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Combined Analysis of Metabolome and Transcriptome Reveals *Bauhinia variegata*—Specific Floral Scent Profile and Key Aroma Components

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Abstract

Bauhinia variegata is a plant with considerable application potential owing to its combined ornamental, edible, aromatic, and medicinal values. However, research on this species remains limited and superficial both domestically and internationally, and systematic investigation of the volatile organic compounds (VOCs) emitted from its flowers is still lacking. Through integrated metabolome and transcriptome analyses, this study provides the first comprehensive characterization of the VOC composition, floral scent profile, key aroma components, and the molecular mechanisms underlying VOC variation during anthesis in *Bauhinia variegata* floral buds and fully opened flowers. A total of 1,214 volatile compounds were identified across buds and flowers, including 239 odor-active compounds and 37 differential odor-active compounds. Flavor statistics revealed that the floral scent profile of *Bauhinia variegata* is dominated by fruity, sweet, floral, green, woody, herbal, citrus, phenol, fresh, and spicy. Compared to floral buds, the majority of differential odor-active compounds were markedly up-regulated in fully opened flowers, notably including key floral aroma constituents such as phenylacetaldehyde, rose oxide, beta-ocimene, (Z)-beta-ocimene, 2-methylbenzaldehyde, and melon heptenal. Conversely, (R)-(+)-citronellal, which possesses defensive functions, and the bitter-tasting compound 1-methyl-4-nitro-benzene were significantly down-regulated in flowers, reflecting an ecological strategy shift from a defense-oriented mode at the bud stage to an attraction-oriented mode at anthesis. The up-regulation of Phenylalanine/histidine ammonia-lyase, Acyl-CoA synthetase, and Squalene synthetase genes, together with the down-regulation of Copper amine oxidase, O-methyltransferase, and Aldo/keto reductase genes, synergistically promoted the accumulation of floral aroma compounds such as phenylacetaldehyde and facilitated the floral transition. This study provides an important theoretical foundation for understanding the ecological interactions between *Bauhinia variegata* floral scent and its pollinators, as well as the molecular mechanisms governing floral scent formation. Furthermore, it contributes to the application of *Bauhinia variegata* in landscape beautification, edible flower utilization, and fragrance development.

Keywords: *Bauhinia variegata*; floral VOCs; floral scent profile; metabolome; transcriptome

1. Introduction

Bauhinia belongs to the family Fabaceae (Leguminosae) and is named for its characteristic bilobed leaves resembling the hooves of a sheep or camel. The genus *Bauhinia* is highly diverse in species richness and morphological variation, comprising trees, shrubs, and lianas. It encompasses over 300 known species, with commonly reported species including *B. variegata, B. blakeana, B. purpurea, B. championii, B. galpinii, B. integrifolia, B. kockiana, B. malabarica, B. phoenicea, B. racemosa, B. retusa, B. scandens, B. tomentosa, B. acuminata, and B. vahlii* [1]. *Bauhinia* species are predominantly distributed across tropical and subtropical regions, including Asia, South America, and Africa.

Within Asia, *Bauhinia* is primarily found in countries such as India, China, Thailand, Malaysia, Vietnam, Myanmar, and the Himalayan region [1].

Flowers, with their appealing colors, fragrances, flavors, and rich nutritional profiles, have a long-standing tradition of culinary use among people worldwide, including in Asia, ancient Greece and Rome, Medieval Europe, France, Victorian England, and the Middle East. Edible flowers include rose, marigold, gerbera, chrysanthemum, jasmine, tuberose, gladiolus, carnations, *Bauhinia variegata*, *Bombax ceiba* and *Sesbania grandiflora*, among which *Bauhinia* is included [2–4]. Beyond its ornamental value and traditional dietary uses, the genus *Bauhinia* also possesses significant medicinal properties. Various tissues of *Bauhinia* species, including *Bauhinia variegata*, have been reported to exhibit antimicrobial, anti-inflammatory, antioxidant, nephroprotective, hepatoprotective, anticancer, and antidiabetic activities and are widely utilized in traditional folk medicine in countries such as India [5–9].

In China, the genus *Bauhinia* is mainly distributed in the southern regions, including Hong Kong, Taiwan, Yunnan, Guangdong, Guangxi, and Hainan. Bauhinia blakeana is the city flower of Hong Kong and is extensively used in urban landscaping across various regions due to its high ornamental value, characterized by profuse flowering, pleasant fragrance, and a long blooming period. The tradition of consuming flowers in China is as ancient as its history and culture, particularly in Yunnan Province, where flowers of *Bauhinia variegata L.*, *Buddleja officinalis Maxim.*, *Gmelina arborea Roxb.*, *Smilax zeylanica L.*, and *Musa spp.* have rich records of culinary use [10].

Floral VOCs play critical roles in attracting pollinators and seed dispersers, defending against herbivores, protecting against plant pathogens, mediating plant-plant communication, and shielding plants from abiotic stresses. Additionally, they hold substantial economic importance in the perfume, cosmetics, food, beverage, and pharmaceutical industries [11–13]. Different plant species exhibit species-specific floral volatile profiles, which are crucial for their reproduction and environmental adaptation [14].

Bauhinia possesses considerable application potential in ornamental horticulture, edible flower production, fragrance development, and medicine. However, research on this genus, both domestically and internationally, remains relatively superficial, thereby limiting its further application and promotion. To date, a systematic investigation of the VOCs emitted from the flowers of *Bauhinia variegata* is still lacking. This study provides, for the first time, a comprehensive characterization of the volatile organic compound composition, floral scent profile, and key aroma components of *Bauhinia variegata* floral buds and fully opened flowers. This work establishes a crucial theoretical foundation for a deeper understanding of the ecological interactions between *Bauhinia variegata* floral scent and its pollinators, as well as the molecular mechanisms governing floral scent formation. Concurrently, it contributes to the application of *Bauhinia variegata* in landscape beautification, edible flower utilization, and fragrance development.

2. Materials and Methods

2.1. Plant Materials

Three groups of *Bauhinia variegata* floral buds and opened flowers were collected from Baoshan University, immediately frozen in liquid nitrogen, and stored at −80 °C until analysis.

2.2. Volatile Organic Compounds Extraction and GC–MS Analysis

VOCs were extracted by headspace solid-phase microextraction (HS-SPME) using a DVB/CWR/PDMS fiber. Separation and detection were performed on a gas chromatograph coupled with a mass spectrometer (GC–MS) equipped with a DB-5MS capillary column [15,16]. Identification was based on mass spectral libraries and retention indices.

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2.3. Relative Odor Activity Value Calculation

Odor is primarily determined by volatile compounds. Since human sensitivity varies among different substances, the contribution of a compound to odor is influenced not only by its concentration but also by its odor threshold. Odor thresholds and odor descriptors were retrieved from public databases. The relative odor activity value (ROAV) was calculated to evaluate the contribution of individual volatiles to the overall odor, with compounds exhibiting ROAV ≥ 1 considered as Odor-Active Compounds [17–19].

2.4. Differential VOCs Selection and KEGG Pathway Enrichment

Differential VOCs between bud and flower stages were identified using orthogonal partial least squares discriminant analysis (OPLS-DA) combined with variable importance in projection (VIP > 1) and absolute fold change ($|\mathrm{Log}_2\mathrm{FC}| \geq 1$). Identified metabolites were annotated against the KEGG database and subjected to pathway enrichment analysis [20].

2.5. RNA Extraction and Transcriptome Sequencing

Total RNA was extracted from bud and flower samples. After quality control, cDNA libraries were constructed and sequenced. Clean reads were de novo assembled using Trinity. Functional annotation of unigenes was performed using GO, COG, and KEGG databases. Gene expression levels were quantified as fragments per kilobase of transcript per million mapped reads (FPKM), and differentially expressed genes (DEGs) were identified using DESeq2 (for samples with biological replicates) or edgeR, with significance thresholds of corrected P-value and $|\mathrm{log}_2\mathrm{foldchange}|$ [21–25].

2.6. Statistical Analysis

Multivariate statistical analyses and data visualisation were conducted using the Metware Cloud platform (https://cloud.metware.cn).

3. Results

3.1. Identification of VOCs in Floral Buds and Flowers of Bauhinia variegata

A total of 1,214 volatile metabolites were detected in *Bauhinia variegata* floral buds (Figure 1C) and flowers (Figure 1A,B) using gas chromatography–mass spectrometry (GC–MS). Detailed information, including compound names, chemical classes, molecular formulas, and CAS registry numbers for these 1,214 volatiles, is provided in Table S1. The classification and proportional distribution of these volatiles are illustrated in Figure 1D and Table 1. Among the detected volatiles, the following categories were identified: Terpenoids (300), Esters (221), Ketones (119), Heterocyclic compounds (115), Alcohols (95), Hydrocarbons (74), Phenols (58), Acids (55), Aldehydes (51), Aromatics (45), Amines (25), Ethers (26), Nitrogen compounds (15), and Sulfur compounds (12).

