA. Similarly, we found that 36 miRNAs have only one target lncRNA; while the remaining 40 miRNAs have more than two target lncRNAs. This indicated that most lncRNAs identified in present study were regulated as targets of multiple miRNAs.

As a novel regulatory factor, eTMs can effectively inhibit miRNAs functions by binding to miRNAs, which indirectly affects the expression level of miRNA targets. And some lncRNAs can be involved in the regulation of miRNAs as eTMs. In the present study, two lncRNAs in transcriptome dataset were predicted to be potential eTMs (LTCONS_00026271 and LTCONS_00020084) for two miRNAs (novel_miR44 and miR169d-5p_1) involved in JA/MeJA biosynthesis and signal transduction pathway, respectively (Figure 14a). Moreover, we found that novel_miR44 targets *LOX*, and miR169d-5p_1 targets *ACX* (Additional file 10: Table S10).

To further explore the regulatory networks of eTMs with miRNAs and target genes, the expression patterns of these eTMs (LTCONS_00026271 and LTCONS_00020084), miRNAs (novel_miR44 and miR169d-5p_1), and target genes (*LOX*: MTCONS_00093153 and *ACX*: MTCONS_00008303) were detected in the qRT-PCR analyses. According to qRT-PCR analyses (Figure 14b), the expression levels of LTCONS_00026271 and *LOX* gene both significantly increased in SW compared to FL and IW, whereas the expression level of novel_miR44 in SW is significantly lower than that of FL and IW. In addition, LTCONS_00020084 and *ACX* gene were also up-regulated in SW than that in FL and IW, while miR169d-5p_1 was down-regulated in SW than that in FL and IW. On the basis of these results, we found negative relationships between eTMs and miRNAs, as well as miRNAs and targets. Conversely, the expressions of eTMs and target genes were positively correlated. And the lncRNAs-miRNAs-mRNAs regulatory network in JA/MeJA biosynthesis and signal transduction pathway was also constructed (Figure 14c). Together, these results suggested that lncRNAs may affect the expressions of related mRNAs through miRNAs.

Discussion

The expression levels of identified DE-lncRNAs and their target genes involved in flavonoid metabolism were associated with the low contents of total flavonoid and catechins in the solar-withered leaves

In the previous studies [28], tea flavor is greatly influenced by the manufacturing process. Withering is the first indispensable processing for improving flavors in oolong tea. During this processing, the content of some tea flavor-related compounds changed. Among these flavor-related compounds, flavonoid and catechins are the representative factors to evaluate the flavor quality of oolong tea [34, 35]. However, the contents of flavonoid and catechins in fresh leaves and withered leaves of oolong tea had not been determined. Thus, we detected the total flavonoid and total catechins, including 8 individual catechins, in FL, IW, and SW, respectively.

In this study, the leaves of SW had lower levels of total flavonoid, total catechins, and individual catechins in FL vs. SW, and IW vs. SW. Further analysis found that 5 individual catechins (EGC, CG, GCG, ECG, and EGCG) were all present the lowest contents in SW than that in FL and IW. However, the molecular mechanism underlying with the different contents of total flavonoid and catechins in solar-withered leaves and indoor-withered leaves remains unclear. Moreover, many research reported that lncRNAs play important regulatory roles at the transcript level of genes [69-71]. Conversely, studies on lncRNAs in tea plants are still in their infancy, and there is no research on the regulatory mechanisms of lncRNAs in tea processing. Thus, identifying and characterizing the lncRNAs that are present in withering processing are benefit to understand their roles for solar-withering.

To explore the regulatory mechanisms of lncRNAs in withering processing, we performed KEGG pathway enrichment analysis on target genes of DE-lncRNAs. The results of KEGG analyses revealed that the flavonoid metabolic pathway were one of the representative pathways among FL, IW, and SW. In lncRNAs regulation analysis, 10 regulatory relationships of lncRNAs and mRNAs involved in flavonoid metabolism analyzed by qRT-PCR in FL, IW, and SW, including 7 lncRNAs-mRNAs regulatory pairs (LTCONS_00054003-*4CL*, LTCONS_00060939-*CHI*, LTCONS_00031811-*FLS*, LTCONS_00001863-*CCR*, LTCONS_00090121-*CAD*, LTCONS_00030131-*HCT*, and LTCONS_00101116-*HCT*) that had positively regulated and other 3 regulatory pairs (LTCONS_00056216-*F3H*, LTCONS_00044497-*F3'H*, and LTCONS_00000233-*CAD*) had negatively regulated between lncRNAs and their target genes.

