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Changes in alkaloids and their related metabolites during the processing of 'Qiancha 1′ white tea based on transcriptomic and metabolomic analysis

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ABSTRACT

The regulatory mechanism underlying alkaloid metabolism during the withering process of' Qiancha 1' white tea remains poorly understood. Analyze the changes in metabolites and gene expression during the processing of white tea using metabolomics and transcriptomics.

The results showed that a total of 1,542 metabolites and 60,987 genes were identified in the sample. Among them, there are 353 differential metabolites and 12 alkaloids and their related differential metabolites.129 key differentially expressed genes related to alkaloid metabolism were identified, and the expression of most genes exhibited a significant negative correlation with the corresponding alkaloids and their related metabolites content. The study elucidated the alkaloids and their related metabolites metabolism pathway and related differential gene regulation mechanism of 'Qiancha 1' white tea during processing, and found that alkaloid metabolites are closely related to amino acid metabolism. This research lays a foundation for understanding the importance of alkaloids and their related metabolite metabolism in the formation of tea flavor.

1. Introduction

Tea can be classified in multiple ways. China mainly produces six types of tea: green tea, black tea, white tea, yellow tea, oolong tea, and dark tea. The above classification is based on the fermentation level of fresh tea leaves (Chen et al., 2020).Tea is one of the most popular beverages in the world, receiving increasing attention due to its unique and pleasant flavor and outstanding health benefits (Huang et al., 2024). Compared with other types of tea, white tea has the simplest processing technology, which only includes two stages: withering and drying. This special processing technology imparts white tea with unique flavor characteristics, mainly presenting a fresh and sweet taste. Withering is the first step in the processing of white tea and plays an important role in the formation of its unique flavor (Zhu et al., 2023). During the withering process, detached leaves are affected by a combination of water loss, temperature, and light. Due to the relatively intact morphological structure of withered leaves, physiological activities such as photosynthesis, transpiration, and plant hormone signal transduction still occur normally in the leaves (Wong et al., 2022; Zeng et al., 2018).

Furthermore, various biochemical reactions and changes in the abundance of secondary metabolites occur during the withering process of tea leaves to maintain the permeability and metabolic balance of leaf cell membranes and cope with the adverse effects of environmental stress (Wang et al., 2019).External stress can induce plants to produce many secondary metabolites, which have the function of helping plants resist external stress (Zhu et al., 2020).Simultaneously, stress can trigger signaling molecules, especially the biosynthesis of plant hormones (Bartwal et al., 2012).These plant hormones serve as primary messengers in signal transduction, regulating the expression levels of genes related to the biosynthesis of metabolites, thereby influencing the changes in metabolite concentrations (Xu et al., 2020).

The metabolites in these tea leaves imparts them with unique flavor and quality. Alkaloids are important secondary metabolites in tea (Wan et al., 2011).Alkaloids mainly exist in plants and have a wide range of biological activities. High alkaloid concentration is positively correlated with bitterness (Qin et al., 2020). Common alkaloids found in nature include caffeine, theobromine, theophylline, methyluric acid, hypoxanthine, xanthine, and uric acid, which are typically found in tea, coffee, and cocoa plants (Jiao et al., 2023, Wan et al., 2011).

Most previous studies have focused on exploring the correlation

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between changes in metabolite profiles during white tea processing and the expression levels of key genes in metabolic pathways. There has been relatively little research on a specific flavor substance category in white tea processing, especially alkaloid metabolites (Wang et al., 2016).The research on tea processing is limited to the determination of secondary metabolite content before and after production process processing (Zhang et al., 2019; Chen et al., 2019).However, there have been no reports on revealing the metabolic patterns of alkaloids during white tea processing from the perspective of transcriptional regulation. At present, the combination of metabolome detection and transcriptome sequencing has been widely applied in the mining of key metabolites and related gene information for the formation of tea flavor quality (Tai et al., 2015; Li et al., 2020; Wu et al., 2014).Wang et al. identified 12 structural genes involved in catechin synthesis using transcriptome sequencing (Wang et al., 2018). The identification of structural genes related to terpenoid biosynthesis and the mapping of metabolic pathways have all been completed (Xu et al., 2018).

'Qiancha 1′ is an independently selected variety in Guizhou Province, China. It is a clonal, shrub type, and mid leaf variety suitable for producing green tea, black tea, and white tea, with excellent tea quality (Yang et al., 2019).However, during the processing of 'Qiancha 1′ white tea, the specific regulatory mechanisms of key alkaloid metabolites and associated genes related to flavor quality formation remain unclear.To reveal the molecular mechanism by which mRNA affects the formation of flavor quality during the withering process of white tea, this study conducted transcriptome sequencing on fresh and withered leaves, identified the metabolic pathways where differentially expressed genes were mainly enriched, identified alternative splicing events and differentially expressed genes, and conducted in-depth analysis of the expression patterns of key differentially expressed genes involved in the formation of flavor quality in 'Qiancha 1′ white tea.

At the same time, the metabolism of alkaloids and their related metabolites during the processing of 'Qiancha 1′ white tea has not been studied. Therefore, this study combines metabolomics and transcriptomics techniques to explore the metabolic patterns and gene regulation mechanisms of alkaloids and their related metabolites during the processing of white tea. This is of great significance for studying the mechanism of quality formation of 'Qiancha 1′ white tea.

2. Materials and methods

2.1. Tea samples

On July 6, 2022, a sample of 'Qiancha 1′ white tea was produced at Langzhu Organic Tea Industry Co., Ltd. in Fenggang County, Guizhou Province. The standard for picking fresh leaves is one bud and two leaves, with an average withering temperature of 28 °C and a drying temperature of 65 °C.Tea samples are dried using a box hot air drying. Samples were taken separately (Fig. 1), including fresh leaves (WD0), withered 6-h leaves (WD6), withered 12-h leaves (WD12), withered 18-h leaves (WD18), withered 24-h leaves (WD24), withered 36-h leaves (WD36), and dried tea leaves (GZ). Three biological replicates were taken from each group of samples.Biological samples are freeze-dried by vacuum freeze-dryer (Scientz-100F). The freeze-dried sample was crushed using a mixer mill (MM 400, Retsch) with a zirconia bead for 1.5 min at 30 Hz. Dissolve 50 mg of lyophilized powder with 1.2 mL 70% methanol solution, vortex 30 s every 30 min for 6 times in total. Following centrifugation at 13523×*g* for 3 min, the extracts were filtrated (SCAA-104, 0.22 μm pore size; ANPEL, Shanghai, China, http://www.anpel.com.cn/) before UPLC-MS/MS analysis.

2.2. Chemicals

Acetonitrile and methanol were both purchased from Merck (Darmstadt, Germany). Formic acid was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd (Shanghai, China). CTAB and

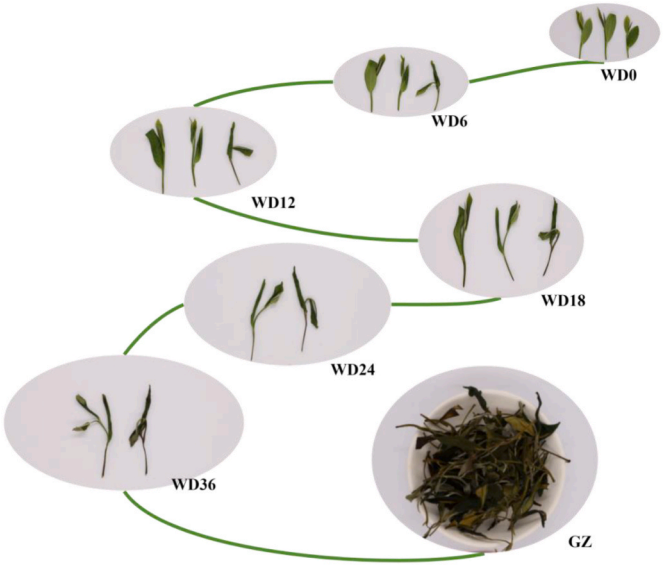


Fig. 1. Sample diagram of white tea processing process.

lithium chloride purchased from Autobio Engineering (Shanghai) Co., Ltd (Shanghai, China). β - mercaptoethanol was purchased from Fuchen (Tianjin) Chemical Reagent Co., Ltd. (Tianjin, China). The pBIOZOL plant total RNA extraction reagent was purchased from Hangzhou Borui Technology Co., Ltd. Polyvinylpyrrolidone (PVP-40) was purchased from amresco company in Hangzhou, Zhejiang, China. (Eden Prairie, United States). Sodium chloride and anhydrous ethanol were purchased from China National Pharmaceutical Group Co., Ltd. (Beijing, China). Tris (1 mol/L), pH 8.0, no RNase, EDTA (0.5 mol/L), pH 8.0, no RNase and no nuclease water were purchased from Thermo Fisher Scientific. Lenexa, United States. The DNA magnetic beads and Hieff NGS ® dual-mode mRNA library kit were purchased from Yisheng Biotechnology (Shanghai) Co., Ltd. (Shanghai, China).