Table 1. Classification, Count, and Proportion of Total Flower VOCs.

Classfication	*Count*	*Percent*
Terpenoids	*300*	*24.71%*
Esters	*221*	*18.20%*
Ketone	*119*	*9.80%*
Heterocyclic compounds	*115*	*9.47%*
Alcohols	*95*	*7.83%*
Hydrocarbons	*74*	*6.10%*
Phenols	*58*	*4.78%*
Acids	*55*	*4.53%*
Aldehydes	*51*	*4.20%*
Aromatics	*45*	*3.71%*

Amines	*28*	*2.31%*
Ethers	*26*	*2.14%*
Nitrogen compounds	*15*	*1.24%*
Sulfur compounds	*12*	*0.99%*

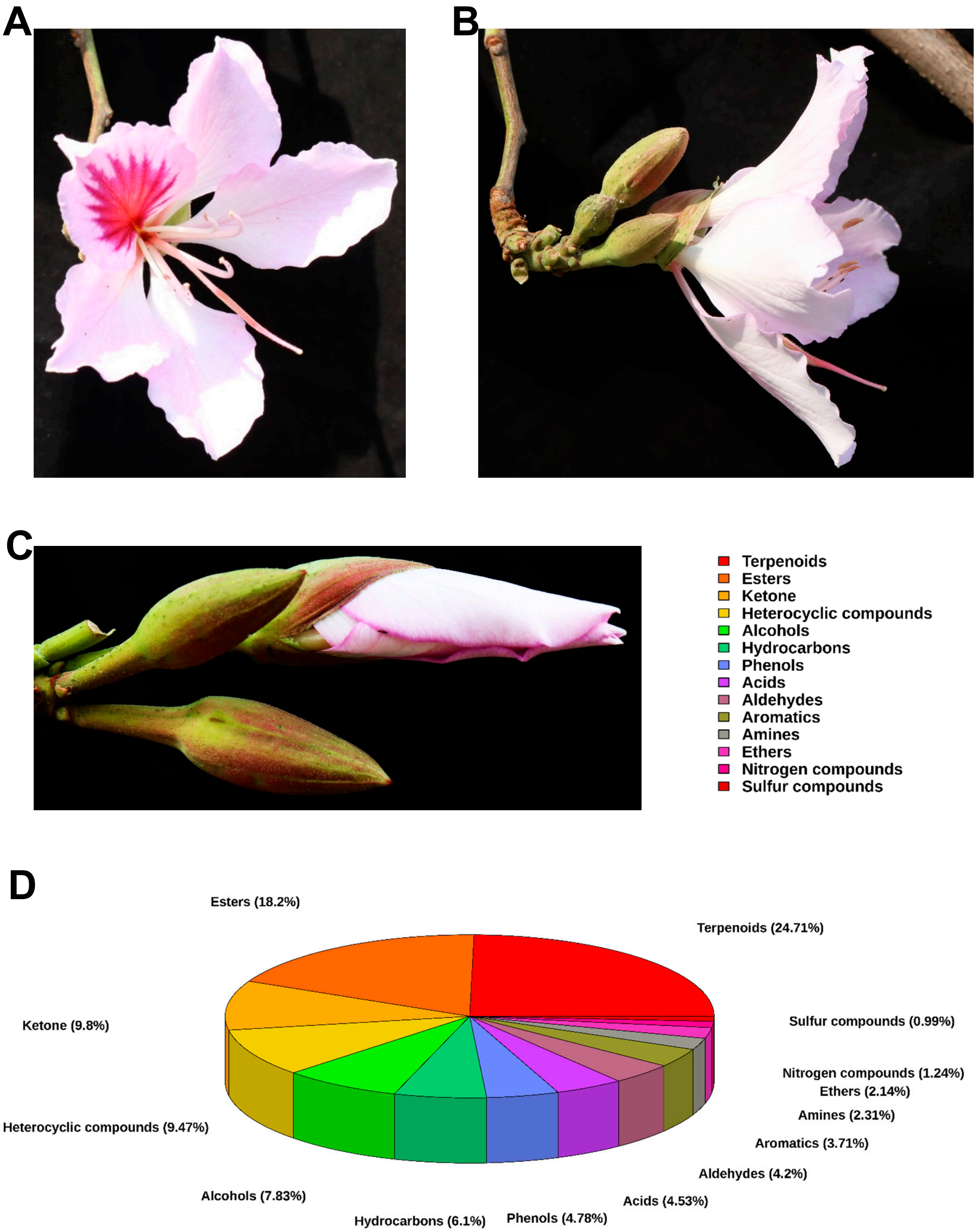


Figure 1. Identification of total flower VOCs of Bauhinia variegata. (A), (B) flowers, with one of the five petals bearing magenta markings; (C) Floral bud; (D) Pie chart depicting the classification and proportional distribution of flower VOCs.

3.2. Floral Scent Profile of Bauhinia variegata and Differential Flavor Analysis Between Floral Buds and Flowers

Odor threshold values and flavor descriptors for the volatile compounds were retrieved from public databases and incorporated into Table S1, which also includes the calculated Relative Odor Activity Values. Volatile compounds exhibiting an ROAV ≥ 1 were considered as Odor-Active Compounds. In floral buds, 211 Odor-Active Compounds were identified and are listed in descending order in Table S2. In flowers, 231 Odor-Active Compounds were identified and are similarly listed in Table S3. Based on the flavor descriptors of these Odor-Active Compounds in buds and flowers, the Floral Scent Profile for floral buds and flowers was constructed and is presented in Figure S2 and Figure S3, respectively. Statistical analysis revealed that the floral scent profile of *Bauhinia variegata* is predominantly characterized by fruity, sweet, floral, green, woody, herbal, citrus, phenol, fresh, and spicy notes.

Integration of the 211 and 231 Odor-Active Compounds in buds and flowers, respectively, yielded a combined set of 239 Odor-Active Compounds; a Venn diagram illustrating the overlap is provided in Figure S4. Among these 239 compounds, 8 were unique to floral buds, including 2-methoxybenzoic acid (CAS: 579-75-9), (4-methylphenyl) acetate (CAS: 140-39-6), 2,6-dichloro-phenol (CAS: 87-65-0), 2,5-dinitro-phenol (CAS: 329-71-5), 1-methoxy-3-methylbenzene (CAS: 100-84-5), and (R)-(+)-citronellal (CAS: 2385-77-5), with herbal being a predominant flavor note among these bud-specific volatiles. Conversely, 28 compounds were unique to fully opened flowers and are detailed in Table S4.

From the combined set of 239 Odor-Active Compounds, 37 were identified as significantly differential between floral buds and flowers and are listed in Table S5. The classification and proportion of these 37 differential compounds are shown in Figure 2A and Table 2. The results show that the predominant classes among the Differential Odor-Active Compounds are Terpenoids (21.6%), Heterocyclic compounds (18.9%), Esters (13.5%), Phenols (10.8%), Aldehydes (8.1%), and Alcohols (8.1%). Notably, compared to the proportions observed in the total metabolite pool, Terpenoids maintained the highest representation among differential compounds, whereas the proportion of Esters decreased, and the proportions of Heterocyclic compounds, Phenols, and Aldehydes increased markedly. The classification and regulation pattern of these 37 Differential Odor-Active Compounds are depicted in Figure 2B. As illustrated, the majority of volatiles were substantially up-regulated in flowers relative to floral buds, with only two compounds, (R)-(+)-citronellal and 1-methyl-4-nitro-benzene, exhibiting significant down-regulation.

Table 2. Classification, Count, and Proportion of Differential Odor-Active Compounds.

