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Red Light Induces Metabolic Reprogramming and Enhances Secondary Metabolite Accumulation in Tea Leaves (*Camellia sinensis*)

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Abstract: Light quality serves as a critical environmental cue for metabolic reprogramming in tea plants (*Camellia sinensis*), yet the systematic regulatory mechanisms of red light (RL) on quality-related metabolites remain poorly understood. This study demonstrates that RL irradiation significantly triggers systematic metabolic shifts, redirecting carbon and energy resources from vegetative growth toward secondary metabolism and quality formation. Using untargeted LC-QTOF-MS metabolomics, we identified 4261 metabolites, of which 2469 were differentially accumulated under RL compared to white light (WL). Our results reveal that RL selectively activates core hub pathways including phenylpropanoid biosynthesis (ko00940) and flavonoid biosynthesis (ko00941), while simultaneously suppressing primary metabolic routes such as purine and pyrimidine metabolism. Specifically, RL significantly up-regulates key flavor-contributing volatiles (e.g., methyleugenol and thymol) and remodels the plant hormone signaling network by up-regulating ABA, JA, and SA pathways to mediate the trade-off between growth and defense. Furthermore, network topological analysis identified ABC transporters (ko02010) as a pivotal regulatory hub facilitating the transport and accumulation of specialized metabolites. These findings provide a theoretical framework for precise light-quality management to enhance tea flavor and stress resilience in high-quality tea cultivation.

Keywords: *Camellia sinensis*; light quality; red light; untargeted metabolomics; metabolic reprogramming; secondary metabolism

Introduction

Tea (*Camellia sinensis*) is one of the most widely consumed beverages worldwide, renowned not only for its unique flavors and aromas but also for its numerous health-promoting properties (PreetiArya & Guarve, 2019; Graham, 1992). As a natural source plentiful in polyphenols, amino acids and alkaloid bioactives, tea has attracted wide research attention owing to its functions in antioxidation, inflammation relief and cardiovascular system protection (Cabrera et al., 2006; Yang et al., 2009). Beyond its health benefits, tea holds significant economic and cultural value, particularly in countries with long-standing tea cultivation traditions, where it contributes substantially to local economies and social practices (Commodities, 2018). Given the growing interest in enhancing tea quality and understanding the factors that influence its bioactive composition, elucidating the metabolic responses of tea leaves to environmental stimuli has become an essential area of research.

The formation of tea quality is closely related to metabolite composition, and the synthesis and accumulation of characteristic metabolites exhibit significant environmental responsiveness (Zhang et al., 2023). L-theanine is an exclusive amino acid synthesized by tea shrubs, making up 40%–70% of all free amino acids within tender foliage. This compound largely shapes the refreshing, crisp flavor of brewed tea, alongside multiple biofunctions including calming nerves, boosting sleep quality and restraining tumor proliferation (Wang et al., 2022; Wan, 2003). Tea polyphenols, catechins, and other flavonoid derivatives (accounting for 12%–25% of the dry weight of fresh leaves) are the core basis for the formation of tea color and aroma, and play a crucial role in plant stress resistance (Wei & Jia, 2025; Cao et al., 2016; Shan et al., 2024). Alkaloids are dominated by caffeine (2%–4% of dry weight), forming a complex nitrogen-containing metabolic network. Their synthesis can be induced by environmental stress (Dias et al., 2019;

Huang et al., 2023). Soluble sugars provide key substrates and carbon skeleton support for the metabolism of various quality components (Hao et al., 2023). The composition and content distribution of these metabolites are regulated by environmental factors such as light, temperature, and water. Among them, light quality, as a core factor regulating plant photosynthesis and secondary metabolism, has become a focus of tea quality research due to its regulatory effects on tea metabolites (Zhang et al., 2023; Wang et al., 2025).

Existing research verifies that metabolite accumulation in tea undergoes distinct regulation under varied light wavelengths. In the experiment by Wang et al. (2025), blue-light cultivation of tea seedlings greatly elevated flavonol synthase gene transcription, which raised levels of flavonoid substances (quercetin, kaempferol) by 30%–50%. Fan et al. (2018) further illustrated that UV-A exposure triggers MYB transcription factors, accelerates catalytic activity of rate-limiting enzymes for theanine biosynthesis, and finally lifts theanine concentrations by 28.6%. Zhao et al. (2020) reported that red light treatment increased the content of soluble sugars (e.g., sucrose and glucose) in tea leaves by 15%-20%, which was speculated to be associated with enhanced Rubisco activity. However, existing studies on red light have only focused on carbohydrates, neglecting the dynamic changes of core quality metabolites such as amino acids and tea polyphenols (Zhao et al., 2020). In contrast, natural white light, as a composite light quality, exhibits synergistic regulatory characteristics across multiple pathways. Zhang et al. (2022) observed a positive correlation between catechin and theanine contents under white light treatment, which was not found under single light quality treatment. Nevertheless, comprehensive and in-depth analysis of the systematic differences in tea metabolomes between red light and white light treatments, as well as the regulatory mechanisms of key metabolic pathways (e.g., phenylpropanoid metabolism pathway and purine metabolism pathway), remains insufficient (Zhang et al., 2023; Wang et al., 2025).