2.3. LC-MS/MS metabolite analysis method

The data acquisition instrument system mainly includes Ultra Performance Liquid Chromatography (UPLC) (ExionLC ™ AD, https://sciex.com.cn/)Tandem mass spectrometry (MS/MS) and Applied Biosystems 6500 QTRAP, https://sciex.com.cn/). The liquid phase conditions mainly include: chromatographic column: Agilent SB-C18 1.8 μ m, 2.1 mm * 100 mm; Mobile phase: A phase is ultrapure water (with 0.1% formic acid added), B phase is acetonitrile (with 0.1% formic acid added); Elution gradient: At 0.00 min, the proportion of phase B was 5%. Within 9.00 min, the proportion of phase B increased linearly to 95% and remained at 95% for 1 min, 10.00–11.10 min. The proportion of phase B decreased to 5% and equilibrated at 5% for 14 min; Flow rate 0.35 mL/min; Column temperature of 40 °C; Injection volume 2 μ L. The mass spectrometry conditions mainly include: the temperature of electric spray ion source (ESI) is 500 °C; Ion spray voltage (IS) 5500 V (positive ion mode)/-4500 V (negative ion mode); Ion source gas I (GSI), gas II (GSII), and curtain gas (CUR) are set to 50, 60, and 25 psi, respectively, with collision induced ionization parameters set to high. QQ scanning uses MRM mode and sets collision gas (nitrogen) to medium. By further optimizing the declustering potential (DP) and collision energy (CE), the DP and CE of each MRM ion pair were completed. Monitor a specific set of MRM ion pairs at each stage based on the metabolites eluted during each period.

2.4. Screening of differential metabolites

Univariate statistical analysis methods include parametric tests and

non parametric tests. Multivariate statistical analysis methods include principal component analysis, partial least squares discriminant analysis, etc. Based on the OPLS-DA(Orthogonal Partial Least Squares-Discriminant Analysis)results, the variable importance in projection (VIP) of the obtained multivariate analysis OPLS-DA model can preliminarily screen metabolites with differences between different varieties or tissues. At the same time, the P-value or fold change of univariate analysis can be combined to further screen for differential metabolites. If there are ≥3 biological replicates, a combination of fold change value, *P* value, and VIP value of the OPLS-DA model is used to screen for differential metabolites. Screening criteria:

For comparison between two groups.

(1) Select metabolites with VIP ≥1. The VIP value represents the strength of the impact of inter group differences of corresponding metabolites on the classification discrimination of each group of samples in the model. It is generally believed that metabolites with VIP ≥1 have significant differences (Chen et al., 2021).
(2) Select metabolites with fold change ≥2 and fold change ≤0.5. If the difference in metabolites between the control group and the experimental group is more than twice or less than 0.5, the difference is considered significant (Chen et al., 2021).

For multiple group comparisons.

(1) Select metabolites with VIP ≥1.
(2) Select metabolites with P-value<0.05 in ANOVA. If there is a statistically significant difference in metabolites between different groups, the difference is considered significant (Mo et al., 2019).

2.5. Transcriptome analysis RNA detection and library construction

Agarose gel electrophoresis: analyze the integrity of RNA and whether there is DNA contamination; Qubit 2.0 fluorescence meter: high-precision measurement of RNA concentration; Agilent 2100 Bioanalyzer: Accurately detects RNA integrity.

There are two main ways to obtain mRNA: one is to utilize the structural feature that most mRNA in eukaryotes has polyA tails, and enrich mRNA with polyA tails using Oligo (dT) magnetic beads; The second is to remove ribosomal RNA from total RNA to obtain mRNA.

Subsequently, a fragmentation buffer was added to break the RNA into short fragments. Using the short fragment RNA as a template, the first strand cDNA was synthesized using random hexamers primers. Then, buffer, dNTPs (dUTP, dATP, dGTP, and dCTP), and DNA polymerase I were added to synthesize the second strand cDNA. The double strand cDNA was purified using AMPure XP beads. The purified double stranded cDNA was subjected to terminal repair, followed by the addition of an A tail and ligation of sequencing adapters. Then, fragment size selection was performed using AMPure XP beads, and finally PCR enrichment was performed to obtain the final cDNA library. After the construction of the library is completed, the quality of the library is tested. Only when the test results meet the requirements can machine sequencing be carried out. The testing method is as follows.

(1) Use Qubit2.0 for preliminary quantification, and use Agilent 2100 to detect the insert size of the library. Only when the insert size meets expectations can the next experiment be carried out.
(2) The Q-PCR method accurately quantifies the effective concentration of the library (library effective concentration>2 nM) and completes the library inspection. After passing the library inspection, different libraries are pooled according to the target offline data volume and sequenced using the Illumina platform.

2.6. Transcriptome sequencing and analysis

Sequencing of library fragments was performed on the Illumina HiSeq sequencing platform using Metwell Biotechnology Co., Ltd. After filtering the raw data, checking the sequencing error rate, and examining the GC content distribution, clean reads were obtained for subsequent analysis. Using HISAT2 to perform sequence alignment between clean reads and the reference genome (CSS_ChrLev_20200506_Genome.fas), obtain positional information on the reference genome or gene, as well as unique sequence feature information of the sequencing sample. The reads count of genes is performed using featureCounts, and FPKM (Fragments Per Kilobase of transcript per Million fragments mapped) is calculated. DESeq2 is used to determine differentially expressed genes (DEGs), and after differential analysis, multiple hypothesis testing (P-value) correction is required using the Benjamini Hochberg method to obtain the false discovery rate (FDR). The screening criteria for differentially expressed genes are | log2 fold change | ≥ 1 and FDR<0.05. Further KEGG enrichment analysis of DEGs was performed using clusterProfiler software.

2.7. Verification of transcription levels of differentially expressed genes

Using TransScript reagent kit ® Uni All in One First Strand cDNA Synthesis SuperMix (TRANS, China) will reverse transcribe extracted RNA to synthesize cDNA. Real time quantitative polymerase chain reaction (qRT PCR) amplification of key differentially expressed genes was performed using the SCI1000-G PCR gradient gene amplifier (SCILOGEX, China). Using GAPDH as an internal reference gene, the relative quantification method 2- Δ Δ Ct algorithm (Livak and Schmittgen, 2001) was employed to detect the expression levels of key differentially expressed genes in different withering treatments. Three biological replicates were conducted for each experiment. The relevant primer information can be found in Table S1.

3. Results

3.1. Profile of overall changes in metabolites during the processing of 'Qiancha 1' white tea

Through extensive targeted metabolomics, a total of 1542 metabolites were identified in the processing samples of 'Qiancha 1' white tea, among which flavonoids accounted for the highest proportion (24.58%) and terpenoids accounted for the lowest proportion (1.88%) (Fig. 2A). Through PCA analysis (Fig. 2B), it was found that with the passage of withering time, the difference separation of samples with different withering times gradually became obvious, and the separation of dried tea leaves from other samples was the most significant. Indicating good separation of samples from different groups, similar situations between parallel samples, and reliable samples. In order to identify which differential metabolites caused the separation phenomenon, metabolites with VIP ≥1 and P-value<0.05 in ANOVA were selected for comparison of the entire sample group. 353 metabolites with significant changes in content were discovered.