Classfication	*Count*	*Percent*
Ethers	*1*	*2.7%*
Ketone	*1*	*2.7%*
Nitrogen compounds	*1*	*2.7%*
Amines	*2*	*5.4%*
Aromatics	*2*	*5.4%*
Alcohols	*3*	*8.1%*
Aldehydes	*3*	*8.1%*
Phenols	*4*	*10.8%*
Esters	*5*	*13.5%*
Heterocyclic compounds	*7*	*18.9%*
Terpenoids	*8*	*21.6%*

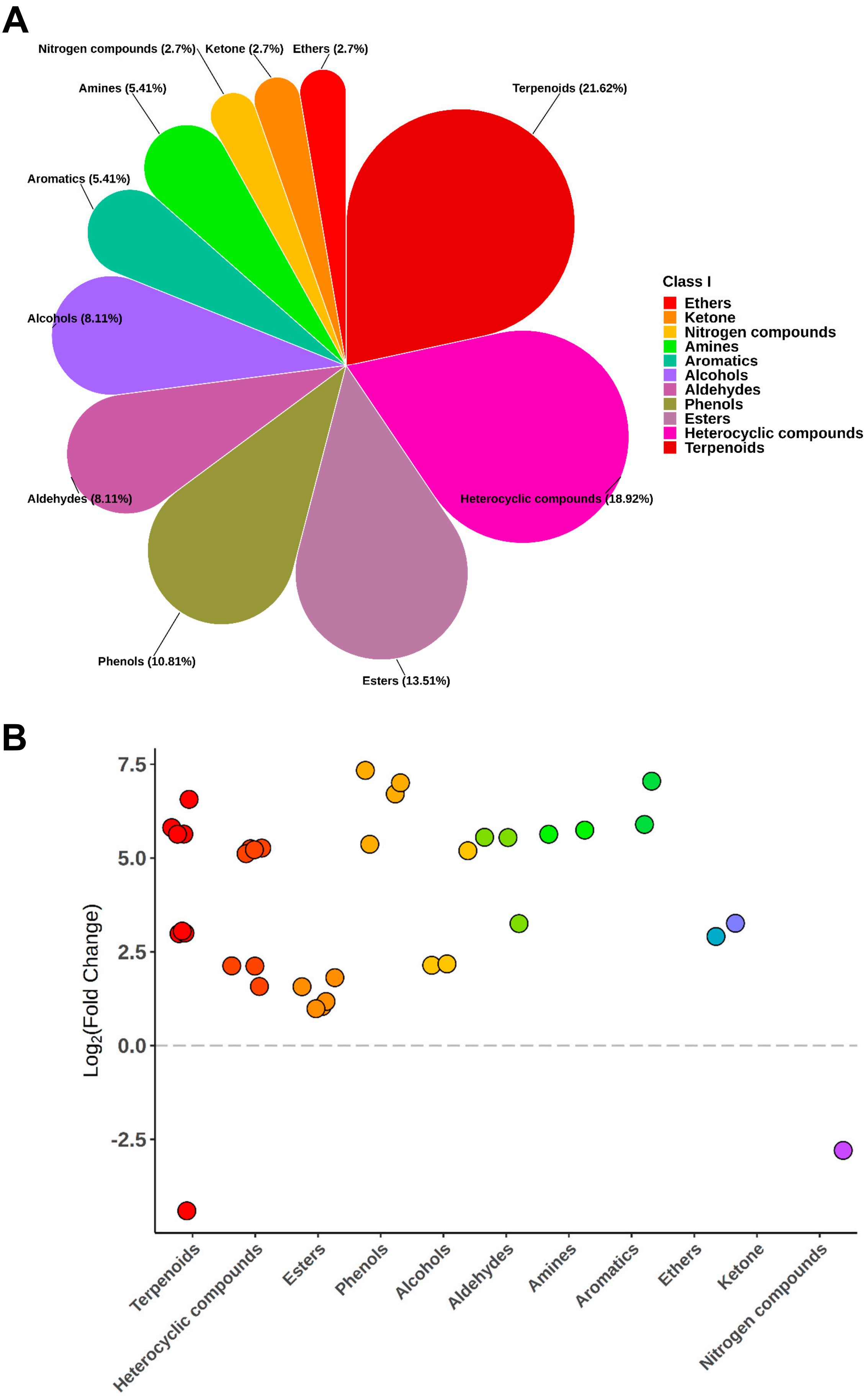
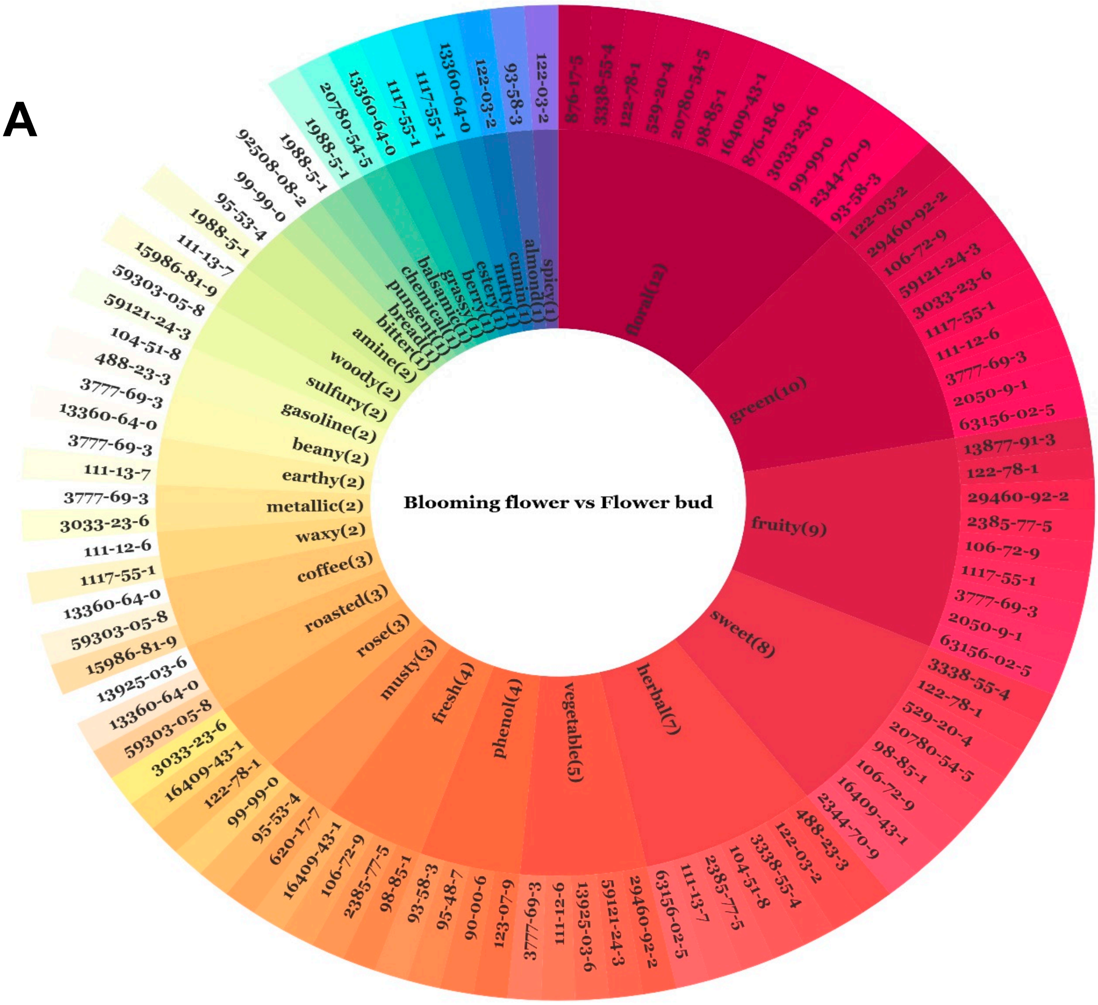

A
Nitrogen compounds (2.7%)
Ketone (2.7%)
Ethers (2.7%)
Amines (5.41%)
Terpenoids (21.62%)
Aromatics (5.41%)
Alcohols (8.11%)
Aldehydes (8.11%)
Heterocyclic compounds (18.92%)
Phenols (10.81%)
Esters (13.51%)
Class I
Ethers
Ketone
Nitrogen compounds
Amines
Aromatics
Alcohols
Aldehydes
Phenols
Esters
Heterocyclic compounds
Terpenoids
B
Log2(Fold Change)
7.5
5.0
2.5
0.0
-2.5
Terpenoids
Heterocyclic compounds
Esters
Phenols
Alcohols
Aldehydes
Amines
Aromatics
Ethers
Ketone
Nitrogen compounds

Figure 2. Characterization of Differential Odor-Active Compounds. (A) Pie chart illustrating the classification of Differential Odor-Active Compounds; (B) Scatter plot of Differential Odor-Active Compounds. The horizontal axis represents volatile compound classes, with differently colored dots denoting distinct compound categories. The vertical axis indicates the $\log_2$ fold change in abundance between fully opened flowers and floral buds, where values above zero denote up-regulation and values below zero denote down-regulation.