Compared with IW, the down-regulated lncRNAs (LTCONS_00054003 and LTCONS_00060939) in SW inhibited the normal expression of their related target genes (*4CL* and *CHI*). Moreover, 5 up-regulated lncRNAs (LTCONS_00031811, LTCONS_00030131, LTCONS_00101116, LTCONS_00001863, and LTCONS_00090121) in SW promote the highly expressed in their target genes (*FLS*, *HCT*, *CCR* and *CAD*). Moreover, it has been reported that some lncRNAs can negatively regulate the expression of target genes [72-74]. In our study, 3 pairs of negatively regulated lncRNAs-mRNAs were also found in the flavonoid metabolic pathway. High expression of LTCONS_00056216 and LTCONS_00044497 suppressed the expression of *F3H* and *F3'H* gene *CAD* gene in SW, while low expression of LTCONS_00000233 in SW promote the highly expressed in *CAD* gene.

Among these genes in flavonoid metabolism, *4CL* is a key gene in the flavonoid metabolic pathway of plant, which is responsible for catalyzing coumarate and its derivatives, providing the substrate for the synthesis of flavonoid and other substances in downstream of this metabolism [75, 76]. Many studies

have shown that high expression of *4CL* gene would promote the flavonoid biosynthesis [77, 78], and down-regulation of *4CL* gene would reduce the accumulation of flavonoid [79]. In our research, the low expression of LTCONS_00054003 inhibit *4CL* expression in SW. Similarly, the expression levels of *4CL* gene in *Populus tomentosa* [80] and *Paulownia tomentosa* [81] are also regulated by related lncRNAs. Besides, previous research has shown that *4CL* is a key gene affecting the production of catechins in tea [82]. It indicated that the expression level of *4CL* was positively correlated with the content of total flavonoid and catechins, and the low expressions of LTCONS_00054003 and *4CL* may be not conducive to accumulation of these compounds in SW. In the flavonoid metabolic pathway, *CHI* is located downstream of *4CL*, and the main role of *CHI* gene is to catalyze the intramolecular cyclization of chalcone into naringenin in plants [83, 84]. In various tissues of grapevine, the expression pattern of *CHI* was positively correlated with the total flavonoid accumulation [85]. In addition, it has been reported that withering is conducive to decreasing the expression of *CHI* and accumulation of catechins [37]. Furthermore, recent research showed that EGCG content was suppressed in tea leaves under drought stress, and the biosynthesis of EGCG were positively associate with the expression of *CHI* gene in response to drought [86]. Consistent with this, we observed that the transcript levels of *CHI* and EGCG content were both present at a lower level in SW than in IW. Besides, studies have found that some lncRNAs have the regulatory effects on the transcript level of *CHI* gene [71]. Also, we found that the LTCONS_00060939 and *CHI* were both present at lower expression level in SW than in IW. It inferred that high expression level of LTCONS_00060939 is a key factor in inhibiting the expression of *CHI* gene. During the solar-withering processing, the withered leaves will suffer from different types of stress, including drought, heat, and UV/light radiation, while the indoor withered leaves are mainly affected by drought stress [29, 87]. The solar-withering accelerates the dehydration process of tea leaves, and the degree of drought stress in solar-withered leaves were deeper than that in indoor-withered leaves. Thus, we suggested that solar-withering is beneficial to reduce the expression of *CHI* gene and EGCG content. Other 2 genes in the flavonoid metabolism were *F3H* and *F3'H*. Previous studies have shown that the catechins content in tea plant were positively correlated with the transcript levels of *F3H* and *F3'H* [88, 89]. In this research, there was a negative correlation between the expression levels lncRNA and these two genes. Therefore, the high expression of LTCONS_00056216 and LTCONS_00044497 in SW were not conducive to the normal expression of *F3H* and *F3'H* compared to SW, which also decreased the content of total flavonoid and catechins in SW.

Downstream of the flavonoid metabolic pathway, *FLS* is a branching gene responsible for converting dihydroflavonols to flavonols. At the same time, it competes with DFR for the substrate dihydroflavonols, and *DFR* is beneficial to promote the synthesis of catechins. Meanwhile, it has a competitive relationship with catechins biosynthesis. A previous research showed that *FLS* expression was negatively correlated with catechins biosynthesis, especially with EGCG [90]. Thus, we inferred that low expression of *FLS* in IW had little effect on catechins biosynthesis, while high expression of *FLS* in SW inhibited catechins accumulation. And highly expressed lncRNA (LTCONS_00031811) may positively regulate *FLS* gene, which may be responsible for the high expression of *FLS* in SW. The remaining three genes (*CCR*, *CAD*, and *HCT*) belong to the lignin biosynthesis pathway, and their abundance directly affects the lignin