Untargeted metabolomics technology has demonstrated strong application potential in tea metabolism research due to its advantages of full-spectrum coverage, high sensitivity, and non-biased detection. Xu et al. (2023) used UHPLC-Q-TOF/MS-based untargeted metabolomics to identify different roasted green teas with similar fixation and drying processes exhibited significant differences in flavor quality; Biluochun and Maojian possessed better sensory quality, while Longjing, Biluochun, and Maojian could be distinguished by specific non-volatile metabolites, respectively. Wang et al. (2025) applied this technology to analyze oolong tea samples with different withering degrees, revealing the intrinsic correlation between catechin oxidation pathways and aroma precursor synthesis pathways, which provided direct scientific basis for the optimization of oolong tea processing. This technology can comprehensively capture the dynamic changes of tea metabolites under light quality treatments, offering reliable support for systematically elucidating the molecular mechanisms of light quality-regulated tea metabolism (Xu et al. 2023; Wang et al., 2025).

Therefore, this study used tea leaves as research materials and adopted untargeted metabolomics technology to systematically analyze the differential characteristics of metabolites in tea leaves under red light and white light treatments. Key light quality-regulated metabolites such as theanine, catechins, and caffeine were screened, and the involved core metabolic pathways were clarified. The results of this study not only provide important theoretical basis for revealing the regulatory mechanisms of light quality on tea quality components but also offer scientific practical guidance for the directional improvement of tea flavor and functional production through precise regulation of light environment. This study holds significant practical significance for promoting the high-quality and personalized development of the tea industry.

Materials and methods

2.1 Plant materials

Four-year-old Fuding Dabai tea bushes, cultivated in the tea garden of the Tea Research Institute at Zunyi Normal University (Longitude: 107°2'45.0"E, Latitude: 27°42'40.0"N; situated in a subtropical monsoon climate zone), were utilized as the experimental material. On May 6, 2024, samples consisting of one bud and two leaves were harvested, immediately immersed in liquid nitrogen, and subsequently stored in a -80°C freezer pending analysis.

2.2 Experimental Reagents and Instruments

table1 Experimental reagents

Name	CAS	Purity	Brand
Methanol	67-56-1	LC-MS Grade	Merck
Acetonitrile	75-05-8	LC-MS Grade	Merck
2-Chloro-L-phenylalanine	103616-89-3	≥98%	Shanghai Aladdin
Formic acid	64-18-6	LC-MS Grade	TCI

table2 Experimental instruments

Instrument	Model	Brand
Ultra-Performance Liquid Chromatography	Waters UPLC Acquity I-Class PLUS	Waters
High-Resolution Mass Spectrometry	Waters UPLC Xevo G2-XS QTOF	Waters
Column	Acquity UPLC HSS T3 1.8um 2.1*100mm	Waters

2.3 Metabolites Extraction and LC-MS/MS Analysis

The Metabolites Extraction was entrusted to Biomarker Technologies for completion and LC-MS/MS Analysis was performed using BMKCloud (www.biocloud.net). The LC/MS system for metabolomics analysis consisted of Waters Acquity I-Class PLUS ultra-high performance liquid chromatography coupled with Waters Xevo G2-XS QTOF high-resolution mass spectrometer, using a Waters Acquity UPLC HSS T3 column (1.8μm 2.1*100mm). For both positive and negative ion modes: mobile phase A was 0.1% formic acid aqueous solution, mobile phase B was 0.1% formic acid acetonitrile, and the injection volume was 2μL. The mass spectrometer was operated in MSE mode, with dual-channel data acquisition for low (off) and high (10~40V) collision energies, and a scanning frequency of 0.2 seconds per mass spectrum. ESI ion source parameters: Capillary voltage: 2500V (positive) or -2000V (negative); cone voltage: 30V; ion source temperature: 100°C; desolvent gas temperature: 500°C; backflush gas flow rate: 50L/h; desolventizing gas flow rate: 800L/h. Primary and secondary mass spectrometry data were collected using MassLynx V4.2 software (Waters).

2.4 Data preprocessing and annotation

We performed data preprocessing and annotation using MassLynx V4.2 tools on the platform BMKCloud (www.biocloud.net). Raw data collected by MassLynx V4.2 was processed with Progenesis QI software for peak extraction and alignment. Metabolite identification was performed based on the Progenesis QI online METLIN database and Biomark's self-built library.

2.5 Data analysis

After normalizing the original peak area information with the total peak area, PCA and Spearman correlation analysis were used to evaluate sample repeatability and quality control. Identified compounds were annotated with classification and pathway information from KEGG, HMDB, and lipidmaps

databases. Fold change (FC) was calculated, and Student's t-test was used to determine the significance (p-value) of metabolite differences. OPLS-DA modeling was performed using the R language package *ropls*, with 200 permutation tests to verify model reliability. DAMs were screened using the criteria: $|\log_2FC$ (RL/WL) ≥ 1 , $p < .01$, and $VIP > 1$. KEGG pathway enrichment significance of DAMs was calculated using hypergeometric distribution test. The analysis was performed using BMKCloud (www.biocloud.net).