Figure 3A presents a differential flavor wheel constructed based on the 37 Differential Odor-Active Compounds. Among the down-regulated volatiles, 1-methyl-4-nitro-benzene was the sole contributor to a bitter note, while (R)-(+)-citronellal was a key contributor to the herbal note. Compared to floral buds, the primary differential flavor notes in flowers included floral, sweet, fruity, and herbal. Key differential metabolites influencing floral and sweet notes comprised rose oxide (CAS: 876-17-5), (Z)-beta-ocimene (CAS: 3338-55-4), phenylacetaldehyde (CAS: 122-78-1), 2-methylbenzaldehyde (CAS: 529-20-4), (epoxyethyl)-benzene (CAS: 96-09-3), alpha-methylbenzyl alcohol (CAS: 98-85-1), and 4-phenyl-2-butanol (CAS: 2344-70-9). Differential metabolites impacting fruity notes included beta-ocimene (CAS: 13877-91-3), 2-isobutyl pyrazine (CAS: 29460-92-2), and melon heptenal (CAS: 106-72-9). Those affecting herbal notes included 4-isopropylbenzaldehyde (CAS: 122-03-2), 1,2,3,4-tetramethyl benzene (CAS: 488-23-3), butylbenzene (CAS: 104-51-8), and 2-octanone (CAS: 111-13-7).



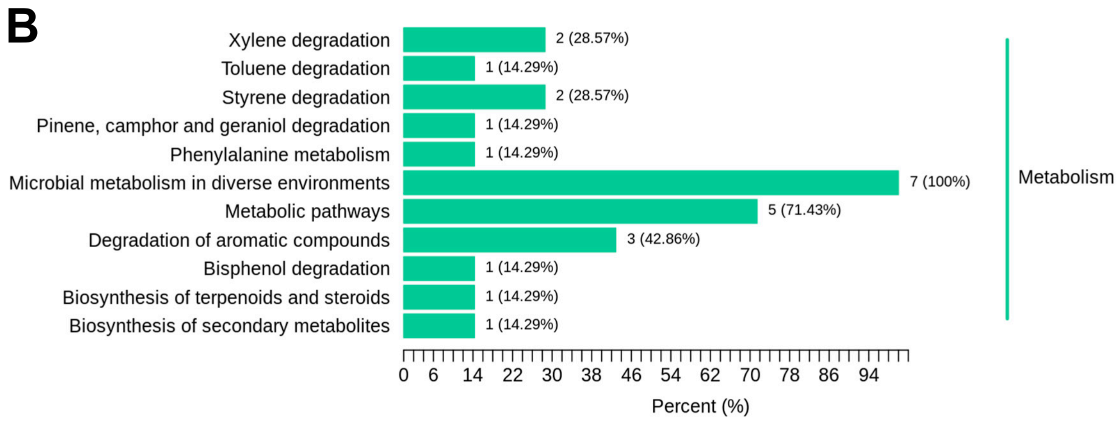


Figure 3. Flavor analysis and KEGG enrichment analysis of Differential Odor-Active Compounds. (A) Flavor wheel of Differential Odor-Active Compounds. The inner ring of the flavor wheel denotes the subjects of flavor comparison—fully opened flowers versus floral buds. The middle ring represents distinct flavor categories, with different colors indicating different flavor notes; flavors are arranged clockwise in descending order based on the number of metabolite species contributing to each note. The outer ring displays the CAS registry numbers of individual odor-active compounds. Within a given flavor category, individual metabolites are arranged according to the magnitude of their differential abundance, with darker shading indicating a greater fold change. (B) Bar chart depicting KEGG pathway enrichment of Differential Odor-Active Compounds.

3.3. KEGG Pathway Enrichment Analysis of Differential Odor-Active Compounds

The 37 identified Differential Odor-Active Compounds were subjected to KEGG pathway enrichment analysis. Among these, 19 compounds were assigned KEGG metabolite IDs, but only 7 were further annotated with KEGG pathway IDs. Detailed KEGG annotation information is recorded in Table S6. Summary information for these 7 metabolites is presented in Table 3, which includes (epoxyethyl)-benzene, 2-methylbenzaldehyde, phenylacetaldehyde, 2-methylphenol, (R)-(+)-citronellal, 4-isopropylbenzaldehyde, and 4-ethylphenol.

Table 3. Seven Metabolites Annotated to KEGG Pathways.

CAS	Compounds	Class	Formula
96-09-3	(Epoxyethyl)-benzene	Heterocyclic compound	C_8H_8O
529-20-4	2-methylbenzaldehyde	Aldehyde	C_8H_8O
122-78-1	phenylacetaldehyde	Aldehyde	C_8H_8O
95-48-7	2-methylphenol	Phenol	C_7H_8O
2385-77-5	(R)-(+)-citronellal	Terpenoids	$C_{10}H_{18}O$
122-03-2	4-isopropylbenzaldehyde	Terpenoids	$C_{10}H_{12}O$
123-07-9	4-ethylphenol	Phenol	$C_8H_{10}O$

The KEGG enrichment analysis results for these seven metabolites are displayed in Figure 3B. The metabolites were associated with pathways including Xylene degradation, Toluene degradation, Styrene degradation, Pinene, camphor and geraniol degradation, Phenylalanine metabolism, Degradation of aromatic compounds, Bisphenol degradation, and Biosynthesis of terpenoids and steroids. Detailed information on the enriched pathways and their corresponding metabolites is provided in Table 4.

Table 4. Statistical Summary of KEGG Pathway Enrichment Analysis for Differential Odor-Active Compounds.

Kegg pathway	*Ko_ID*	*Count*	*IndexList*	*CIDList*
Styrene degradation	*ko00643*	*2*	*96-09-3;122-78-1*	*C20782;C00601*
Metabolic pathways	*ko01100*	*5*	*96-09-3;529-20-4;122-78-1;95-48-7;122-03-2*	*C20782;C07214;C00601;C01542;C06577*
Microbial metabolism in diverse environments	*ko01120*	*7*	*96-09-3;529-20-4;122-78-1;95-48-7;2385-77-5;122-03-2;123-07-9*	*C20782;C07214;C00601;C01542;C09848;C06577;C13637*
Xylene degradation	*ko00622*	*2*	*529-20-4;122-03-2*	*C07214;C06577*
Degradation of aromatic compounds	*ko01220*	*3*	*529-20-4;95-48-7;122-03-2*	*C07214;C01542;C06577*
Phenylalanine metabolism	*ko00360*	*1*	*122-78-1*	*C00601*
Toluene degradation	*ko00623*	*1*	*95-48-7*	*C01542*
Pinene, camphor and geraniol degradation	*ko00907*	*1*	*2385-77-5*	*C09848*
Biosynthesis of terpenoids and steroids	*ko01062*	*1*	*2385-77-5*	*C09848*
Biosynthesis of secondary metabolites	*ko01110*	*1*	*2385-77-5*	*C09848*
Bisphenol degradation	*ko00363*	*1*	*123-07-9*	*C13637*

3.4. Expression Analysis of Significantly Differentially Expressed Genes

Analysis of the transcriptomic data identified significantly differentially expressed genes (DEGs) within the KEGG pathways associated with the Differential Odor-Active Compounds. Figure 4 presents a heatmap illustrating the expression patterns of these DEGs. A total of 23 significant DEGs were identified in the Phenylalanine metabolism pathway, 3 in Biosynthesis of terpenoids and steroids, 8 in Styrene degradation, and 6 in Degradation of aromatic compounds. No significant DEGs were detected in the Xylene degradation, Toluene degradation, Pinene, camphor and geraniol degradation, or Bisphenol degradation pathways.

Basic information on the DEGs enriched in the relevant pathways is summarized in Table 5, with detailed expression data provided in Table S7. Within the Phenylalanine metabolism pathway, six Copper amine oxidase genes were identified (five down-regulated, one up-regulated), along with five Acyl-CoA synthetase genes (all up-regulated), three Phenylalanine/histidine ammonia-lyase genes (all up-regulated), two Tyrosine aminotransferase genes (one up-regulated, one down-regulated), two O-methyltransferase genes (both down-regulated), two 3-Hydroxyacyl-CoA dehydrogenase genes (one up-regulated, one down-regulated), one up-regulated 4-Hydroxyphenylpyruvate dioxygenase gene, one up-regulated Macrophage migration inhibitory factor gene, and one up-regulated Cytochrome P450 CYP2 subfamily gene. In the Biosynthesis of terpenoids and steroids pathway, three Squalene synthetase genes were up-regulated. In the Styrene degradation pathway, seven Carbon-nitrogen hydrolase genes were all up-regulated, and one Glutathione S-transferase gene was up-regulated. In the Degradation of aromatic compounds pathway, four Aldo/keto reductase family protein genes were all down-regulated, and one Alcohol dehydrogenase, class III gene was down-regulated. Additionally, the Cytochrome P450 CYP2 subfamily gene (Cluster-17537.2) was up-regulated and is also associated with Phenylalanine metabolism.