accumulation in plants [91]. In phenylpropanoid metabolism, lignin biosynthesis pathway has parallel status with flavonoid metabolic pathway. Many studies have found that the content of lignin is positively correlated with the expression of *CCR*, *CAD*, and *HCT* genes, and up-regulation of these genes may benefit to the increase of lignin production [92-94]. Similar to that in *Arabidopsis thaliana* [95], lignin metabolism may also compete for same precursors substance with flavonoid metabolism in tea plant. Therefore, compared with IW, the higher transcript level of *CCR*, *CAD*, and *HCT* in SW may transfer the metabolic flux to lignin biosynthesis and suppressed flavonoid biosynthesis. This would reduce the amount of precursor substance for catechins biosynthesis in SW, and it may explain that high levels of total flavonoids and catechins can occur in SW, but not in IW.

Together, the above results suggest that high transcript levels of *FLS*, *CCR*, *CAD*, and *HCT*, and seven up-regulated lncRNAs (LTCONS_00056216, LTCONS_00044497, LTCONS_00031811, LTCONS_00001863, LTCONS_00090121, LTCONS_00030131, and LTCONS_00101116), and low transcript levels of *4CL*, *CHI*, *F3H*, and *F3'H*, and three down-regulated lncRNAs (LTCONS_00054003, LTCONS_00060939, and LTCONS_00000233) in SW closely linked with the low contents of total flavonoid and catechins in the solar-withered leaves. Thus, we suggested that, due to the regulation of related lncRNAs, the gene-expression related to flavonoid biosynthetic pathway was inhibited and the expression of related genes involved in lignin biosynthetic pathway was increased, thereby reducing total flavonoid and catechins in SW, especially the accumulation of galloylated catechins. Combined with our sensory evaluation, the reduction of galloylated catechins and flavonoid content is beneficial to reduce bitter and astringent tastes and moderately improve the palatability of tea infusion. Therefore, the solar-withering is conducive to the formation of weak astringent and mellow tastes of oolong tea.

The expression levels of identified DE-lncRNAs and their target genes involved in terpenoid metabolism were connected with the high contents of terpenoid volatiles in the solar-withered leaves

The terpenoid is one of the important components in oolong tea, and its content is positively correlated with the aroma quality of oolong tea [28]. Some gene families involved in the terpenoid metabolic pathway were expanded in the tea genome, which indicated that terpenoid was a crucial factor in aroma formation of tea [46]. On the basis of volatile determination, the terpenoid volatiles, including β -ocimene, limonene, γ -terpinene, α -farnesene, and β -myrcene, were also significantly increased in SW than that in IW. To investigate the differences in terpenoid volatiles between IW and SW, we further analyzed the transcript level of DE-lncRNAs and their target genes related to terpenoid metabolism via qRT-PCR. In this research, we identified 9 regulatory relationships of lncRNAs and mRNAs in terpenoid metabolism, including 3 lncRNAs-mRNAs pairs (LTCONS_00093140-*DXS*, LTCONS_00012676-*CMK*, and LTCONS_00092790-*PMK*) with the positive regulatory relationships and other 6 lncRNAs-mRNAs pairs (LTCONS_00002173-*HDS*, LTCONS_00078708-*HDR*, LTCONS_00039845-*GGPPS*, LTCONS_00025739-*AACT*, LTCONS_00091745-*MVK*, and LTCONS_00043160-*TPS*) with the negative regulatory relationships. Then, the qRT-PCR results confirmed that LTCONS_00093140, LTCONS_00012676, and LTCONS_00092790 were up-regulated in SW compared with IW. Also, their related target genes (*DXS*, *CMK*, and *PMK*) were present at a higher transcript level in SW than in IW. The other 6 lncRNAs

(LTCONS_00002173, LTCONS_00078708, LTCONS_00039845, LTCONS_00025739, LTCONS_00091745, and LTCONS_00043160) in SW were all present at a low transcript level, and related target genes in SW also showed a high transcript level than that in IW. Therefore these lncRNAs have the negative regulatory effect on the expression of their target genes (*HDS*, *HDR*, *GGPPS*, *AACT*, *MVK*, and *TPS*).