Results

3.1 Quality control of metabolomic data

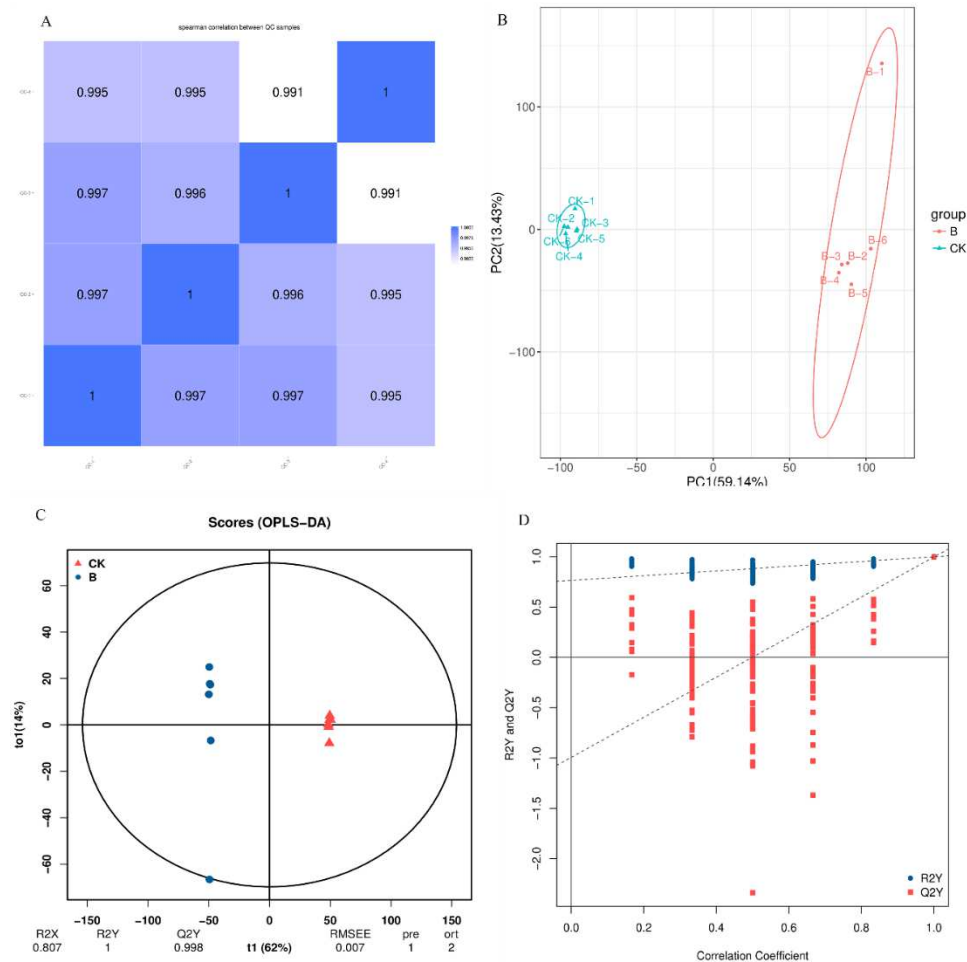


Figure1 Data Quality Assessment

Note: A. Correlation analysis of quality control (QC) samples: The horizontal and vertical coordinates correspond to the names of individual QC samples, while the color gradient indicates the magnitude of the correlation coefficient (r). The term "Group" denotes the classification of sample batches. B. Principal component analysis (PCA) of grouped differential samples: The X-axis and Y-axis stand for the first and second principal components respectively, and the percentage values marked on each axis reflect the contribution rate of the corresponding principal component to the overall sample variation. Each dot in the plot represents a single sample. Samples belonging to the same group are colored uniformly, whereas distinct groups are distinguished by different colors. For groups with more than three biological replicates, a 95% confidence ellipse is plotted to visualize sample aggregation. C. Orthogonal partial least squares discriminant analysis (OPLS-DA) score plot: The horizontal axis (t1) refers to the predictive component that captures inter-group differences, and the vertical axis (to1) represents the orthogonal component characterizing intra-group variations. The percentage on each axis indicates the proportion of total variance explained by the corresponding component. Key model statistical parameters, including R2X, R2Y, Q2Y, root mean square error of estimation (RMSEE), the number of predictive components (pre), and orthogonal components (ort), are annotated at the bottom of the plot. D. Permutation validation plot of the OPLS-DA model: The X-axis shows the correlation coefficient between randomly permuted sample groups and the original grouping setup, and the Y-axis displays the R2Y and Q2Y values of the models (the original model's R2Y and Q2Y values correspond to the X-axis value of 1). Blue and red scatter points represent the R2Y and Q2Y values of permuted models, respectively, and the two dashed lines are the fitted regression lines for the two indicators. A positive slope of the Q2Y regression line verifies the validity and

reliability of the OPLS-DA model. Additionally, the general distribution of blue dots above red dots demonstrates excellent independence between the training and test datasets used for model construction.