For the comparison between the two groups, metabolites with VIP ≥1 and fold change ≥2 and fold change ≤0.5 were selected as significantly different metabolites. The volcano plot (Fig. 2C) shows that compared with fresh leaves of 'Qiancha 1', 16 up-regulated differential metabolites and 23 down regulated differential metabolites were found in withered leaves (6h). Compared with withered leaves (6h), withered leaves (12h) upregulated 35 differential metabolites and downregulated 11 differential metabolites. Compared with withered leaves (12h), withered leaves (18h) upregulated 40 differential metabolites and downregulated 18 differential metabolites. Compared with withered leaves (18h), withered leaves (24h) upregulated 20 differential metabolites and downregulated 6 differential metabolites. Compared with withered leaves (24h), withered leaves (36h) upregulated 61 differential

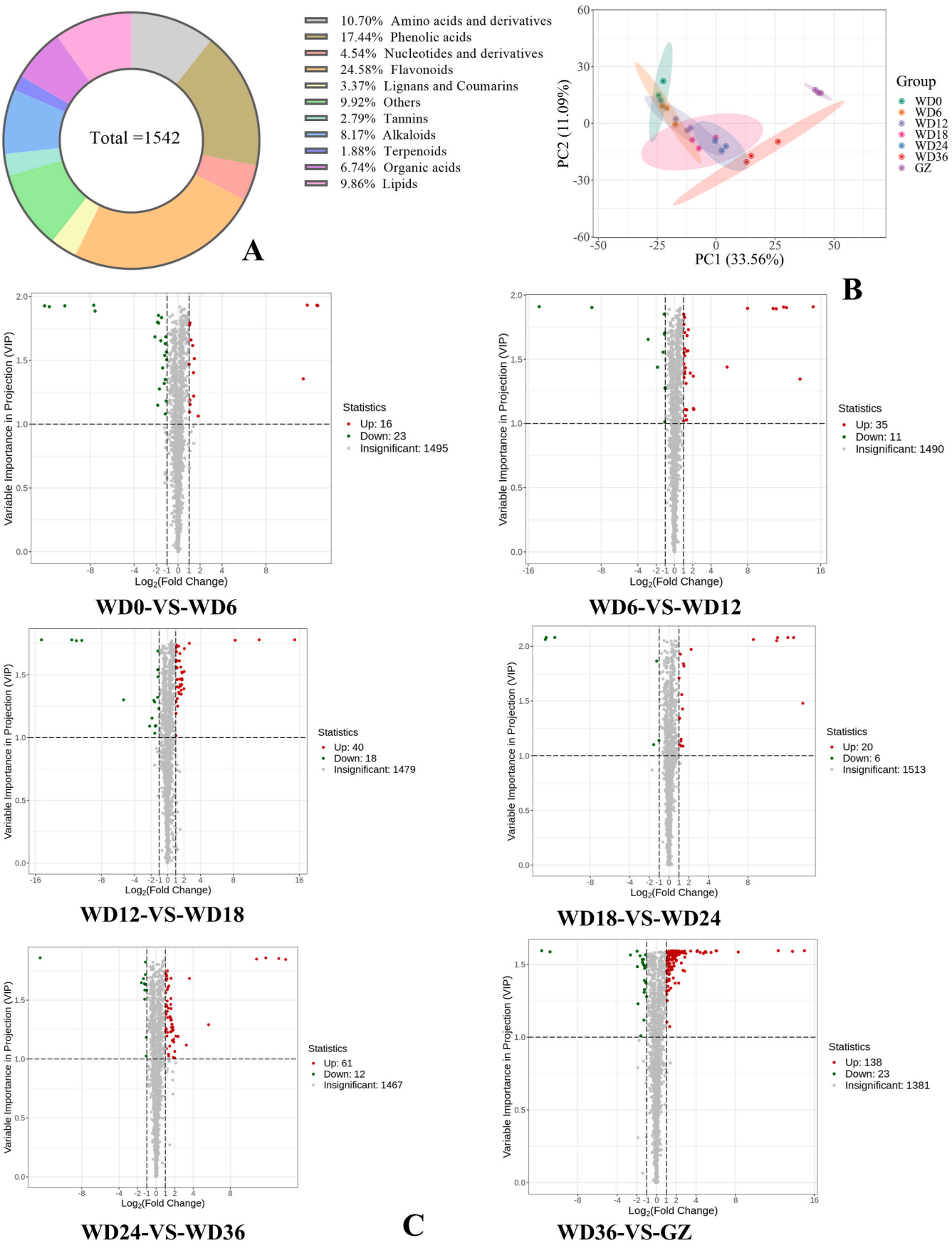


Fig. 2. Summary of Metabolite Changes. (A circular diagram of metabolite category composition, B PCA diagram of samples during processing; C Volcano plots of different metabolites compared at different times during white tea withering).

metabolites and downregulated 12 differential metabolites. Compared with withered leaves (36 h), the dried white tea product upregulated 138 differential metabolites and downregulated 23 differential metabolites.

From this, it can be seen that as the withering process progresses, the number of up-regulated differential metabolites in 'Qiancha 1′tea leaves remains greater than the number of down regulated differential metabolites after 6 h of withering. Especially in the drying stage, the number of up-regulated differential metabolites was as high as 138, while the number of down regulated differential metabolites was only 12, indicating that the final formation of white tea quality during the processing of 'Qiancha 1′ white tea may be related to the accumulation of these significant differential metabolites. Through identification, these differential metabolites can be classified into 11 categories, including amino acids and their derivatives, phenolic acids, nucleotides and their derivatives, flavonoids, lignans and coumarins, tannins, terpenes, organic acids, lipids, alkaloids, and others. This indicates that during the processing of 'Qiancha 1′ white tea, withering and drying cause changes in metabolites, which have an impact on the quality of white tea.

3.2. Changes in alkaloids and their related metabolites

In this test, a total of alkaloids and their related metabolites were identified, among which 12 key differential alkaloids and their related metabolites were determined through screening (as described in the

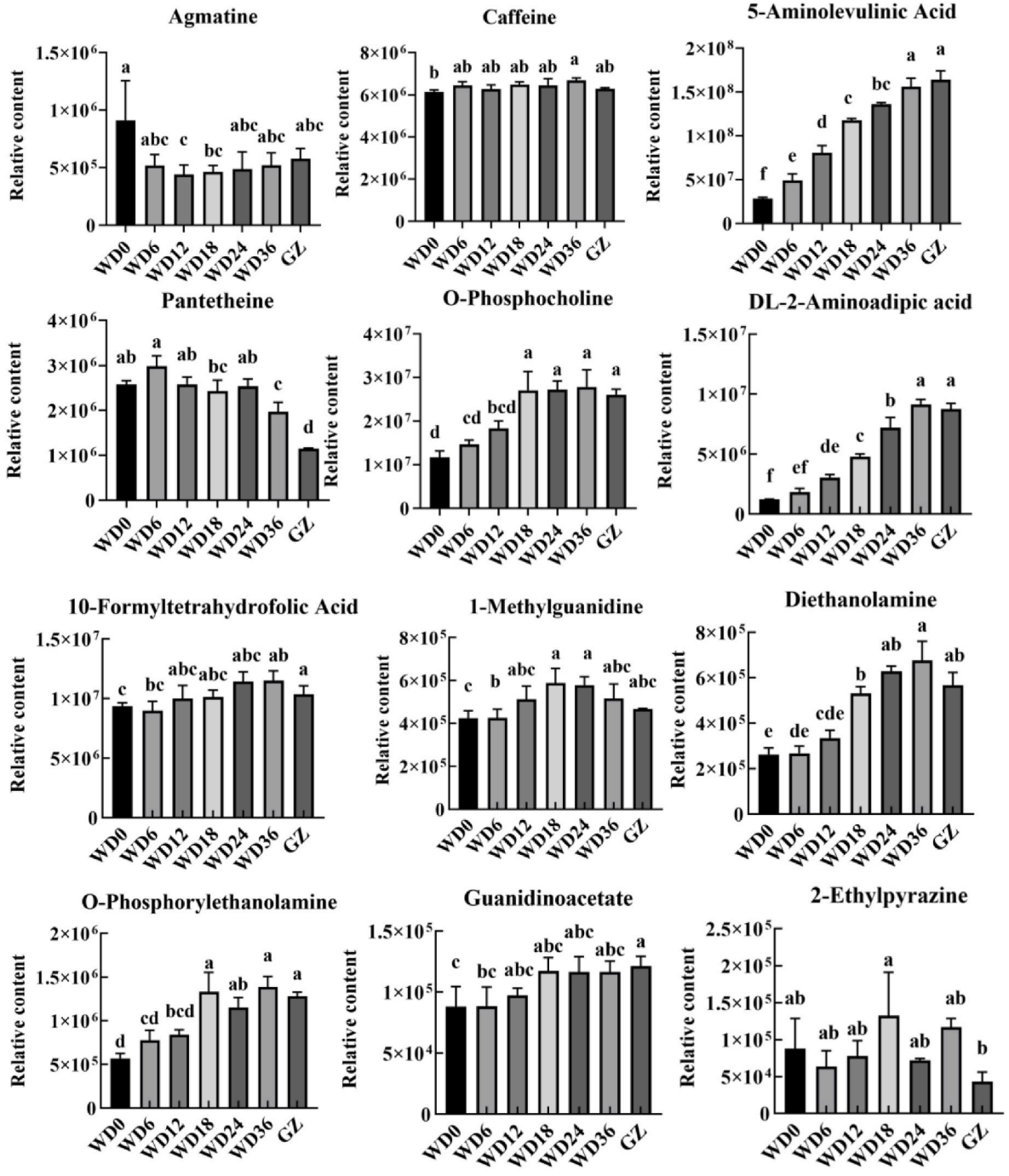


Fig. 3. Relative content changes of alkaloids and their related metabolites during processing.

previous section), including agmatine, caffeine, 5-aminolevulinic acid, pantetheine, o-phosphocholine, dl-2-aminoadipic acid, 10-formyltetrahydrofolate, 1-methylguanidine, diethanolamine, o-phosphoethanolamine, guanidinoacetate and 2-ethylpyrazine. This indicates that these 12 alkaloids and their related differential metabolites have an impact on the quality changes of 'Qiancha 1′white tea during processing.