Among these genes in terpenoid metabolism, *DXS*, *CMK*, *HDS*, and *HDR* genes belong to methylerythritol phosphate (MEP) pathway, other 3 genes (*AACT*, *MVK*, and *PMK*) belong to mevalonate (MVA) pathway [37, 42]. In the MVA pathway, acetyl-CoA acts as a substrate, while pyruvate and glyceraldehyde-3-phosphate (GA-3P) were used as the metabolic substrate in the MEP pathway. However, the final products of these two pathways are isopentenyl diphosphates (IPP). Moreover, the biosynthesis of IPP will directly affect the yield of downstream terpenoids [96]. Additionally, several studies have shown that the expression levels of these genes involved MEP pathway are positively correlated with the yield of terpenoids [30, 97, 98]. Consistent with this, transcript levels of these target genes related to terpenoid metabolic pathway showed that the high expression of 7 upstream genes (*DXS*, *CMK*, *HDS*, *HDR*, *AACT*, *MVK*, and *PMK*) in SW than in IW was regulated by related lncRNAs. Thus, we inferred that high content of terpenoid metabolites such as γ -Terpinene, α -farnesene and β -myrcene in SW may be due to the transcript level of these lncRNAs and their target genes related to terpenoid metabolism, which enhanced the metabolic flux of terpenoid biosynthesis and their precursors, especially IPP. The remaining two target genes, *GGPPS* and *TPS*, are located downstream of the terpenoid metabolism. *GGPPS* is known to be a key gene that catalyze the biosynthesis of carotenoid from geranylgeranyl diphosphate (GGPS) [99, 100]. And carotenoids can be further degraded into aromatic compounds such as β -ionone, which has a significant contribution to the flavor of tea because of its low olfactory threshold [66, 101, 102]. In our study, the content of β -ionone in SW was significantly higher than that in IW. After being negatively regulated by LTCONS_00039845, the transcript level of *GGPPS* gene was also up-regulated in SW compared to IW. It indicated that the expression level of *GGPPS* gene is positively correlated with the content of β -ionone, while the expression level of LTCONS_00039845 is a factor that negatively regulates the content of β -ionone. Thus the high level of *GGPPS* gene may function as a profound role in biosynthesis of β -ionone. In addition, sunlight and UV treatments were beneficial for the accumulation of β -ionone in plants [103-105]. Consistent with sunlight and UV caused by solar-withering, we also suggested that solar-withering was also a factor in which the contents of β -ionone in SW is higher than IW. And β -ionone is one of the characteristic aroma components of oolong tea, its high content in SW also indicated that solar-withering is more conducive to the formation of high quality aroma in oolong tea. *TPS* is an indispensable gene directly catalyzing the formation of the most abundant and structurally diverse terpenoids in tea aroma, and high expression of *TPS* facilitates the conversion of the precursors into various terpenoids [106, 107]. In present study, highly expressed LTCONS_00043160 inhibits the expression of *TPS* gene in IW. However, LTCONS_00043160 was in low expression level in SW, which had little effect on the expression level of *TPS* gene. Therefore, the expression level of *TPS* gene in SW was higher than IW. Meanwhile, the content of terpenoid volatiles were present at a high level in SW than in IW. We inferred that the content of terpenoid volatiles will be affected by the expression level of the *TPS* gene and its related lncRNA.

Above all, SW had the 9 up-regulated genes (*DXS*, *CMK*, *HDS*, *HDR*, *AACT*, *MVK*, *PMK*, *GGPPS*, and *TPS*), 3 up-regulated lncRNAs (LTCONS_00093140, LTCONS_00012676, and LTCONS_00092790), 6 down-regulated lncRNAs (LTCONS_00002173, LTCONS_00078708, LTCONS_00039845, LTCONS_00025739, LTCONS_00091745, and LTCONS_00043160) involved in terpenoid metabolism compared with IW. In addition, lncRNAs in plants are recognized as emerging regulatory components involved in most secondary metabolism [108-111]. Consistent with previous research, terpenoid metabolism in withering processing were both not only affected by the expression level of related genes, but also by the changes in the expression level of related lncRNAs. And it is indicated that the synergism between lncRNAs and target genes was related to terpenoid metabolism in oolong tea withering. After the solar-withering processing, the oolong tea products have the floral and fruity aroma. However, after indoor withering, the oolong tea products has the characteristics of calm and dull aroma [33]. Combined with the expression levels of lncRNAs and their target genes related to terpenoid metabolism, high contents of terpenoids may beneficial for the formation of high quality aroma in SW than in IW. Moreover, compared with IW, the content of β -ionone in SW increased significantly, while the content of (Z)-3-hexenal and (E)-2-hexenal decreased significantly. And β -ionone has floral aroma and is one of the characteristic aroma components of oolong tea. However, (Z)-3-hexenal and (E)-2-hexenal are the most important components of grass flavor, which is an undesirable aroma in tea [36]. Therefore, we concluded that after the solar-withering processing, high levels of terpenoid and β -ionone, as well as low levels of (Z)-3-hexenal and (E)-2-hexenal, are beneficial for SW to have the light grass flavor, and slightly floral and fruity aromas in SW. And this would be one of the reasons for why the solar-withering is more conducive to the formation of higher quality aroma than the indoor-withering.