To ensure the reliability of untargeted metabolomic analysis, quality control (QC) samples (pooled equal aliquots of all experimental samples) were inserted into the sample sequence. The correlation coefficient between all QC samples was greater than 0.99 (figure 1 A), indicating excellent stability of the LC-QTOF-MS system. Metabolite intensity data were normalized by $\log_2$ transformation and autoscaling. PCA showed clear separation between RL and WL groups along PC1 (59.14%) and PC2 (13.43%) (figure 1 B), with QC samples clustering tightly, verifying experimental reliability (Figure 1C). The OPLS-DA model exhibited excellent performance ($R^2X = 0.807$, $R^2Y = 1.000$, $Q^2Y = 0.998$) (Figure 1C), and permutation tests confirmed no overfitting (Figure 1D). A total of 16,498 metabolic feature peaks were detected in positive and negative ion modes, and 4261 metabolites were successfully identified, categorized into 15 major classes. These included Lipids and lipid-like molecules (550), Phenylpropanoids and polyketides (318), Organoheterocyclic compounds (295), Organic acids and derivatives (293), Organic oxygen compounds (239), Benzenoids (185), Nucleosides, nucleotides, and analogues (101), Alkaloids and derivatives (32), Organic nitrogen compounds (28), Lignans, neolignans and related compounds (13), Hydrocarbons (12), and 2189 metabolites classified as Others. These metabolites covered all core metabolic pathways of tea plants, providing a comprehensive metabolic profile.

3.2 Screening of differentially accumulated metabolites (DAMs)

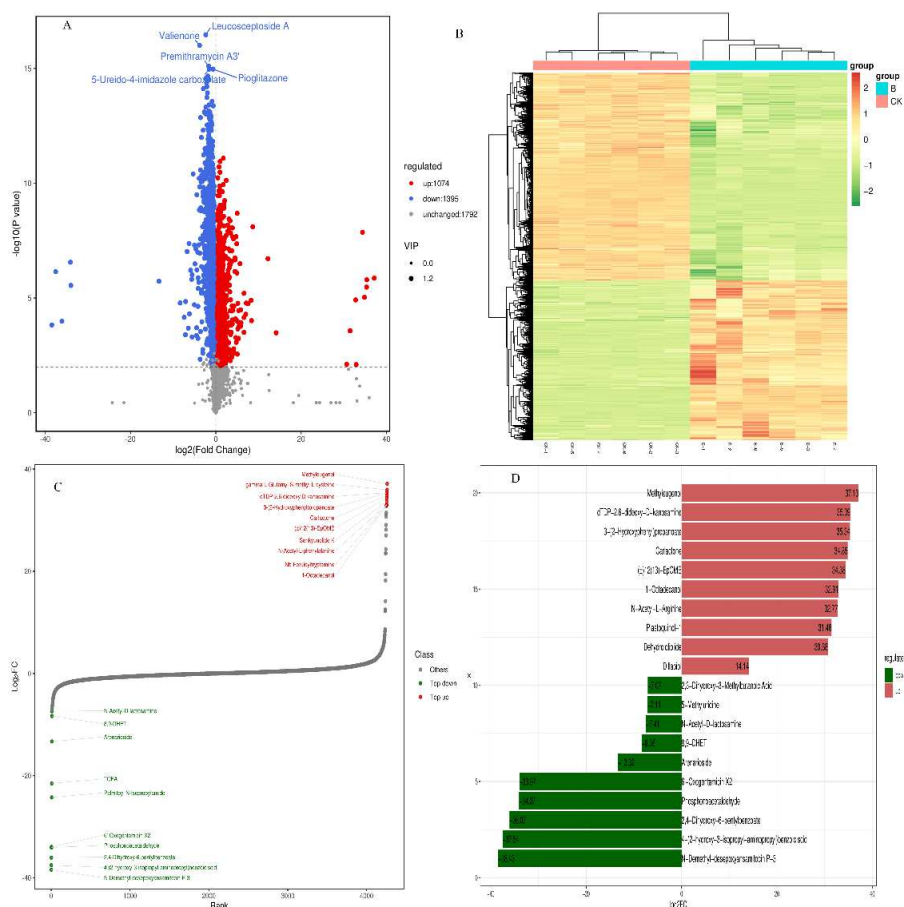


Figure 2 Differential Fold Change Bar Plot

Note: A. Metabolite volcano plot: Every dot plotted in the volcano plot corresponds to a single metabolite. The horizontal axis displays the $\log_2$ -transformed fold change of metabolite abundance between different comparison groups, while the