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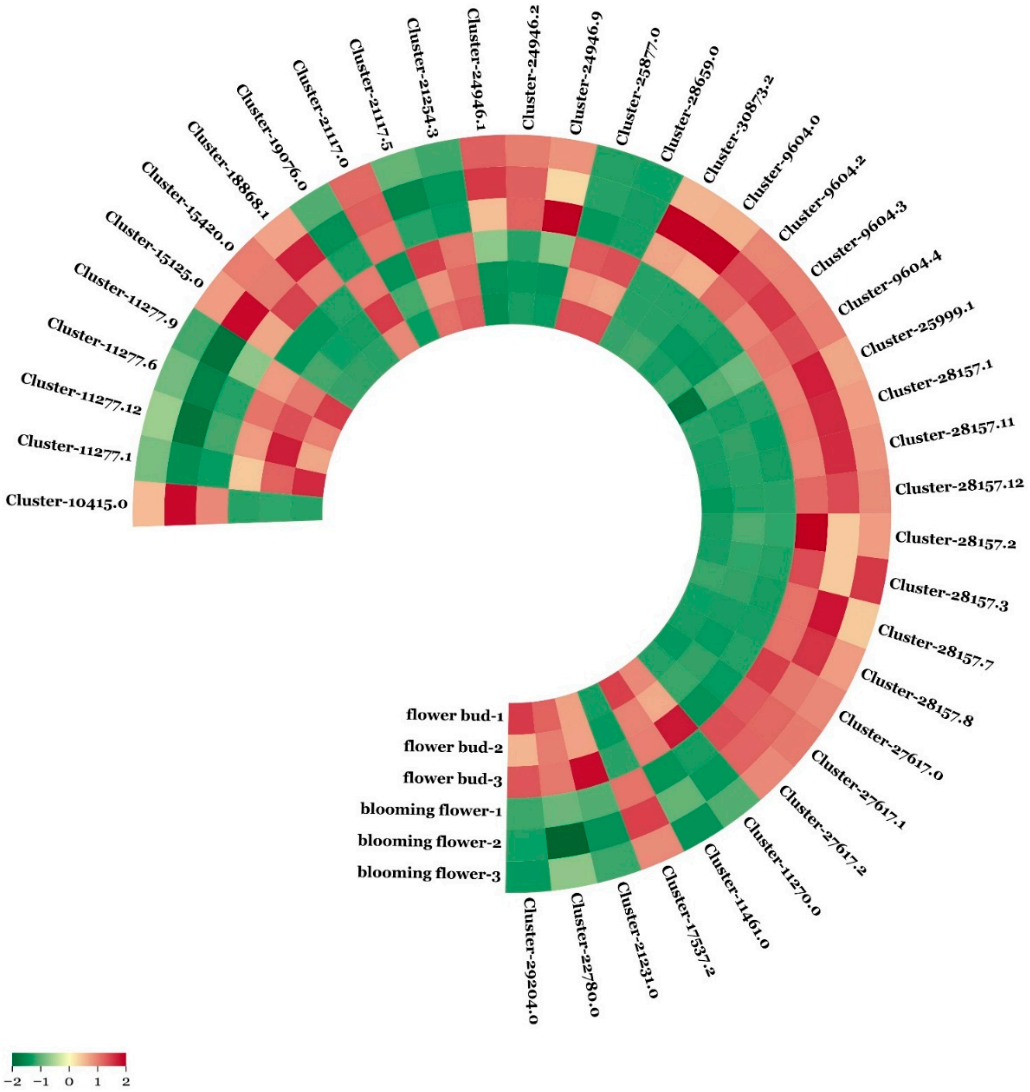

Figure 4. Analysis of differentially expressed genes. In the heatmap, the inner ring represents the floral bud group, and the outer ring represents the fully opened flower group. Red coloration indicates up-regulation, while green coloration indicates down-regulation, with deeper color intensity corresponding to greater magnitude of differential expression.

Table 5. Information on Differentially Expressed Genes Associated with Relevant KEGG Pathways.

KEGG pathway	K-ID, EC number and KOG	Regulated	Gene ID
Phenylalanine metabolism（ko00360）	K00276, [EC:1.4.3.21], Copper amine oxidase	Up	Cluster-10415.0
		Down	Cluster-11277.1; Cluster-11277.12; Cluster-11277.6; Cluster-11277.9; Cluster-19076.0
	K01904, [EC:6.2.1.12], Acyl-CoA synthetase	Up	Cluster-30873.2; Cluster-9604.0; Cluster-9604.2; Cluster-9604.3; Cluster-9604.4
	K10775, [EC:4.3.1.24], Phenylalanine/histidine ammonia-lyase	Up	Cluster-24946.1; Cluster-24946.2; Cluster-24946.9

	K00815, [EC:2.6.1.5], Tyrosine aminotransferase	Up	Cluster-18868.1
		Down	Cluster-25877.0
	K00588, [EC:2.1.1.104], O-methyltransferase	Down	Cluster-21254.3; Cluster-28659.0
	K00074, [EC:1.1.1.157], 3-Hydroxyacyl-CoA dehydrogenase	Up	Cluster-21117.0
		Down	Cluster-21117.5
	K00457, [EC:1.13.11.27], 4-Hydroxyphenylpyruvate dioxygenase	Up	Cluster-15125.0
	K07253, [EC:5.3.2.1], Macrophage migration inhibitory factor	Up	Cluster-15420.0
	K00487, [EC:1.14.14.91], Cytochrome P450 CYP2 subfamily	Up	Cluster-17537.2
Biosynthesis of terpenoids and steroids（ko01062）	K02291, [EC:2.5.1.32], Squalene synthetase	Up	Cluster-27617.0; Cluster-27617.1; Cluster-27617.2
Styrene degradation（ko00643）	K13035, [EC:3.5.5.4 4.2.1.65], Carbon-nitrogen hydrolase	Up	Cluster-28157.1; Cluster-28157.2; Cluster-28157.3; Cluster-28157.7; Cluster-28157.8; Cluster-28157.11; Cluster-28157.12
	K01800, [EC:5.2.1.2], Glutathione S-transferase	Up	Cluster-25999.1
Degradation of aromatic compounds（ko01220）	K00002, [EC:1.1.1.2], Aldo/keto reductase family proteins	Down	Cluster-11270.0; Cluster-11461.0; Cluster-29204.0
	K22374, [EC:1.1.1.285], Aldo/keto reductase family proteins	Down	Cluster-21231.0
	K00487, [EC:1.14.14.91], Cytochrome P450 CYP2 subfamily	Up	Cluster-17537.2
	K00121, [EC:1.1.1.284 1.1.1.1], Alcohol dehydrogenase, class III	Down	Cluster-22780.0

3.5. Metabolite-Gene Correlation Analysis

Correlation networks illustrating the relationships between Differential Odor-Active Compounds and significantly differentially expressed genes within the aforementioned four pathways are presented in Figure 5. As shown in Figure 5A, within the Phenylalanine metabolism pathway, the up-regulated metabolite phenylacetaldehyde (CAS: 122-78-1) exhibited a positive correlation with 14 up-regulated genes and a negative correlation with 9 down-regulated genes. As depicted in Figure 5B, within the Biosynthesis of terpenoids and steroids pathway, the down-regulated metabolite (R)-(+)-citronellal (CAS: 2385-77-5) was negatively correlated with three up-regulated genes. Figure 5C demonstrates that within the Styrene degradation pathway, the two up-regulated metabolites, (epoxyethyl)-benzene (CAS: 96-09-3) and phenylacetaldehyde, were positively correlated with eight up-regulated genes. Finally, Figure 5D shows that the three up-regulated metabolites, 4-isopropylbenzaldehyde (CAS: 122-03-2), 2-methylbenzaldehyde (CAS: 529-20-4), and 2-methylphenol (CAS: 95-48-7), were negatively correlated with five down-regulated genes and positively correlated with one up-regulated gene.