Solar light, high contents of JA and MeJA, and eTMs regulatory mechanism are benefit to the accumulation of terpenoid in solar-withered leaves

Light is a major environmental factor affecting the accumulation of terpenoid metabolites, and expression patterns of numerous genes involved in terpenoid metabolic pathway are regulated by light [112-114]. On the basis of previous research [115-118], light has a positive effect on the transcript levels of *DXS*, *CMK*, *HDS*, and *HDR* genes. In present study, the transcript levels of *DXS*, *CMK*, *HDS*, and *HDR* were significantly increased in SW than that in IW. It can be seen that solar light has the ability to induce high expression of these genes in the tea withering. Meanwhile, there are a large number of light-specific *cis*-acting elements in the promoter region of most genes in the MEP pathway [118]. After solar light stimulation, the expression of *DXS*, *CMK*, *HDS*, and *HDR* genes is activated and the expression level of these genes and related terpenoid content are up-regulated in solar-withering. At the same time, the solar-withering has stronger light intensity than the indoor-withering, which promotes the up-regulation of related genes in the MEP pathway and enhances the metabolic flow of terpenoids, thereby increasing the content of terpenoid volatiles in SW. It can therefore be concluded that the whole MEP pathway in withering processing is light-stimulated. Additionally, light can also induce significant expression of *AtGGPPS11* downstream of terpenoid metabolism in *A. thaliana* seedlings [96], and the expression of *TPS* in tea plant were regulated by light [32]. Consistent with it, we also observed that the *GGPPS* and *TPS* were present a high transcript level in SW than IW, and β -ionone and terpenoid contents were highly co-

associated with the transcript level of *GGPPS* and *TPS*, respectively. The lack of sufficient light illumination in the indoor-withering, the expression level of related genes in terpenoid metabolism is present at a low level in IW than SW, which is not conducive to the accumulation of related terpenoid volatiles. Thus, solar-withering is benefit for up-regulating the transcript level of *DXS*, *CMK*, *HDS*, *HDR*, *GGPPS*, and *TPS* and increasing the accumulation of the β -ionone and terpenoid volatiles in SW, which may be the reason for the floral and fruity aroma of oolong tea after solar-withering processing.

Phytohormones are important bioactive molecules, which act as signal chemicals to regulate the synthesis of related volatiles that have been demonstrated [38, 119]. Over the past decade, some studies have shown that jasmonate is benefit to the formation of terpenoid volatiles [120-122]. However, little is known about the specific role of jasmonate in tea processing. To investigate the effects of JA and MeJA on the tea aroma in withering processing, we detected the contents of JA and MeJA in FL, IW and SW, respectively. In present study, the content of JA and MeJA both showed the a significantly higher level in SW compared with IW and FL. Moreover, 7 regulatory relationships of lncRNAs and mRNAs involved in JA/MeJA biosynthesis and signal transduction pathway were identified. Among these, 4 lncRNAs-mRNAs regulatory relationships (LTCONS_00040667-*LOX*, LTCONS_00032547-*OPR*, LTCONS_00064473-*ACX*, and LTCONS_00061187-*MFP2*) that had positively regulated and other 3 regulatory relationships (LTCONS_00087608-*AOS*, LTCONS_00035664-*AOC*, and LTCONS_00087182-*ACX*) had negatively regulated between lncRNAs and their target genes. In JA/MeJA biosynthesis, a series of genes are involved, including *LOX*, *AOS*, *AOC*, *OPR*, *ACX*, and *MFP2* [123]. Among these genes, *LOX* is the first step of linolenic acid utilization for JA/MeJA biosynthesis, and up-regulated *LOX* gene in plants will induce the accumulation of jasmonate in response to various stresses [124]. In our research, highly expressed LTCONS_00040667 promote the expression of *LOX* gene in SW, and JA and MeJA were also present at a high level in SW. It is indicated that the transcript level of LTCONS_00040667 and *LOX* gene were highly associated-with the contents of JA and MeJA, and high expression of LTCONS_00040667 and *LOX* gene were benefit to JA/MeJA biosynthesis in SW.