Fig. 3 shows the relative content changes of each alkaloid and its related metabolites, with the relative content being the peak area of the chromatographic peak corresponding to each metabolite.The relative content of Agmatine and 2-Ethylpyrazine decreased under processing, but there was no significant difference.The relative content of caffeine significantly increased after 36 h of withering compared to 0 h, and decreased during drying, but there was no significant difference. The relative contents of 5-aminolevulinic acid, dl-2-aminoadipic acid, 10-formyltetrahydrofolic acid, diethanolamine, o-phosphatlethanolamine, and guanidinoacetate showed an increasing trend under processing, and the relative contents significantly increased under drying compared to the samples that had been wilted for 0 h. The relative content of Pantetheine significantly decreases with the processing progress. This shows,in the processing of white tea, withering has an impact on the relative content of alkaloids and their related metabolites, and under the heat effect of later drying, it also has a regulatory effect on the content of alkaloids and their related metabolites.

3.3. Gene expression analysis during the withering process of white tea

Differential metabolite KEGG functional enrichment analysis was performed on 6 groups of withered tea samples, and it was found that differential metabolites were enriched in pathways such as ABC transporters, amino acid biosynthesis pathways, carbon metabolism, alanine, aspartate, and glutamate metabolism, cysteine and methionine metabolism, photosynthetic metabolism, and other pathways (Fig. 4A). This indicates that a large-scale metabolic reprogramming event may occur during the withering process of 'Qiancha 1′ to adapt to the environment.

Therefore, we performed transcriptome sequencing on withered tea samples.This project completed transcriptome sequencing analysis of 6 groups of withered tea samples during the withering process, and obtained 134.77 Gb of Clean Data (Table S2). The Clean Data of each sample reached 6 Gb, and the Q30 base percentage was 93% or above. Based on the alignment results, analyses for alternative splicing prediction and the identification of novel genes were performed. Furthermore, gene expression level analysis was conducted according to alignment results. Genes exhibiting differential expression were identified based on their expression levels across different samples, followed by functional annotation and enrichment analysis.

From the overall distribution of gene expression levels in the samples, a total of 60987 genes were detected in this transcriptome sequencing. During the process from 0 h to 36 h of withering, the distribution of gene expression levels in the samples was relatively concentrated without significant dispersion, and it can be seen that the overall gene expression level was higher in the early stage of withering (Fig. 4BC). The density plot illustrated the trends in abundance for all samples fluctuating with expression levels, clearly indicating that the range of gene expression levels in all samples was consistent (Fig. 4D), thereby demonstrating the reliability of the transcriptomic analysis data for this study.

By comparing the expressed genes at different withering times, differentially expressed genes between different withering times of tea were screened. In this study, the threshold for significant differences is $|\log2foldchange| \geq 1$, and $p < 0.05$ is used as the screening criterion. The number of differentially expressed genes is shown in Fig. 4E. Compared with withering for 0 h, 2674 differentially expressed genes were screened after tea withering for 6 h, including 1015 up-regulated differentially expressed genes and 1659 down regulated differentially expressed genes. Compared with withering for 6 h, 2360 differentially expressed genes were screened after 12 h of tea withering, including 761 up-regulated differentially expressed genes and 1599 down regulated differentially expressed genes. Compared with withering for 12 h, 2848 differentially expressed genes were screened after tea withering for 18 h, including 878 up-regulated genes and 1970 down regulated differentially expressed genes. Compared with withering for 18 h, 403 differentially expressed genes were screened after 24 h of tea withering, including 254 up-regulated differentially expressed genes and 149 down regulated differentially expressed genes. Compared with withering for 24 h, 653 differentially expressed genes were screened after tea withering for 36 h, including 470 up-regulated differentially expressed genes and 183 down regulated differentially expressed genes. As the withering process progresses, it was found that the number of differentially expressed genes in tea leaves is highest between 12 and 18 h of withering, indicating that metabolic activity in tea leaves is more active during this withering time range. At the end of the withering time, the number of differentially expressed genes decreases significantly, which may mean that metabolic activity in tea leaves gradually decreases.

Some studies have shown that alternative splicing is an important means of post transcriptional regulation of gene expression, and it can play an important role in plant coping with stress by producing specific alternative splicing bodies (Simpson et al., 2010). Therefore, an in-depth analysis was conducted on the alternative splicing events during the withering process of tea leaves. Based on the relative transcriptional abundance of alternative splicing genes (FPKM), differential splicing genes were analyzed and identified in five comparison groups: WD0 vs WD6, WD6 vs WD12, WD12 vs WD18, WD18 vs WD24, and WD24 vs WD36 (Table S3). Among them, the number of differentially spliced genes identified in WD24 vs WD36 was 470, which was higher than the number of differentially spliced genes found in other groups, indicating that the treatment significantly induced the production of a large number of differentially spliced genes during the late withering period from 24 h to 36 h. Analysis of alternative splicing types for all differentially spliced genes revealed that the number of genes undergoing skipped Exon alternative splicing was the highest, indicating that wilting not only affects changes in the number of alternative splicing, but also affects the expression level of alternative splicing transcripts, resulting in a large number of differentially spliced genes.

The above results indicate that withering treatment not only directly affects the expression levels of key genes in the synthesis pathway of related flavor metabolites, but may also participate in regulating alternative splicing events, resulting in the formation of different transcripts of structural genes in the metabolism of related flavor substances, indirectly affecting the expression levels of related genes, and thus affecting the formation of tea flavor.

Therefore, the transcriptional expression changes of alkaloid and their related metabolites during the withering process deserve further attention.

3.4. Gene mining related to the metabolism of alkaloid metabolites

Among them, through comparison with the KEGG database, it was found that the metabolism of alkaloids and their related metabolites is mainly concentrated in the pentose phosphate pathway; Among these metabolic pathways, caffeine wetabolism; glycine, serine, and threonine metabolism; porphyrin metabolism; biosynthesis of cofactors; abc transporters; pantothenate and coa biosynthesis; glycerofhydrolipid metabolism; lysine biosynthesis; lysine degradation; 2-oxidized acid metabolism; biosynthesis of amino acids one carbon pool by folate; aminoacyl rna biosynthesis; carbon metabolism; biosynthesis of cofactors (Table S4).

At the same time, we excavated the differentially expressed genes under different withering times and found that 129 common key differentially expressed genes were enriched in these metabolic pathways, excluding the duplicated genes in each comparison group, indicating that these 129 key differentially expressed genes are involved in the metabolism of alkaloids and their related metabolites metabolites.