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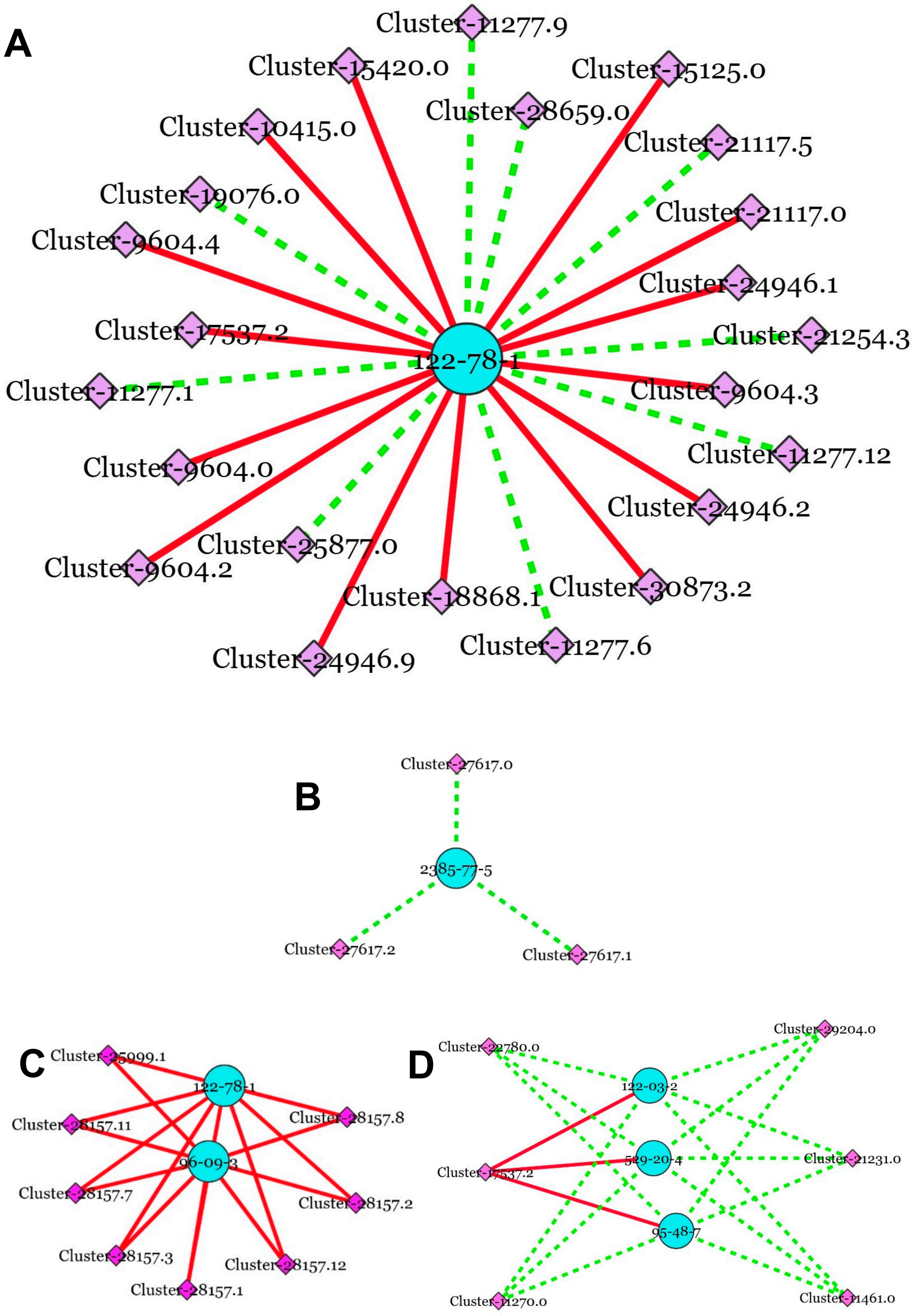
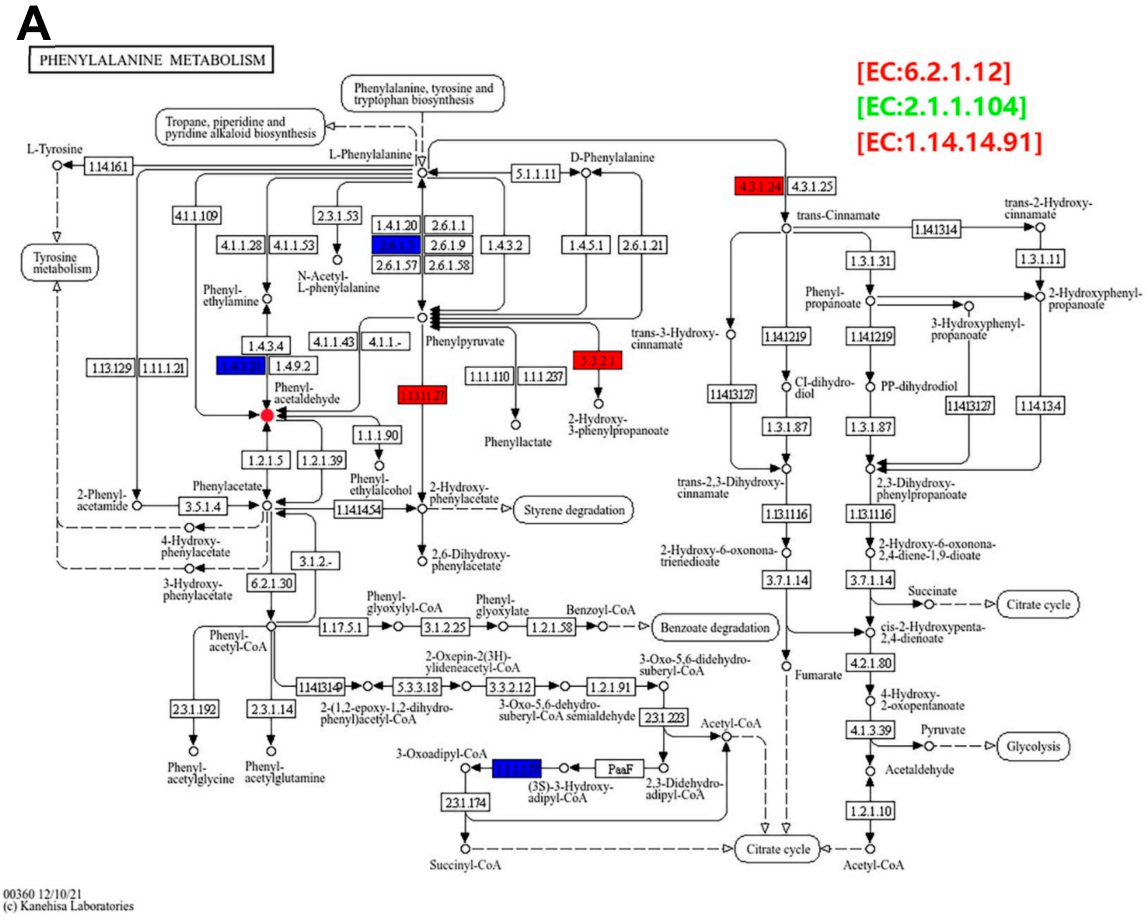


Figure 5. Metabolite-gene correlation analysis. (A), (B), (C), and (D) depict correlation networks between Differential Odor-Active Compounds and significantly differentially expressed genes enriched in the KEGG pathways ko00360 (Phenylalanine metabolism), ko01062 (Biosynthesis of terpenoids and steroids), ko00643 (Styrene degradation), and ko01220 (Degradation of aromatic compounds), respectively. In the networks, cyan circles represent metabolites, purple diamonds represent genes, red solid lines denote positive correlations, and green dashed lines denote negative correlations.

3.6. Integrated Analysis of Differential Metabolites and Genes in KEGG Pathways

The results of the integrated KEGG pathway analysis for differential metabolites and genes are displayed in Figure 6. Figure 6A illustrates the Phenylalanine metabolism pathway, where phenylacetaldehyde was identified as a significantly up-regulated metabolite. The pathway map indicates that during *Bauhinia variegata* anthesis, the predominant metabolic flow within this pathway is directed toward phenylacetaldehyde. The majority of genes encoding Copper amine oxidase [EC:1.4.3.21], which mediates the interconversion between phenylacetaldehyde and phenylethylamine, were down-regulated, suggesting attenuated flux between these two metabolites. Furthermore, all genes encoding Phenylalanine/histidine ammonia-lyase [EC:4.3.1.24], which catalyzes the formation of trans-cinnamate, were up-regulated, indicating that trans-cinnamate and its downstream metabolites represent an important branch of metabolic flow. Additionally, the up-regulation of 4-Hydroxyphenylpyruvate dioxygenase [EC:1.13.11.27] directs metabolic flux toward the Styrene degradation pathway. Figure 6B depicts the Styrene degradation pathway, which is interconnected with Phenylalanine metabolism. Significantly up-regulated metabolites in this pathway included phenylacetaldehyde and (S)-styrene oxide, accompanied by up-regulation of a gene encoding Glutathione S-transferase [EC:5.2.1.2]. The pathway map indicates that the principal fate of the accumulated phenylacetaldehyde is conversion to phenylacetate, feeding back into Phenylalanine metabolism, or diversion toward pathways such as the Citrate cycle (TCA cycle) via the action of Glutathione S-transferase.