In the allene oxide synthase (*AOS*) pathway, 5 genes (*AOS*, *AOC*, *OPR*, *ACX*, and *MFP2*) played the key roles in JA/MeJA biosynthesis, and high expression level of *AOS*, *AOC*, *OPR*, *ACX*, and *MFP2* were contribute to the accumulation of JA and MeJA [40, 123, 125, 126]. In tea withering processing, highly expressed LTCONS_00032547, LTCONS_00064473, and LTCONS_00061187 promote up-regulation of *OPR*, *ACX*, and *MFP2* gene in SW by positive regulation, respectively. The other 3 lncRNAs (LTCONS_00087608, LTCONS_00035664, and LTCONS_00087182) in SW were all present at a low transcript level, and they have a negative regulatory effect on the expression of their target genes (*AOS*, *AOC*, and *ACX*), so these target genes also showed a high transcript level in SW than that in IW. Therefore, these lncRNAs promote the up-regulation of JA/MeJA-related genes in SW through multiple regulatory mechanisms. Previous studies have demonstrated that jasmonate biosynthesis pathway in plants could be induced under abiotic stress, and a large number of jasmonate-related metabolites play the important roles in plants defense and plant-environment interactions [127, 128]. Similarly, the solar-withered leaves will suffer from different types of stress, including drought, heat, and UV/light radiation, while the indoor withered leaves are mainly affected by drought stress. Therefore, the degree of abiotic

stress on the withered leaves in the solar-withering is higher than that in the indoor-withering. In addition, the accumulation of jasmonate in plants is increased by activating the expression of genes related to the JA/MeJA biosynthetic pathway in response to the damage caused by biotic or abiotic stress [129, 130]. In present study, JA/MeJA biosynthesis-related genes were positive correlation with the content of JA and MeJA. In SW, we inferred that up-regulated JA/MeJA biosynthesis-related genes would promote the accumulation of JA and MeJA, and then high levels of jasmonate activate downstream terpenoid metabolic pathway and increase the synthesis of related terpenoid volatiles.

Previous study has shown that some lncRNAs can act as eTMs to bind to miRNAs, thereby inhibiting miRNAs from cleavage of target genes, and this reveal a new mechanism by which lncRNA regulates miRNAs in plants [10]. However, the lncRNAs as eTMs have not been identified in tea plant, and the regulation of miRNAs and mRNAs by eTMs is still unclear. On the analysis of transcriptom dataset, 2 lncRNAs (LTCONS_00026271 and LTCONS_00020084) are found to be potential eTMs for 2 miRNAs (novel_miR44 and miR169d-5p_1) involved in the JA/MeJA biosynthesis and signal transduction pathway, respectively. Further analysis of the target genes of these 2 miRNAs revealed that novel_miR44 targets the *LOX* gene and miR169d-5p_1 targets the *ACX* gene. Compared with IW, the LTCONS_00026271 and *LOX* gene were present at a higher transcript level in SW, while the expression level of novel_miR44 were down-regulated in SW. Similarly, the expression level of miR169d-5p_1 in SW was significantly lower than that of IW, and the LTCONS_00020084 and *ACX* gene consistently showed significant high expression levels in SW compared to IW. Consistent with previous research [11, 131], Highly expressed lncRNA acts as a sponge to bind a large number of miRNAs, thereby protecting coding-genes from the cleavage of miRNAs. In tea withering processing, high transcript level of LTCONS_00026271 and LTCONS_00020084 acting as the eTMs to promote the expression levels of *LOX* and *ACX* genes by decoying relevant miRNAs. And the high transcription level of *LOX* and *ACX* genes are benefit to the accumulation of jasmonic acid, and activated the terpenoid metabolic pathway through JA/MeJA signal transduction to promote the increase of terpenoid volatiles in SW. Thus, the eTMs regulatory mechanism (LTCONS_00026271-novel_miR44-*LOX* and LTCONS_00020084-miR169d-5p_1-*ACX*) in SW were also a crucial factor in increasing the content of terpenoid.

In total, solar light, high contents of JA and MeJA, and eTMs regulatory mechanism are benefit to terpenoid metabolism during tea withering. Among them, it is speculated that solar light, high content of JA and MeJA could have an accumulative effect on the accumulation of terpenoid. The content of MeJA and JA in IW are both significantly higher than FL, so the content of related genes and metabolites in terpenoid metabolism were present at a higher level than FL. Compared with IW, SW not only has higher content of JA and MeJA, but also has higher intensity of solar light. Therefore, the high transcript level of terpenoid metabolism-related genes and high content of terpenoid metabolites in SW are both significantly higher than IW. Additionally, eTMs regulatory mechanism in SW were also a crucial factor in increasing the content of JA, MeJA, and terpenoid. Therefore, solar light, high contents of JA and MeJA, and eTMs regulatory mechanism in SW are all contribute to the accumulation of aroma-related terpenoid. And this also confirms the view of "Kàn qīng shài qīng" that the solar-withering is more contribute to the high quality aroma of tea compared to the indoor-withering.