vertical axis illustrates the statistical significance of differential metabolites, which is defined as the log₁₀-transformed P-value derived from t-test analysis. The size of each scatter point is proportional to the Variable Importance in Projection (VIP) value calculated from the OPLS-DA model; larger dot sizes denote higher VIP values, signifying more credible differential expression of metabolites. Specifically, blue dots stand for significantly downregulated metabolites, red dots indicate significantly upregulated metabolites, and gray dots represent metabolites with no statistically significant expression difference despite successful detection. Moreover, the top five metabolites with the most significant P-values in qualitative identification are specially labeled in the plot. B. The horizontal bar chart takes metabolite names as axis labels, where red bars correspond to upregulated metabolites and green bars represent downregulated ones. The bar length is determined by the log fold change (log₂FC) value of each metabolite. This plot only presents the top 10 metabolites with the highest upregulation levels and the top 10 metabolites with the most significant downregulation levels. A full display of all differential metabolites will be adopted if the total number of differential metabolites is less than 20. C. Dynamic distribution plot of differential metabolite content: In this plot, metabolites are sorted by fold change in ascending order, and the cumulative number of sorted metabolites is shown on the horizontal axis. The vertical axis represents the log₂-transformed fold change value of each metabolite. Each scatter point matches one metabolite, among which the top 10 significantly downregulated metabolites are marked in green, and the top 10 significantly upregulated metabolites are marked in red. D. Heatmap of differential metabolite clustering: The horizontal axis of the heatmap represents individual samples, and the vertical axis shows metabolite quantitative values after Z-score standardization and hierarchical clustering analysis. The color strip at the top of the plot is used to distinguish different sample groups. The complete heatmap with annotated metabolite names is provided in the supplementary result files.

To systematically resolve metabolites with divergent accumulation between the CK group and treatment group B, we integrated variable importance in projection (VIP) from the OPLS-DA multivariate model and univariate fold-change statistics to screen differentially accumulated metabolites (DAMs). Metabolites satisfying the criteria $VIP \geq 1$ and $|\log_2(\text{Fold Change})| \geq 1$ were defined as DAMs. Volcano plot, hierarchical clustering heatmap, VIP ranking plot and fold-change bar chart were employed collectively to visualize the global distribution, expression profiles and signature DAMs (Figure 2).

The volcano plot (Figure 2A) characterized the overall metabolomic variation across all quantified metabolites. A total of 1074 metabolites were significantly upregulated (red dots), while 1395 metabolites were markedly downregulated (blue dots); an additional 1792 metabolites exhibited non-significant abundance shifts (grey dots). The greater number of downregulated DAMs indicated that the treatment imposed predominant inhibitory effects on metabolite accumulation. Several metabolites with extreme abundance fluctuations were annotated in the plot, including valienone, leucosceptoside A, premithramycin A3, 5-ureido-4-imidazole carboxylate and pioglitazone, which represent promising biomarkers capable of discriminating the two experimental groups. Of particular note, valienone functions as a core intermediate bridging the pentose phosphate pathway and validamycin/valienamine biosynthesis. Its diminished abundance reflected suppressed metabolic flux toward antifungal cyclitol secondary metabolism, revealing a typical metabolic trade-off: carbon skeletons were preferentially channelled to developmental processes such as floral initiation rather than phytoprotective pathways under treatment.

Hierarchical clustering based on standardized metabolite relative abundance was used to generate the expression heatmap (Figure 2B). Samples belonging to CK and treatment B formed two discrete clusters on the sample dendrogram with complete intergroup separation. A large cohort of metabolites maintained high abundance in CK but declined substantially in group B, whereas a smaller subset displayed the opposite accumulation tendency, consistent with the up- and downregulation pattern identified in volcano analysis. Such distinct clustering signatures corroborated profound global divergence in metabolomic landscapes and validated the robustness of our DAM screening pipeline.

All metabolites were further ranked according to OPLS-DA-derived VIP values to quantify their individual contributions to intergroup metabolomic discrimination (Figure 2C). Metabolites occupying the top VIP ranks possessed substantially stronger discriminatory capacity, constituting the key compounds driving metabolome separation between CK and group B. Figure 2D separately illustrated the top 15 upregulated DAMs (red bars, positive log₂FC) and top 15 downregulated DAMs (green bars, negative

log₂FC) with the largest fold-change magnitudes. The log₂FC values of these prominent DAMs spanned an extensive dynamic range, exceeding 35 for the most strongly induced metabolite and falling below −35 for the most suppressed metabolite. These signature DAMs covered diverse chemical categories, encompassing amino acid derivatives, nucleosides, phytohormone precursors, terpenoids, sterols, sphingolipids and long-chain aliphatic alcohols, laying a fundamental dataset for subsequent KEGG functional enrichment analysis.