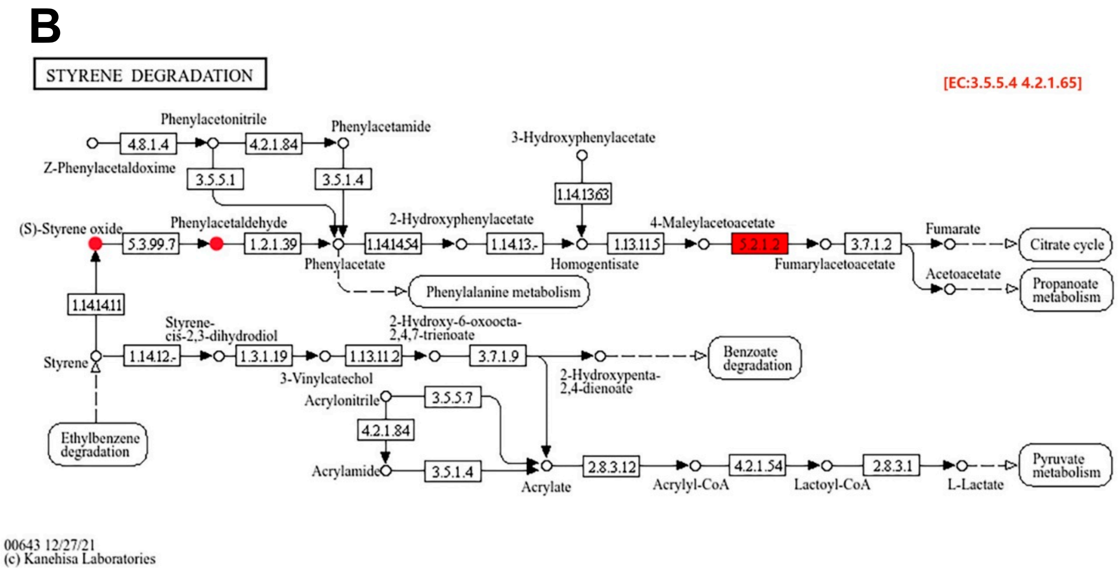


Figure 6. Metabolite-gene KEGG pathway analysis. (A) Phenylalanine metabolism pathway map; (B) Styrene degradation pathway map. In the diagrams, circular nodes represent metabolites, while rectangular boxes denote enzymes encoded by the corresponding genes. Red coloration indicates up-regulation, green coloration indicates down-regulation, and blue coloration signifies that the genes encoding the respective enzyme exhibit both up-regulated and down-regulated expression.

Pathway maps for Biosynthesis of terpenoids and steroids and Degradation of aromatic compounds are provided in Figure S5 and Figure S6, respectively; however, the differential metabolites and genes enriched in these pathways are not explicitly represented within the provided pathway diagrams.

3.7. Quantitative Changes of Key Floral Aroma Components Between Floral Buds and Flowers

As illustrated in Figure 7, (R)-(+)-citronellal (CAS: 2385-77-5) and 1-methyl-4-nitro-benzene (CAS: 99-99-0) were the two volatiles significantly down-regulated in flowers. The mean detected level of (R)-(+)-citronellal decreased from 19,305 in buds to 914 in flowers, representing an approximate 21-fold reduction. Similarly, the level of 1-methyl-4-nitro-benzene declined from 20,244 in buds to 3,313 in flowers, an approximate 6-fold reduction. Key floral aroma components of *Bauhinia variegata*, primarily characterized by floral, sweet, and fruity notes, were markedly up-regulated in fully opened flowers. Specific fold-changes (Flower/Bud) in detected levels for these key constituents were as follows: rose oxide (CAS: 876-17-5) increased from 240 to 13,515 (approx. 56-fold); (Z)-beta-ocimene (CAS: 3338-55-4) increased from 150,244 to 7,511,069 (approx. 50-fold); phenylacetaldehyde (CAS: 122-78-1) increased from 43,098 to 2,022,482 (approx. 47-fold); 2-methylbenzaldehyde (CAS: 529-20-4) increased from 77,305 to 3,603,884 (approx. 47-fold); (epoxyethyl)-benzene (CAS: 96-09-3) increased from 9,220 to 374,284 (approx. 41-fold); alpha-methylbenzyl alcohol (CAS: 98-85-1) increased from 77,900 to 2,917,678 (approx. 37-fold); 4-phenyl-2-butanol (CAS: 2344-70-9) increased from 18,911 to 81,247 (approx. 4-fold); beta-ocimene (CAS: 13877-91-3) increased from 150,244 to 7,511,069 (approx. 50-fold); 2-isobutyl pyrazine (CAS: 29460-92-2) increased from 23,513 to 910,966 (approx. 39-fold); and melon heptenal (CAS: 106-72-9) increased from 69,107 to 657,655 (approx. 10-fold).

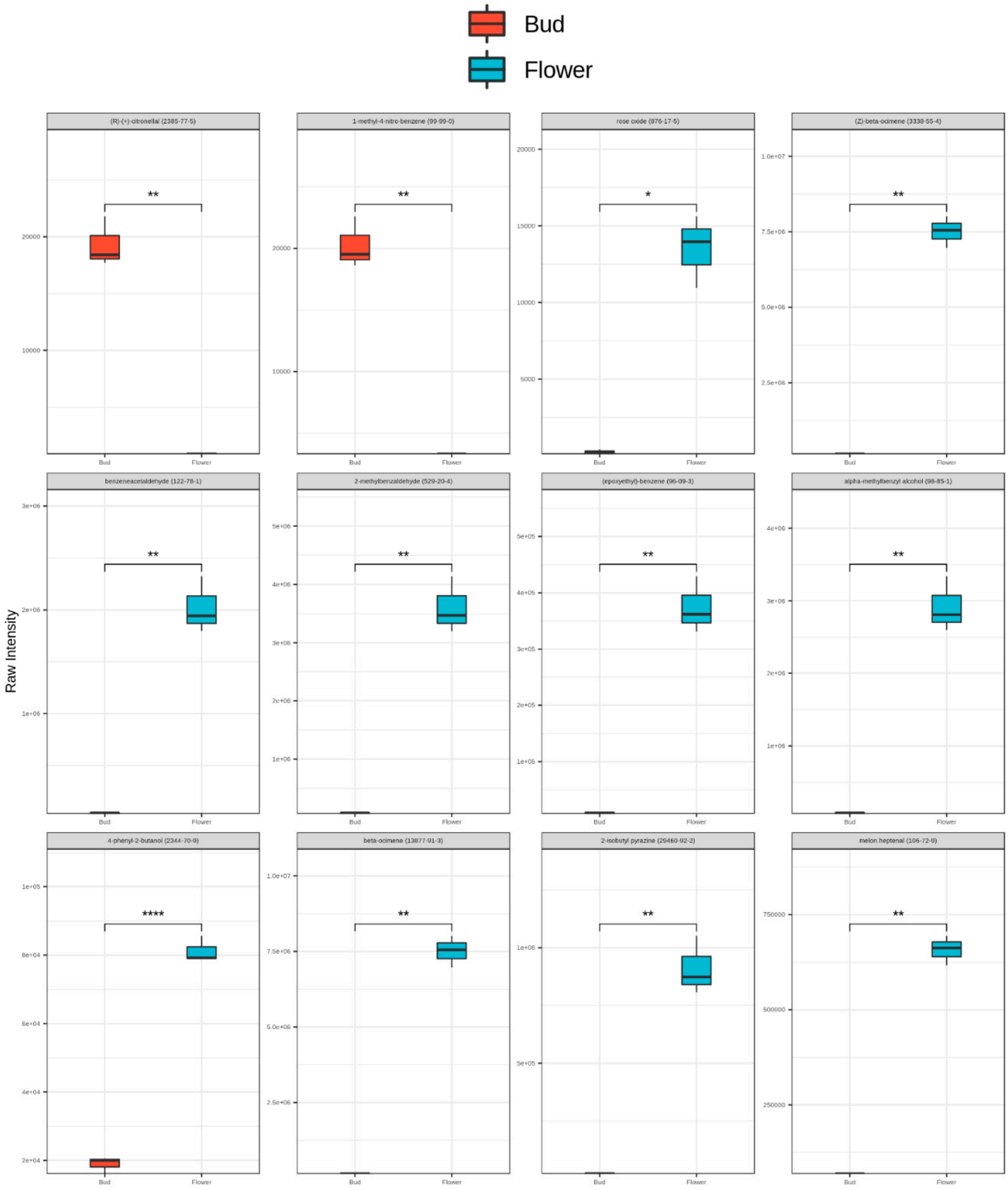


Figure 7. Abundance of key volatile metabolites. Red bars represent the measured abundance of the corresponding metabolites in floral buds, while blue bars represent the measured abundance in fully opened flowers. Asterisks indicate statistically significant differences: *, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.0001$.