Conclusions

Using transcriptome and phytochemical analyses, we found that SW exhibited lower contents of total flavonoid, catechins (EGC, CG, GCG, ECG, and EGCG) and (Z)-3-Hexenal, (E)-2-Hexenal, as well as higher contents of terpenoid volatiles and β -Ionone compared with IW and FL. Additionally, we also analyzed the expression profiles of DE-lncRNAs and their target genes involved in flavonoid metabolism, terpenoid metabolism, and JA/MeJA biosynthesis and signal transduction pathway that were differentially expressed among FL, IW, and SW. The results suggested that the expression of DE-lncRNAs and their target genes involved in the three pathways might be related to the low content of total flavonoid, total catechins (EGC, CG, GCG, ECG, and EGCG), and high content of terpenoid metabolites in SW. And lncRNAs regulatory mechanism play a key role in affecting the accumulation of related secondary metabolites in solar-withered leaves. Moreover, solar light, high content of JA and MeJA could have an accumulative effect on the accumulation of terpenoid. Therefore, the high transcript level of terpenoid metabolism-related genes and high content of terpenoid metabolites in SW are both significantly higher than IW. Further analyses of lncRNAs revealed the eTMs regulatory mechanism (LTCONS_00026271-novel_miR44-*LOX* and LTCONS_00020084 -miR169d-5p_1-*ACX*) in SW were also a crucial factor in increasing the content of terpenoid. Combined with the the expression of DE-lncRNAs and their target genes, our study revealed low contents of total flavonoid, catechins (EGC, CG, GCG, ECG, and EGCG) and (Z)-3-Hexenal, (E)-2-Hexenal, as well as high contents of terpenoid volatiles and β -Ionone in SW were contribute to the formation of the weak astringent and mellow tastes, light grass flavor, and slightly floral and fruity aromas. This findings also confirm the view of "Kàn qīng shài qīng" that the solar-withering is more contribute to the high quality flavor of oolong tea compared to the indoor-withering (Figure 15).

Abbreviations

nt: nucleotide; lncRNA: long non-coding RNA; miRNA: microRNA; mRNA: messenger RNA; FPKM: expected fragments per kilobase of transcript per million fragments mapped; qRT-PCR: quantitative real-time polymerase chain reaction; bp: base pair.

Declarations

Ethics approval and consent to participate

The eight-year-old tea plants (*C. sinensis* cv. Tieguanyin) was cultivated at Fujian Agriculture and Forestry University, Fuzhou, Fujian Province, China (E 119°14', N 26°05'). No specific permits were required for plant collection. The study did not require ethical approval or consent as no endangered or protected plant species were involved.

Consent for publication

Not applicable.

Availability of data and materials

All data presented in this study are provided either in the manuscript or additional files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YQG, ZXL, and CZ designed the work; YQG, CZ, and STZ performed the experiments and wrote the paper; YQG, CZ, SSZ, HFF, XJC, XZL, and YLL analyzed the data; CZ, STZ, CZZ, and LC helped to perform the sequence analysis and revised the paper carefully. All authors have read and approved the manuscript.

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Table

Due to technical limitations, Table 1. Relative contents of top 15 volatile components in FL, IW, and SW (%), is only available as a download from the supplemental file section.

Supplemental File Descriptions

Additional file 1: Table S1. Primers used for qRT-PCR analyses.

Additional file 2: Table S2. Summary of sequencing reads after filtering.

Additional file 3: Table S3. DEGs related to flavonoid metabolic pathway in FL, IW, and SW.

Additional file 4: Table S4. Summary of lncRNA-mRNA pairs related to flavonoid metabolic pathway in FL, IW, and SW.

Additional file 5: Table S5. DEGs related to terpenoid metabolic pathway in FL, IW, and SW.

Additional file 6: Table S6. DEGs related to JA/MeJA biosynthesis and signal transduction pathway in FL, IW, and SW.

Additional file 7: Table S7. Summary of lncRNA-mRNA pairs related to terpenoid metabolic pathway in FL, IW, and SW.

Additional file 8: Table S8. Summary of lncRNA-mRNA pairs related to JA/MeJA biosynthesis and signal transduction pathway in FL, IW, and SW.

Additional file 9: Table S9. Summary of lncRNA-miRNA pairs related to flavonoid metabolic pathway, terpenoid metabolic pathway, and JA/MeJA biosynthesis and signal transduction pathway in FL, IW, and SW.