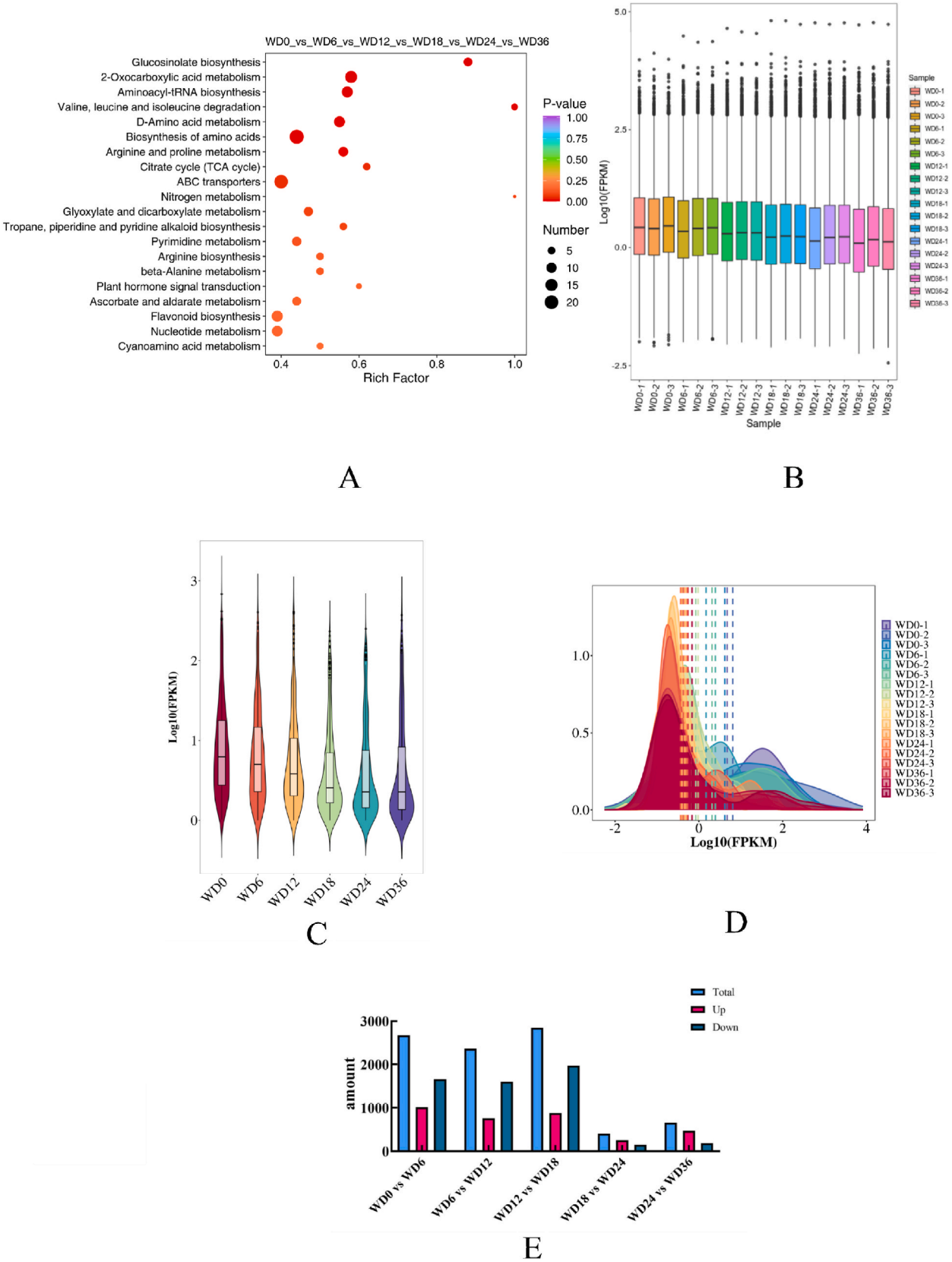


Fig. 4. A Enrichment analysis of metabolites KEGG during tea withering process, B Box plot of gene expression levels in each sample, C Violin plot of gene expression levels in each sample, D Density plot of gene expression levels in each sample,E Differential gene number statistics Analysis of alternative splicing events during the withering process of white tea.

And through comparison, it was found that the details are shown in Table S5 and Fig. 5A. In comparison with fresh leaf samples after withering for 6 h, a total of 60 key differentially expressed genes were identified, including 21 upregulated key differentially expressed genes and 39 downregulated key differentially expressed genes. In the comparison between withering for 12 h and withering for 6 h, a total of 48 key differentially expressed genes were identified, including 12 upregulated key differentially expressed genes and 36 downregulated key differentially expressed genes. In the comparison between the samples withered for 18 h and withered for 12 h, a total of 41 key differentially expressed genes were identified, including 12 up-regulated key differentially expressed genes and 29 down regulated key differentially expressed genes. In the comparison between the samples withered for 24 h and withered for 18 h, there was a total of one key differentially expressed gene, with a trend of upregulation. In the comparison between samples withered for 36 h and 24 h, a total of 11 key differentially expressed genes were identified, including 8 up-regulated key differentially expressed genes and 3 down regulated key differentially expressed genes. It was found that as the withering process progressed, the number of differentially expressed genes related to alkaloid metabolism showed a decreasing trend, and between 18 h and 24 h of withering, there was only one differentially expressed gene related to alkaloid metabolism, CSS0022536 (hypothetical protein HYC85). Combined with KEGG enrichment analysis of differentially expressed genes (Fig. 5), it was found that during the withering process, the metabolism related to alkaloid substances in tea showed a relatively active state in the first 6 h, and the metabolic activity related to alkaloid substances gradually decreased in tea during the time range of 18 h–24 h.

In order to investigate the mechanism of alkaloid metabolism during the withering process of fresh leaves, we analyzed the correlation between changes in 12 key differential alkaloids and their related metabolites contents and changes in the expression levels of related gene transcripts during withering. Pearson's correlation analysis between the expression levels of differentially expressed genes related to the above pathways and the relative content of these alkaloids and their related metabolites showed that the expression of most genes was significantly negatively correlated with the corresponding alkaloids and their related metabolites content (Fig. 6). In the metabolism of caffeine, there is also a significant negative correlation between gene expression and caffeine content.

For example, CSS0015110 and CSS30170 show a significant negative correlation with most alkaloids and their related metabolites. Some genes, such as CSS0022536, CSS0002094, CSS0030403, and CSS0044622, are significantly negatively correlated with Pantetheine, but significantly positively correlated with 5-Aminolevulinic Acid, O-Phosphocholine,DL-2-Aminoadipic acid,10-Formyltetrahydrofolic Acid, Diethanolamine,O-Phosphorylethanolamine,Guanidinoacetate. There is a significant positive correlation, indicating that the expression changes of these genes play an important role in the changes in alkaloid and their related metabolites content.

3.5. Validation and expression analysis of differentially expressed genes

In order to verify the reliability of the identified differential gene transcription events, some differential genes with high correlation with alkaloids and their related metabolites and high absolute FC values in group comparison were selected for qRT PCR. The results showed (Fig. 7) that the expression patterns of differentially expressed genes during tea withering were consistent with those in the transcriptome dataset, confirming the reliability and rationality of high-throughput sequencing data and subsequent analysis.

4. Discussion

Based on the changes in alkaloids and their related metabolites in this study, the metabolic pathways of these metabolites in the KEGG database were screened. The speculated metabolic pathways of caffeine and its related metabolites are shown in Fig. 8. In caffeine metabolism, one pathway metabolizes to theobromine under the action of ndmA (CSS0011494), with ndmA exhibiting the highest expression in fresh leaves, the lowest at 12 h of withering, and subsequently lower than fresh leaves at 36 h of withering. The concentration of theobromine decreases with withering and drying processes. Theobromine continues to metabolize to 3-methylxanthine, with its levels decreasing throughout the withering and drying processes, and further metabolizes to xanthine under the action of ndmB (Zhou et al., 2020).The xanthine content is highest at 12 h of withering, but decreases with the withering and drying process.

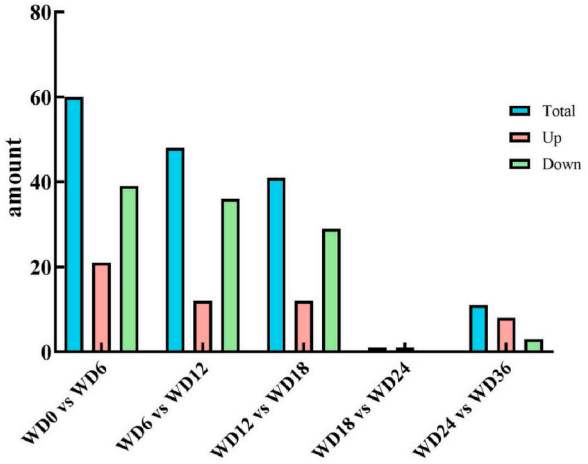
Another pathway is caffeine metabolism to theophylline. The content of theophylline gradually increases from fresh leaves to 24 h of withering, but gradually decreases during 36 h of withering and drying. The accumulation of theophylline content during the early withering process may be related to the decrease in caffeine content, and the accumulation in the later stage may be related to the metabolism of 3-methylxanthine (Zhou et al., 2020). The content of theophylline decreases at 36 h of withering and during the drying process, with theophylline metabolizing to 1-methylxanthine under the action of ndmB. The concentration of 1-methylxanthine increases during withering, but it was not detected in the dried samples. Simultaneously, 1-methylxanthine can also be metabolized to xanthine under the action of ndmA (CSS0011494) (Algharrawi et al., 2015; Baek et al., 2022).

Xanthine is metabolized into xanthine nucleoside, and the content of xanthine nucleoside decreases with withering, but increases in dry samples. Xanthine nucleoside is metabolized into 7-methylxanthine under the action of XMT, and finally participates in the synthesis of caffeine (Suzuki and Takahashi, 1975). The content of xanthine increases with the process of withering and drying, while the content of 7-methylxanthine decreases with the process of processing. The decrease in 7-methylxanthine content may be related to the accumulation of caffeine content. Caffeine can also be metabolized into 1,3, 7-tnmethyluric acid under the action of cdhA, and then participate in Glyoxylate metaboism.