4. Discussion

4.1. *Bauhinia variegata*–Specific Floral Scent Profile and Key Aroma Components

Floral volatile organic compound composition varies considerably among plant species, giving rise to distinct, species-specific floral scent profiles. For instance, key aroma components in rose include phenethyl alcohol, citronellol, heneicosane, pentadecane, eugenol, methyleugenol, and geraniol [26–28]. In tulip, principal constituents comprise eucalyptol, d-limonene, linalool, trans-β-ocimene, α-pinene, α-farnesene, caryophyllene, geranyl acetone, β-ionone, benzaldehyde, acetophenone, 3,5-dimethoxytoluene, benzyl alcohol, methyl salicylate, 2-phenylethanol, decanal, cis-3-hexenol, cis-3-hexenyl acetate, 2-hexenal, and octanal [12]. In peony, phenylethyl alcohol, β-caryophyllene, linalool, nerol, and (R)-citronellol are among the key contributors [29]. In lavender, linalool, linalyl acetate, 1,8-cineole, and α-terpineol represent the primary aroma-active compounds [30,31].

In the present study, the key aroma components of *Bauhinia variegata* were identified and primarily include rose oxide, (Z)-β-ocimene, phenylacetaldehyde, 2-methylbenzaldehyde, (epoxyethyl)-benzene, α-methylbenzyl alcohol, 4-phenyl-2-butanol, β-ocimene, 2-isobutyl pyrazine, and melon heptenal. The floral scent profile corresponding to these volatiles is predominantly characterized by fruity, sweet, floral, green, woody, herbal, citrus, phenol, fresh, and spicy odor notes.

4.2. Changes in Floral Volatile Organic Compounds Between Floral Buds and Flowers of Bauhinia variegata and Their Ecological Functions

Floral VOCs serve as crucial signals mediating interactions between plants and environmental factors, particularly pollinators [11,12]. During the transition from floral bud to flower, the floral volatiles of *Bauhinia variegata* undergoes pronounced alterations. (R)-(+)-Citronellal, one of the volatiles unique to floral buds, exhibited a marked down-regulation upon anthesis. Citronellal has been reported to possess insect repellent and antimicrobial activities [32,33], implying a significant role in the defense of Bauhinia floral buds against herbivores and pathogens. The concomitant down-regulation of 1-methyl-4-nitro-benzene, a volatile characterized by a bitter taste, suggests its potential involvement in a bud defense strategy analogous to that of citronellal. This transition from a predominantly defense-oriented mode at the bud stage to an attraction-oriented mode at anthesis represents a precisely regulated adaptation to pollinator activity. In Antirrhinum majus, the biosynthesis and emission of methyl benzoate are developmentally regulated [34]. Similarly, in Petunia hybrida, the expression of multiple genes associated with floral scent volatile biosynthesis is synchronously up-regulated during flower opening [35,36].

Floral scent volatiles play an indispensable role in plant flowering and reproduction. Phenylacetaldehyde constitutes a prominent floral aroma component in numerous plant species [37,38], including *Bauhinia variegata*, where its abundance in fully opened flowers was 47-fold higher than in buds, representing a substantial up-regulation. Phenylacetaldehyde also functions as a critical olfactory signal for pollinator attraction, exhibiting strong attractiveness to moths and other insects [39]. Additionally, it is recognized as a principal contributor to tea aroma [37]. Rose oxide, renowned for its intense rosy fragrance, is a key constituent of rose flowers and essential oil-derived fragrance products [40,41]. β-Ocimene is likewise a well-established contributor to aroma and a pollinator attractant, representing a significant component of floral scent in diverse plant taxa [39,42]. Melon heptenal is also a recognized aroma compound with widespread applications in the fragrance industry [43].

4.3. Metabolic Changes and Transcriptional Regulation During Anthesis in Bauhinia variegata

To elucidate the molecular mechanisms underlying changes in aroma composition during *Bauhinia variegata* anthesis, we integrated metabolomic and transcriptomic data to analyze key metabolic pathways. This analysis revealed that expression alterations in several key enzyme-encoding genes are intimately associated with flowering.

Within the phenylalanine metabolism pathway, all three detected *Bauhinia variegata* Phenylalanine/histidine ammonia-lyase genes were up-regulated in fully opened flowers. Phenylalanine ammonia-lyase (PAL) is a pivotal enzyme in phenylalanine metabolism, channeling carbon flux from primary metabolites into phenylpropanoid-derived secondary metabolites, which are crucial for plant growth, development, and environmental adaptation [44]. PAL isoforms are functionally diverse and contribute to various aspects of plant biology. For example, enhanced PAL expression and activity can induce flowering in Pharbitis [45]. Phenylalanine/tyrosine ammonia-lyase participates in anthocyanin biosynthesis in Solanum melongena [46]. In Nicotiana attenuata, phenylalanine ammonia-lyase 4 is involved in the biosynthesis of the floral volatile benzyl acetone, thereby contributing to pollinator attraction [47]. Furthermore, PAL has been implicated in plant resistance to herbivores and pathogens [48,49]. It is therefore plausible that Phenylalanine/histidine ammonia-lyase in *Bauhinia variegata* plays a critical role during anthesis, although its precise function warrants further validation.

Of the six *Bauhinia variegata* Copper amine oxidase genes detected within the phenylalanine metabolism pathway, five were down-regulated in fully opened flowers. Copper amine oxidases participate in alkaloid biosynthesis and, consequently, in plant defense mechanisms [50,51]. The predominant down-regulation of Copper amine oxidase genes during the transition from bud to flower supports the hypothesis of a developmental shift from defense to attraction in *Bauhinia variegata*. Additionally, two *Bauhinia variegata* O-methyltransferase genes identified in this pathway were down-regulated in flowers. O-Methyltransferases exhibit a close association with plant flowering, potentially regulating floral transition and the expression of scent-related genes through epigenetic mechanisms such as DNA methylation [52,53]. Our result provides additional data supporting a regulatory role for O-methyltransferases in flowering. In contrast, all five *Bauhinia variegata* Acyl-CoA synthetase genes detected in the phenylalanine metabolism pathway were up-regulated in flowers. Acyl-CoA synthetases are essential for pollen development and are recognized as important regulators of flowering [54–57].

Within the biosynthesis of terpenoids and steroids pathway, all three detected *Bauhinia variegata* Squalene synthetase genes were up-regulated in flowers. Up-regulation of Squalene synthetase promotes squalene biosynthesis; squalene can be subsequently converted into sterols and, ultimately, into brassinosteroids, which are phytohormones known to promote plant growth and flowering [58,59].

In the degradation of aromatic compounds pathway, all four detected *Bauhinia variegata* Aldo/keto reductase genes were down-regulated in flowers. Aldo/keto reductases catalyze the reduction of aldehydes to their corresponding alcohols. Their down-regulation likely favors the accumulation of aroma-active aldehydes such as phenylacetaldehyde, representing a strategic mechanism by which the plant conserves scent molecules during the flowering phase [60].

5. Conclusions

Through integrated metabolomic and transcriptomic analyses, this study provides the first systematic elucidation of the flower volatile organic compound composition, floral scent profile characteristics, key aroma constituents, and the molecular mechanisms underlying changes in floral volatiles during anthesis in *Bauhinia variegata*. A total of 1,214 volatile compounds were identified across floral buds and flowers, encompassing 239 odor-active compounds and 37 differential odor-active compounds. Flavor statistics revealed that the floral scent of *Bauhinia variegata* is predominantly characterized by fruity, sweet, floral, green, woody, herbal, citrus, phenol, fresh, and spicy. Compared to floral buds, the vast majority of differential odor-active compounds were markedly up-regulated in fully opened flowers, notably including key floral aroma constituents such as phenylacetaldehyde, rose oxide, β-ocimene, (Z)-β-ocimene, 2-methylbenzaldehyde, and melon heptenal. Conversely, (R)-(+)-citronellal, a compound with documented defensive functions, and the bitter-tasting substance 1-methyl-4-nitro-benzene were significantly down-regulated in flowers, reflecting an ecological strategy shift in *Bauhinia variegata* from a defense-oriented mode at the bud stage to a pollinator-attraction mode at anthesis. The up-regulation of genes encoding Phenylalanine/histidine ammonia-lyase, Acyl-CoA synthetase, and Squalene synthetase in flowers, coupled with the down-regulation of genes encoding Copper amine oxidase, O-methyltransferase, and Aldo/keto reductase, synergistically facilitated the accumulation of floral aroma compounds such as phenylacetaldehyde and promoted the floral transition. This study establishes a crucial theoretical foundation for a deeper understanding of the ecological interactions between *Bauhinia variegata* floral scent and its pollinators, as well as the molecular mechanisms governing floral scent formation. Furthermore, it contributes to the application of *Bauhinia variegata* in landscape beautification, edible flower utilization, and fragrance development.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Author Contributions: Yantong Teng designed the study and revised the manuscript; Zhijiao Song conducted the experiments, performed data analysis, and wrote the manuscript; Guixiang Li was responsible for material identification and data analysis; Qing Liu and Wenhua Chen provided critical editing of the manuscript. All authors read and approved the final manuscript.

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