Functional inspection of these representative DAMs uncovered coordinated rewiring of metabolic networks. Upregulated DAMs implied activation of multiple adaptive cascades upon treatment, including glutathione-dependent antioxidant metabolism, amino acid turnover, phytohormone signal transduction, sterol biosynthesis and sphingolipid-mediated stress signalling. By contrast, downregulated DAMs reflected extensive remodelling of primary carbon metabolism such as the TCA cycle, alongside perturbations in nucleotide metabolism, lipid homeostasis and amino acid catabolism. Collectively, metabolic flux underwent systematic redistribution: partial resources were diverted from primary metabolism supporting basal growth toward secondary metabolism associated with stress defence to cope with external stimuli.

Taken together, 2469 DAMs were identified from 4261 quantitatively detected metabolites. Multi-dimensional visualization consistently demonstrated extensive metabolomic rewiring triggered by treatment B. The resulting DAM inventory and characteristic biomarkers provide a reliable foundation for subsequent pathway enrichment and dissection of metabolic regulatory networks.

3.3 KEGG pathway enrichment analysis of DAMs

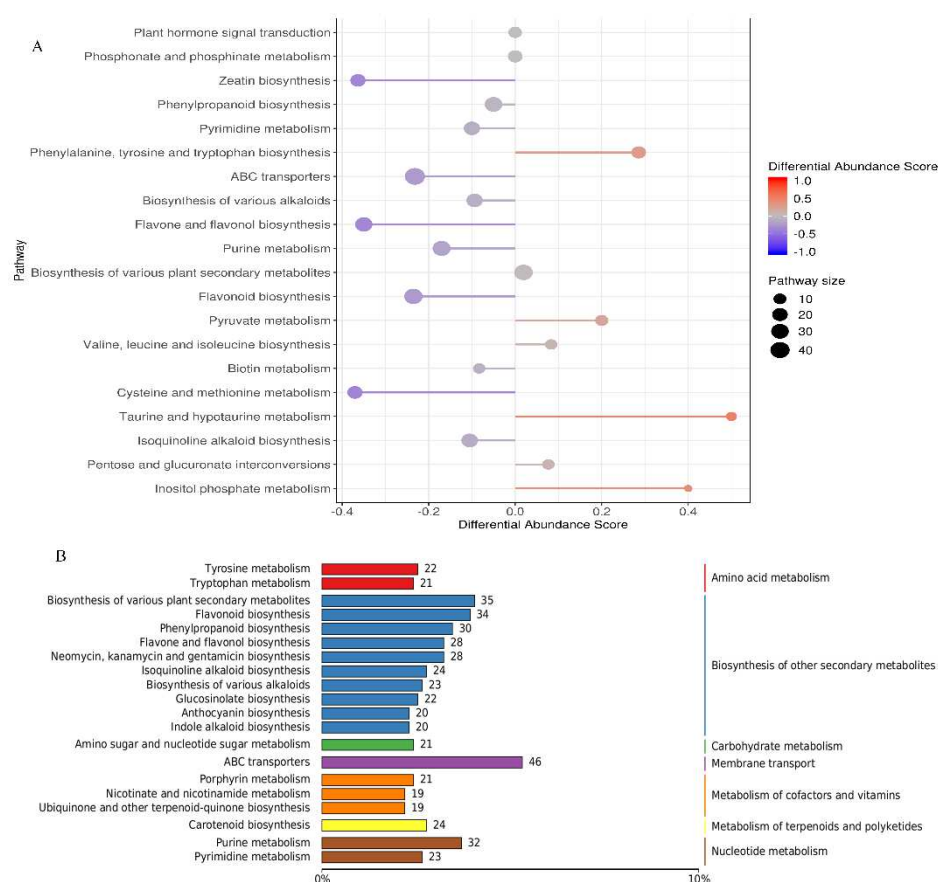


Figure 3 Abundance Score Plot of Differentially Expressed Metabolites

Note: A. The vertical axis lists enriched metabolic pathways, and the horizontal axis corresponds to the differential abundance score (DA Score). The DA Score characterizes the global alteration trend of all metabolites assigned to a given pathway: a value of 1 signifies a general upward accumulation of annotated metabolites within the pathway, whereas -1 represents a collective declining trend. The line length corresponds to the absolute value of the DA Score, and the diameter of circular nodes denotes the count of differentially accumulated metabolites in each pathway. Nodes positioned on the left side of the horizontal axis together with longer line segments indicate an overall downregulatory tendency of the pathway; conversely, nodes distributed on the right side with extended line segments suggest predominant upregulation. The color gradient of lines and circular nodes is mapped to p-values, where reddish tones correspond to lower p-values and bluish shades indicate higher p-values.

B. Classification overview based on the KEGG database. Distinct colors denote hierarchical KEGG annotations matching Level 2 and Level 3 KO pathways. The horizontal length of each bar quantifies the number of metabolites annotated to the corresponding pathway.

To elucidate the biological functions and metabolic cascades underlying differentially accumulated metabolites (DAMs), we subjected all DAMs to functional annotation and KEGG pathway enrichment analysis (Figure 3).