Among other alkaloids and their related metabolites, metabolic pathways revealed through KEGG database comparisons show that their metabolism is mostly associated with amino acid metabolism. (Fig. 8). During this study, diethanolamine is metabolized to O-phosphoethanolamine under the action of ETNK (CSS0050397), and then O-phosphoethanolamine is further metabolized to O-phosphocholine under NMT.Choline can also be metabolized into o-phosphatoline under the action of CK11 (CSS0010774). Throughout the study, the content of diethanolamine accumulates with the duration of withering, showing a slight decrease during drying. The expression level of CK11 (CSS0010774) decreases with the process of withering.The content of O-Phosphatothhanolamine and O-Phosphatoline accumulates with the progress of withering time, slightly decreasing during the drying process, but still much higher than the content in fresh leaves. At the same time, the accumulation of O-Phosphothanolamine content may also be related to the metabolism of serine to O-Phosphothanolamine under the action of ETNK (CSS0028454) (Liu et al., 2021). In this study, the content of serine also increased with the withering process and slightly decreased during the drying process.

Additionally, we found that what was detected in this study 5-aminolevulinic acid and guanidinoacetate can be metabolized from glycine (Bollenbach et al., 2019; Ducamp et al., 2021). glycine can be metabolized to threonine under the action of ITaE (CSS0002094). The content of methionine is highest at 18 h of withering, but it decreases with the progress of processing.Simultaneously, glycine can also be metabolized into serine under the action of glyA (CSS0002204). Glycine can be metabolized under the action of ALAS to 5-aminolevulinic acid, while glycine can be metabolized under the action of GATM guanidinoacetate.

The content of guanidinoacetate increases with the processing



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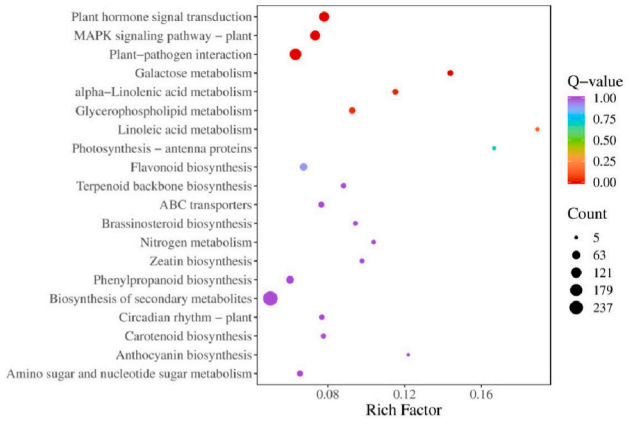
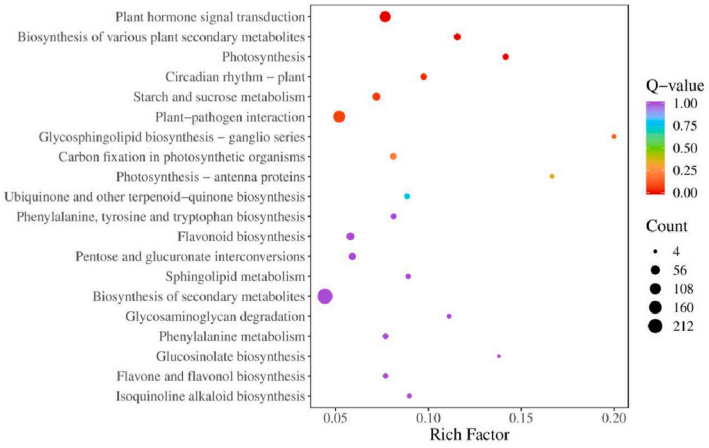
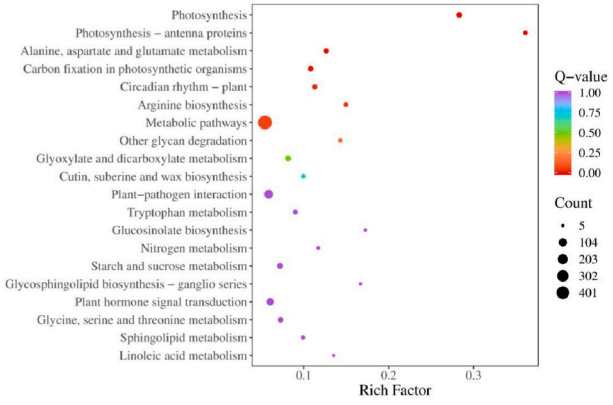


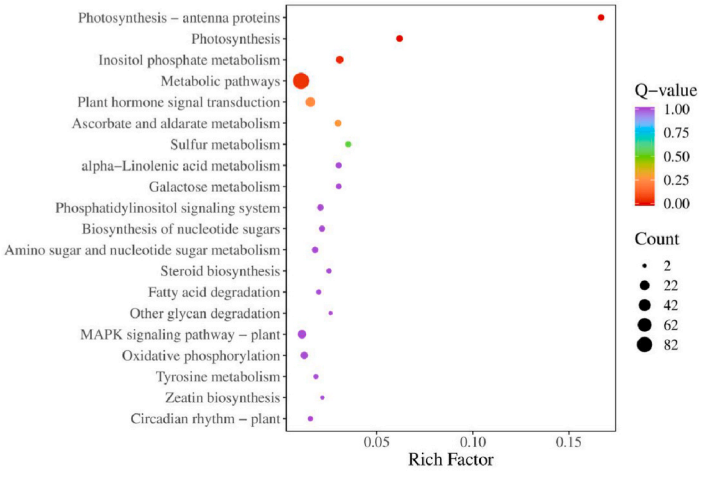
WD0 vs WD6



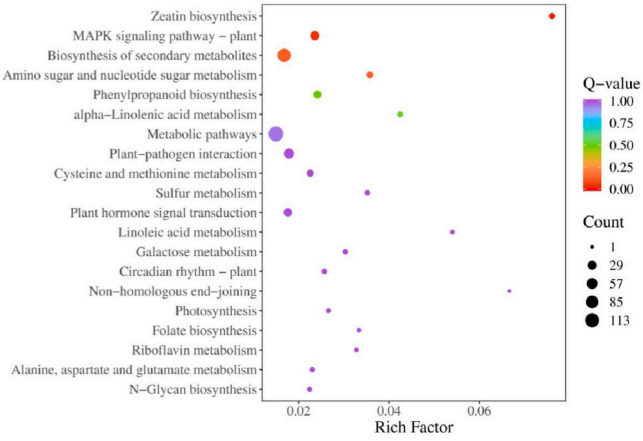
WD6 vs WD12



WD12 vs WD18



WD18 vs WD24



WD24 vs WD36

Fig. 5. A Number of key genes differentially associated with alkaloid metabolism, Functional enrichment analysis of KEGG for differential genes during withering.