Figures

Figure 1

Leaf phenotype of fresh leaves, indoor-withered leaves, and solar-withered leaves.

Figure 2

Contents of total flavonoid, catechins, JA, and MeJA in fresh leaves (FL), indoor-withered leaves (IW), and solar-withered leaves (SW). C: catechin; GC: gallocatechin; EC: epicatechin; EGC: epigallocatechin; CG: catechin gallate; GCG: gallocatechin gallate; ECG: epicatechin gallate; EGCG: epigallocatechin gallate; JA: jasmonic acid; MeJA: methyl jasmonate. Data are means $\pm$ standard deviations (SD). Lowercase letter indicates significant difference ($P < 0.05$); uppercase letter indicates highly significant difference ($P < 0.01$).

Figure 3

Distribution of lncRNA expression level.

Figure 4

Gene ontology (GO) analysis of the target genes of differentially expressed-lncRNAs (DE-lncRNAs) identified in FL vs. IW, FL vs. SW, and IW vs. SW.

Figure 5

Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses of differentially expressed genes (DEGs) identified in FL vs. IW, FL vs. SW, and IW vs. SW. Large rich factor represents high degree of enrichment. Lower q-value represents more significant enrichment of DEGs.

Flavonoid metabolic pathway

Figure 6

Differentially expressed genes (DEGs) involved in flavonoid metabolic pathway. Numbers in parentheses following each gene name indicate number of corresponding DEGs. The expression level of DEGs in FL, IW, and SW are shown by color grids based on FPKM value.

Figure 7

Differentially expressed genes (DEGs) involved in flavonoid metabolic pathway. Numbers in parentheses following each gene name indicate number of corresponding DEGs. The expression level of DEGs in FL, IW, and SW are shown by color grids based on FPKM value.

Figure 8

The relative expression levels of the differentially expressed-lncRNAs (DE-lncRNAs) related to flavonoid metabolic pathway

Figure 9

Expression patterns of (A) differentially expressed genes (DEGs), (B) differentially expressed-lncRNAs (DE-lncRNAs) and their target genes in flavonoid metabolic pathway determined by qRT-PCR. Data are means $\pm$ standard deviations (SD). Lowercase letter indicates significant difference ($P < 0.05$); uppercase letter indicates highly significant difference ($P < 0.01$).

Figure 10

Differentially expressed genes (DEGs) involved in terpenoid metabolic pathway. Numbers in parentheses following each gene name indicate number of corresponding DEGs. The expression level of DEGs in FL, IW, and SW are shown by color grids based on FPKM value.

Figure 11

The lncRNA-mRNA relationships involved in JA/MeJA biosynthesis and signal transduction pathway and terpenoid metabolic pathway. Solid line represents direct action; dashed line represents potential action. Solid line with arrow represents positive regulation; "T" type solid line negative regulation.

Figure 12

The relative expression levels of differentially expressed-lncRNAs (DE-lncRNAs) related to JA/MeJA biosynthesis and signal transduction pathway and terpenoid metabolic pathway.

Figure 13

The relative expression levels of differentially expressed-lncRNAs (DE-lncRNAs) related to JA/MeJA biosynthesis and signal transduction pathway and terpenoid metabolic pathway.

Figure 14

LncRNA acting as endogenous target mimics (eTMs) of miRNAs, qRT-PCR validation of lncRNAs, miRNAs, and mRNAs, and the eTM regulatory network. (A) Two lncRNAs were identified as eTMs of miRNAs. (B) qRT-PCR validation of novel_miR44 with its target gene (LOX) and eTM (LTCONS_00026271), and miR169d-5p_1 with its target gene (ACX) and eTM (LTCONS_00020084). Data are means $\pm$ standard deviations (SD). Lowercase letter indicates significant difference ($P < 0.05$); uppercase letter indicates highly significant difference ($P < 0.01$). (C) The eTM regulatory network involved

in JA/MeJA biosynthesis and signal transduction pathway. The solid line with the arrow represents direct action; dashed line represents indirect negative regulation; "T" type solid line negative regulation.

Figure 15

Schematic diagram of key lncRNAs, genes and metabolites metabolites of flavonoid metabolic pathway, terpenoid metabolic pathway, and JA/MeJA biosynthesis and signal transduction pathway in SW. Red font indicates lncRNA and gene with higher expression level or metabolite with higher content in SW than

in IW; green font indicates lncRNA and gene with lower expression level or phytohormones and metabolite with lower content in SW than in IW. Solid line represents direct action.

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