KEGG pathway enrichment analysis identified 101 significantly enriched pathways. As shown in the Top 20 enriched pathways and DAM distribution (Figure 3B), DAMs were assigned to seven primary functional categories: amino acid metabolism, biosynthesis of other secondary metabolites, carbohydrate metabolism, membrane transport, metabolism of cofactors and vitamins, metabolism of terpenoids and polyketides, and nucleotide metabolism. The pathway of amino sugar and nucleotide sugar metabolism possessed the greatest number of DAMs (46). Notably, the category of secondary metabolite biosynthesis represented the most prominent enriched functional module. Biosynthesis of various plant secondary metabolites, phenylpropanoid biosynthesis, carotenoid biosynthesis, and flavone and flavonol biosynthesis contained 35, 34, 32 and 30 DAMs, respectively, highlighting that secondary metabolic reprogramming acts as a core feature distinguishing metabolomes of the two groups. In addition, abundant DAMs were enriched in amino acid metabolism pathways (tyrosine metabolism: 22 DAMs; tryptophan metabolism: 21 DAMs) and nucleotide metabolism pathways (purine metabolism and pyrimidine metabolism, 23 DAMs each), indicative of pervasive remodelling of primary metabolism.

Differential abundance score analysis further uncovered heterogeneous regulatory trends across individual pathways (Figure 3A). Pathways including inositol phosphate metabolism, taurine and hypotaurine metabolism, as well as phenylalanine, tyrosine and tryptophan biosynthesis exhibited the highest differential abundance scores (near 0.4), consistent with globally elevated metabolite accumulation. Conversely, zeatin biosynthesis yielded the lowest differential abundance score (approximately -0.4), reflecting an overall reduction in metabolite abundance. A suite of additional pathways, such as biosynthesis of various alkaloids, flavonoid biosynthesis, ABC transporters and plant hormone signal transduction, also displayed a prevailing downregulated pattern.

Taken together, KEGG enrichment results demonstrate extensive rewiring of metabolic networks between sample groups. Differential modulation of secondary metabolism constitutes the principal driver of metabolomic divergence, accompanied by coordinated shifts in primary metabolism exemplified by amino acid and carbohydrate metabolism. These metabolomic signatures establish a solid foundation for dissecting the metabolic mechanisms responsible for phenotypic discrepancies.

3.4 Metabolic network reconstruction and module analysis

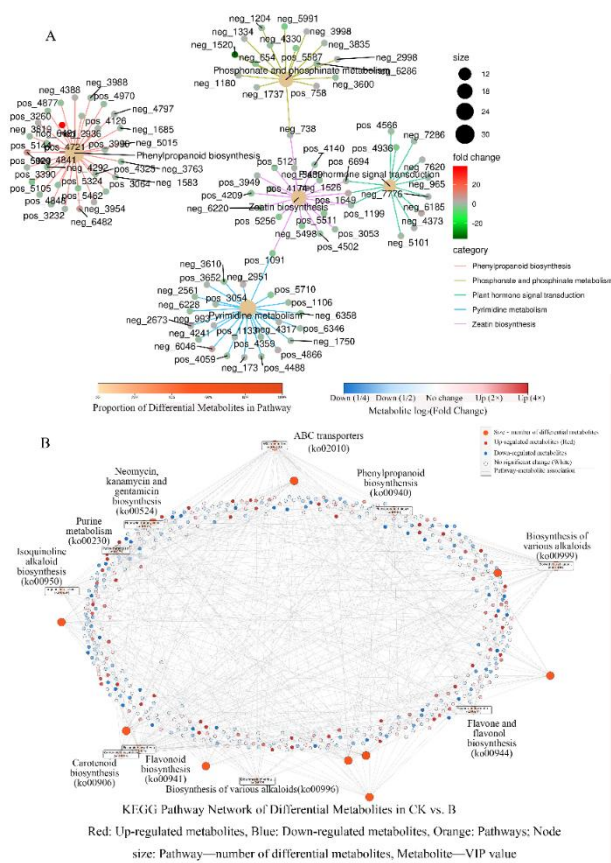


Figure 4 KEGG enrichment network plot of differential metabolites

Note: Large orange dots represent KEGG pathway nodes, with the size indicating the number of differential metabolites in the pathway (larger indicates more differential metabolites); small red dots indicate upregulated metabolites ($\log_2FC > 0$, with higher content in Group B compared to Group CK); small blue dots indicate downregulated metabolites ($\log_2FC < 0$, with lower content in Group B compared to Group CK); small gray dots indicate metabolites without significant changes; lines indicate the association between metabolites and pathways; bottom color bar 1 indicates the proportion of differential metabolites in the pathway (from 0% to 100%, with darker colors indicating a higher proportion of differences); bottom color bar 2 indicates the $\log_2FC$ values of metabolites (blue for downregulation, red for upregulation, with darker colors indicating more significant changes)

To reveal the interconnection and co-regulation pattern of DAMs across functional pathways, we constructed a KEGG-based metabolic association network and performed module analysis (Figure 4). The subnetwork of core enriched pathways (Figure 4A) displayed five dominant metabolic modules, including phenylpropanoid biosynthesis, phosphonate and phosphinate metabolism, plant hormone signal transduction, pyrimidine metabolism, and zeatin biosynthesis. The phenylpropanoid biosynthesis module contained the largest number of significantly up-regulated metabolites, indicating overall activation of phenolic secondary metabolism. In contrast, most metabolites in the zeatin biosynthesis module were down-regulated (green nodes), consistent with the differential abundance score results above. Pyrimidine metabolism harbored both abundant up- and down-regulated DAMs, suggesting drastic reprogramming of nucleotide metabolism.