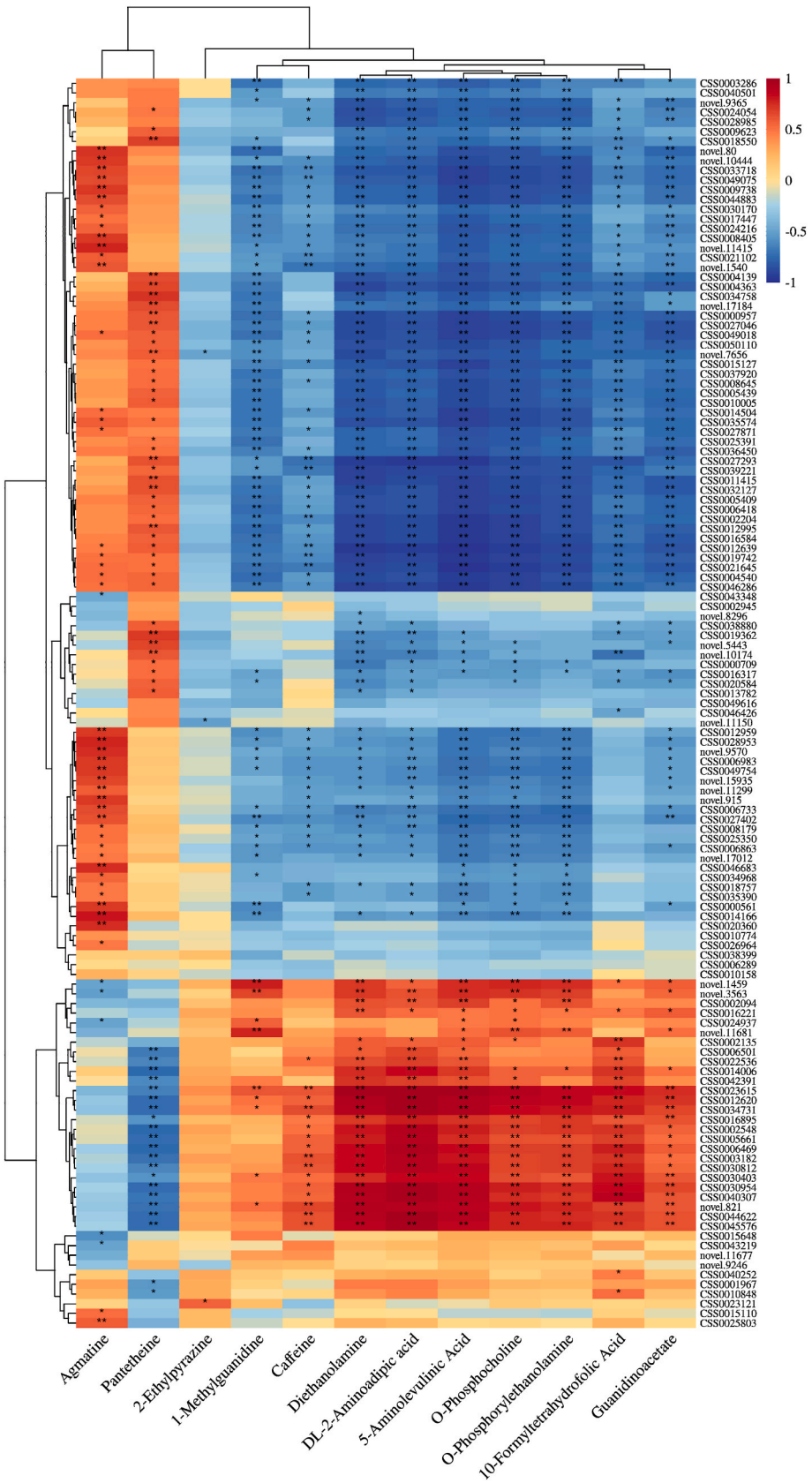


Fig. 6. Correlation of alkaloids with key genes.

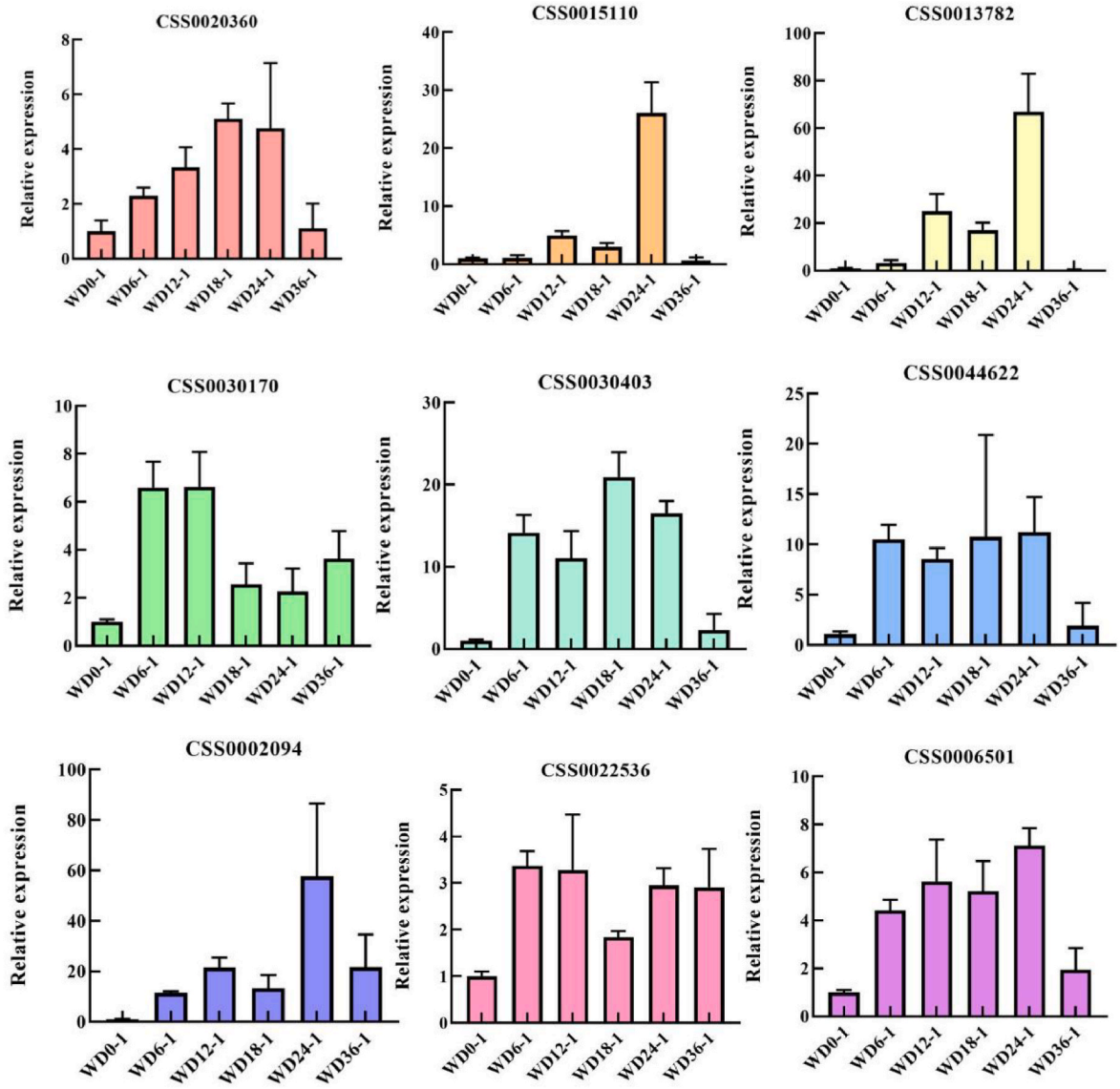


Fig. 7. Expression patterns of key differentially expressed genes during withering process.

progress.Guanidinoacetate is metabolized to arginine under the action of GATM, which is a precursor metabolite of agmatine (Kobayashi et al., 2023). Arginine is metabolized to agmatine under the action of E4.1.1.19 (CSS0006469). The expression level of E4.1.1.19 (CSS0006469) showed an upward trend during withering, while the content of agmatine decreased with processing. Agmatine is then metabolized into putrescine and ultimately participates in tropane, piperidine, and pyridine biosynthesis. Moreover, arginine can also be metabolized into putrescine under the action of ODC1 (CSS0030403), and the expression level of ODC1 (CSS0030403) shows an upward trend during withering. In this study, it was found that the content of putrescine decreased with the processing progress, and Seprmine could also be directly metabolized into putrescine through the action of MPAO (CSS0030360). The expression level of MPAO (CSS0030360) gradually decreased from fresh leaves to 24 h of withering, but increased at 36 h of withering. The content of dl-2-aminoadipic acid also increases with the processing progress. It can be metabolized into lysine under the action of LYS2 and LYS1, and ultimately participates in the biosynthesis of terpenes, piperidine, and pyridine alkaloids. In this related metabolism, the content of glycine decreases with the progress of processing, the content of threonine decreases with the progress of processing, and the content of arginine and lysine increases with the progress of processing.

The content of 10-formyltetrahydrofolic acid increases with the progress of withering time and decreases during the drying process. It participates in cysteine and methioninemetabolizm. Methionine can be metabolized into 10-formyltetrahydrofolic acid under the action of metG (CSS0035920) (Tonetti et al., 2001). The expression level of metG (CSS0035920) was highest at 12 h of withering, but decreased during subsequent withering processes. L-valine and L-Alanine are also involved in metabolism. In this study, the content of L-alanine was highest at 18 h of withering, but as the processing progress decreased, the content of L-valine increased with the processing progress.

From the changes in the content and metabolic patterns of alkaloids and their related metabolites mentioned above, it can be seen that the metabolism of caffeine related alkaloid metabolites during withering and drying processes is related to the biochemical changes of methylation and demethylation, which is consistent with the metabolic patterns of caffeine related metabolites studied by previous researchers (Ashihara et al., 1998). In previous studies on tea flavor, amino acids have often received a lot of attention as key flavor compounds in tea (Cui et al., 2024). The changes in alkaloids are closely related to the flavor of tea(Xiao et al., 2022). Through this study, it was found that the metabolism of alkaloids and their related metabolites is closely related to the metabolism of amino acids (Fig. 8)(Hoshyar et al., 2024).