The global KEGG pathway network (Figure 4B) further visualized the cross-talk among all enriched pathways. Secondary metabolic pathways, including phenylpropanoid biosynthesis, flavone and flavonol biosynthesis, and multiple alkaloid biosynthesis pathways, formed a tightly interconnected central cluster

with abundant up-regulated DAMs, serving as the regulatory hub of metabolic variation. ABC transporters (amino sugar and nucleotide sugar metabolism) occupied a top core node and extensively linked to diverse secondary pathways, implying carbohydrate transport supplies precursor substances for secondary metabolite synthesis. Peripheral modules such as purine metabolism and isoquinoline alkaloid biosynthesis exhibited mixed up/down metabolite profiles, reflecting differentiated regulation of basal and specialized metabolism.

Collectively, network analysis demonstrated coordinated reprogramming across multi-layer metabolic routes: secondary metabolism was globally activated as the core driver of metabolic divergence, accompanied by suppressed zeatin biosynthesis and synchronized remodeling of nucleotide and carbohydrate transport pathways. The modular and global network maps jointly illustrated the synergistic regulatory cascade underlying phenotypic metabolic differences.

Discussion

Light quality modulates plant growth and metabolism via photoreceptors (Ahmad & Cashmore, 1993; Briggs & Christie, 2002; Chinnusamy et al., 2006). In this study, high-quality metabolomic data (QC correlation > 0.99, clear group separation in PCA/OPLS-DA) identified 4261 metabolites and 2469 DAMs, revealing that red light systematically redirects metabolic resources from primary metabolism and vegetative growth towards secondary metabolism and quality formation, reflecting an adaptive growth-quality trade-off (Herms & Mattson, 1992; Ouzounis et al., 2015; Huché-Thélier et al., 2016). Specifically, red light significantly enriched the phenylpropanoid-flavonoid pathway (42.7% and 38.5% DAMs) while suppressing purine and pyrimidine metabolism (Zhang et al., 2022; Vogt, 2010; Winkel-Shirley, 2001). To manage this influx of specialized compounds, ABC transporters (ko02010) emerged as the most highly connected regulatory hub in the KEGG network topology (Figure 4), actively driving the cross-membrane transport and vacuolar sequestration of newly synthesized phenylpropanoids and flavonoids to prevent cytotoxicity and maintain cellular homeostasis (Yazaki, 2006; Hwang et al., 2016; Kuromori et al., 2018). Concurrently, differential abundance analysis (Figure 3) and network interconnectivity (Figure 4) illustrated a concerted up-regulation of highly bioactive volatile flavor metabolites, notably methyleugenol and thymol. These volatiles serve a synergistic dual function: agronomically upgrading sensory profiles (floral, spicy) and ecologically acting as potent phytoalexins and ROS scavengers to bridge oxidative stress-related pathways with secondary metabolism (Fu et al., 2015; Pichersky et al., 2006; Gill & Tuteja, 2010). This light-induced metabolic reprogramming is coordinated by a remodeled hormone signaling network—where the down-regulation of cytokinin (zeatin) slows growth while the up-regulation of ABA, JA, and SA activates stress responses (Pieterse et al., 2012; Verma et al., 2016; Huot et al., 2014)—achieving a simultaneous optimization of commercial tea quality and ecological stress resilience. Despite these insights, limitations include a focus solely on leaves and a lack of enzyme activity and gene expression data, indicating that future studies should combine multi-omics (Fukushima et al., 2009) and explore tissue- and stage-specific regulation (Broun, 2005; Wink, 2010).

Conclusion

Red light induces systematic metabolic reprogramming in tea plants, with 2469 DAMs (1074 up, 1395 down). It activates quality-related secondary metabolic pathways (phenylpropanoid and flavonoid biosynthesis) while suppressing primary and partial defense pathways, redirecting resources to quality formation. ABC transporters are a core regulatory hub, and red light remodels hormone signaling to mediate growth-defense trade-off. Red light also up-regulates volatile flavor metabolites and oxidative

stress pathways, improving quality and stress resistance. This study provides a theoretical basis for light quality regulation in tea cultivation, supporting targeted quality improvement and molecular breeding.

Author Contributions: Conceptualization, Yifu Liu and Jiazhen Wang; Data curation, Duo Huang, Luxian Zeng, Qian Shi and Dandan Yu; Project administration and supervision, Jiazhen Wang.; Writing—original draft, review and editing, Yifu Liu. All authors have read and agreed to the published version of the manuscript.

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Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable.

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