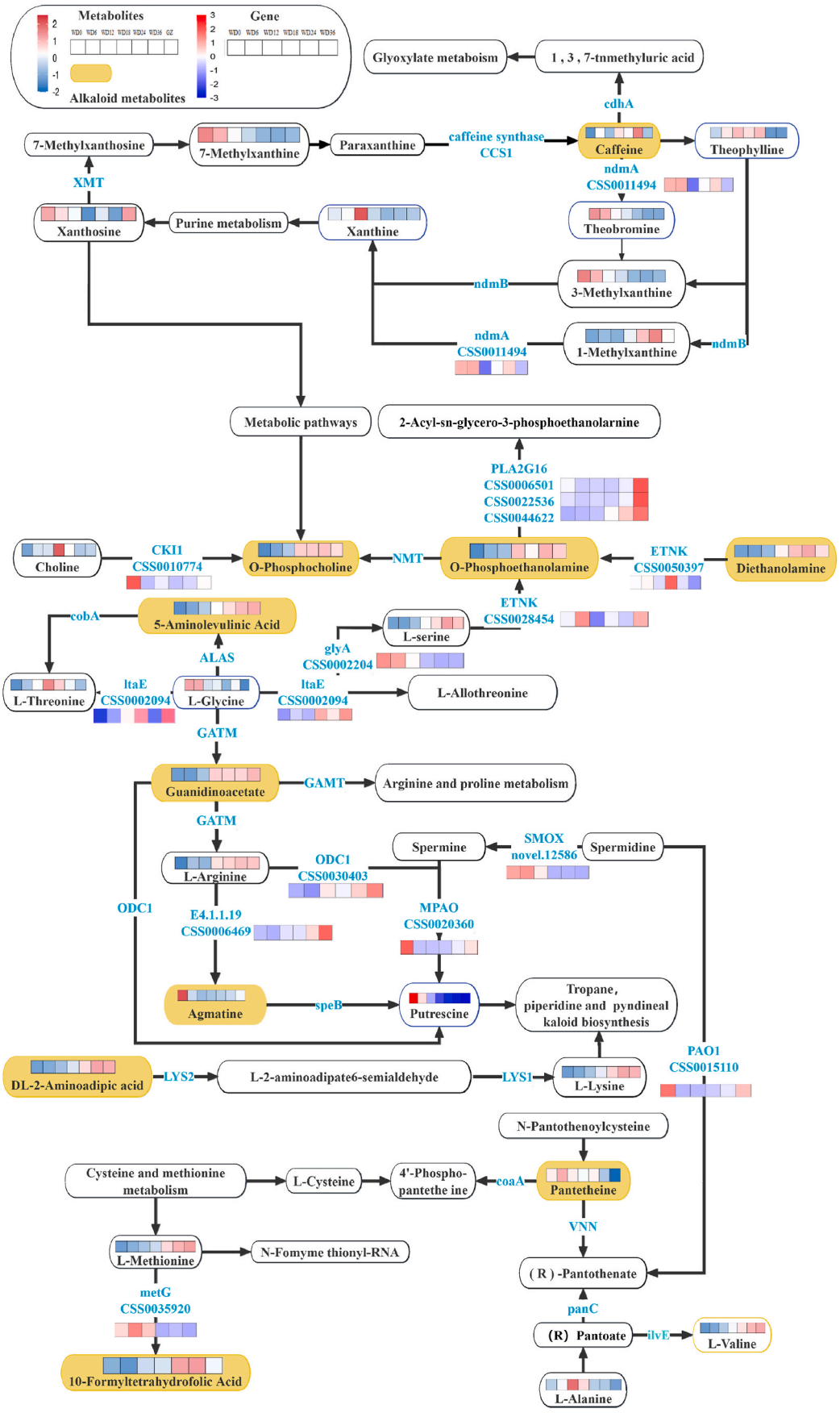


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Fig. 8. Caffeine and others alkaloids metabolite metabolism pathways.ndmB:methylxanthine N3-demethylase [EC:1.14.13.179]; ndmA:methylxanthine N1-demethylase [EC:1.14.13.178]; cdhA:caffeine dehydrogenase subunit alpha [EC:1.17.5.2]; XMT:7-methylxanthosine synthase; E4.1.1.19:arginine decarboxylase; GATM:glycine amidinotransferase; glyA:glycine hydroxymethyltransferase; ltaE:threonine aldolase; ALAS:5-aminolevulinate synthase; GAMT:guanidinoacetate N-methyltransferase; ETNK:ethanolamine kinase; NMT:phosphoethanolamine N-methyltransferase; PHOSPHO1:phosphoethanolamine/phosphocholine phosphatase; ETNK:ethanolamine kinase; speB:agmatinase; LYS2:L-2-aminoadipate reductase; LYS1:saccharopine dehydrogenase; VNN:pantetheine hydrolase; panC:pantoate–beta-alanine ligase; MTFMT:methionyl-tRNA formyltransferase; MTHFD1L:monofunctional C1-tetrahydrofolate synthase, mitochondrial; coaA:type I pantothenate kinase; DAO:D-amino-acid oxidase [EC:1.4.3.3]; cobA: uroporphyrin-III C-methyltransferase; ODC1:ornithine decarboxylase; MPAO:polyamine oxidase; GDP1: glycerol-3-phosphate dehydrogenase; DAD1:phospholipase A1; ilvE:branched-chain amino acid aminotransferase; SMOX: spermine oxidase; PAO1:polyamine oxidase; ODC1:ornithine decarboxylase; PLA2G16:HRAS-like suppressor 3; CKI1:choline kinase; metG:methionyl-tRNA synthetase.

In history, 'alkaloids' referred to nitrogen-containing chemicals in plants with basic structures. This term now includes nitrogen-containing chemical molecules found in plants, bacteria, marine organisms, and fungi. Alkaloids, due to their nitrogen-containing properties, have different structures, making them unique among natural compounds. They act as biological regulators, assisting various low molecular weight biological reactions (Glenn et al., 2013). In plants, they serve as defense mechanisms and communication molecules. Amino acids are important substances that make up the flavor of tea (Xie et al.,2024). Amino acids are the main form of nitrogen for long-distance transportation and tissue distribution of nitrogen assimilates in higher plants. They rely on inner membrane transporters for transmembrane transport in plants to regulate the steady-state balance of amino acids and nitrogen in plants (Jonasson and Shaver, 1999, Näsholm and Persson, 2001). The basic structure and metabolism of amino acids and alkaloids and their related metabolites are closely related to N element, which may be the reason why the alkaloids and their related metabolites pathway is closely related to amino acid metabolism during the processing of 'Qiancha 1′ white tea.

In previous studies, most alkaloids were bitter compounds that contributed to the bitterness in plants (Qin et al., 2020). In this study, 'Qiancha 1′ white tea had a sweet and mellow taste, but the bitterness was not prominent. However, the relative content of alkaloids and their related metabolites did not decrease with the processing process, indicating that in the formation of tea taste, the manifestation of a certain flavor characteristic is not only related to the flavor compounds with this characteristic, but also to the changes of other flavor compounds, comprehensively regulating the final taste quality of tea.

5. Conclusions

In summary, this study explored the metabolic changes during the processing of Qiancha 1 white tea and the transcriptional expression patterns of genes during the withering process, with a focus on the changes in alkaloids and their related metabolites. It was found that the withering and drying processes regulate the metabolism of alkaloids and their related metabolites.Simultaneously, 120 key genes were identified that regulate the metabolism of alkaloids and their related metabolites, and also affect the changes in other metabolites, especially the close relationship between amino acid metabolism and alkaloid metabolism. The study analyzed the metabolic patterns of alkaloids and their related metabolites during the processing of Qiancha 1 white tea, as well as the regulatory mechanisms of related differential genes. Our research results reveal the complexity of the metabolic regulation of alkaloids and their related metabolites in the formation of tea quality, providing new insights into the metabolism of alkaloids and their related metabolites during the processing of fresh leaves after isolation. It also highlights the importance of withering treatment in the metabolism of alkaloids and their related metabolites in the formation of white tea quality.

CRediT authorship contribution statement

Yuqiao Dai: Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ting Yang:** Formal analysis, Data curation, Conceptualization. **Jinglong Luo:** Project administration, Methodology, Investigation. **Shimao Fang:** Validation, Supervision, Software, Resources, Project administration, Methodology. **Tuo Zhang:** Validation, Supervision, Software, Resources. **Qin Li:** Software, Resources, Project administration. **Xiaowei Yang:** Investigation, Funding acquisition, Formal analysis, Data curation. **Qiang Shen:** Resources, Project administration, Funding acquisition. **Beibei Zhang:** Investigation, Funding acquisition. **Ke Pan:** Resources, Project administration, Methodology, Investigation, Funding acquisition.

Declaration of competing interest

We would like to submit the enclosed manuscript entitled "Changes in alkaloid metabolites during the processing of 'Qiancha 1′ white tea based on transcriptional and metabolomic analysis", which we wish to be considered for publication in "LWT-FOOD SCIENCE AND TECHNOLOGY". No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication. I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part.All the authors listed have approved the manuscript that is enclosed.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2025.117435.

Data availability

Data will be made available on request.

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