Melatonin is a pleiotropic molecule that regulates a variety of developmental processes. Floral volatiles are important features of flowers that facilitate flower–visitor interactions by attracting pollinators, structure flower–visitor communities, and play defensive roles against plant and flower antagonists. Aside from their role in plants, floral volatiles are an essential ingredient in cosmetics, perfumes, pharmaceuticals, and flavorings. Herein, integrated metabolomic and transcriptomic approaches were carried out to analyze the changes triggered by melatonin exposure during the Hedychium coronarium flower development stages. Quantitative analysis of the volatiles of H. coronarium flowers revealed that volatile organic compound emission was significantly enhanced after melatonin exposure during the half bloom (HS), full bloom (FB) and fade stage (FS). Under the melatonin treatment, the emission of volatile contents was highest during the full bloom stage of the flower. Variable importance in projection (VIP) analysis and partial least-squares discriminant analysis (PLS-DA) identified 15 volatile compounds with VIP > 1 that were prominently altered by the melatonin treatments. According to the transcriptome sequencing data of the HS, FB, and FS of the flowers, 1,372, 1,510, and 1,488 differentially expressed genes were identified between CK-HS and 100MT-HS, CK-FB and 100MT-FB, and CK-FS and 100MT-FS, respectively. Among the significant differentially expressed genes (DEGs), 76 were significantly upregulated and directly involved in the floral scent biosynthesis process. In addition, certain volatile organic compounds were substantially linked with various DEGs after combining the metabolome and transcriptome datasets. Moreover, some transcription factors, such as MYB and bHLH, were also significantly upregulated in the comparison, which might be related to the floral aroma mechanism. Our results suggested that melatonin increased...
floral aroma production in *H. coronarium* flowers by modifying the expression level of genes involved in the floral scent biosynthesis pathway. These findings serve as a foundation for future research into the molecular mechanisms underlying the dynamic changes in volatile contents induced by melatonin treatment in *H. coronarium*.

**Keywords:** *Hedychium coronarium*, metabolome, transcriptome, melatonin, floral scent

## INTRODUCTION

Melatonin (N-acetyl-5-methoxytryptamine) is a low molecular weight molecule that is ubiquitously present in nature, and it has pleiotropic cellular and physiological activities in several kingdoms (Tan et al., 2012; Zhao et al., 2019). It was first discovered in the cow pineal gland in 1958, where it acts as a neurohormone and contributes to the regulation of numerous physiological processes, including circadian rhythm, appetite, body temperature, immunological system, etc (Lerner et al., 1958; Jan et al., 2009; Carrillo-Vico et al., 2013). Melatonin is also engaged in a variety of cellular functions as an antioxidant, and it has good *in vitro* and *in vivo* free radical scavenging activities (Fischer et al., 2013; Arnao and Hernández-Ruiz, 2015). Furthermore, it is considered a master regulator of plant growth, gene expression developmental regulation and stress alleviation. Melatonin plays a significant role in the metabolism of ethylene, indole-3-acetic acid, gibberellin, cytokinin, and auxin carrier proteins, as well as other plant hormones (Li et al., 2019; Nawaz et al., 2021). It also extends the shelf life and quality of fruits and vegetables and plays a key role as an anti-senior agent in response to numerous abiotic stresses, such as salinity, temperature, drought, UV radiation, and harmful substances (Arnao and Hernández-Ruiz, 2006, 2019). Melatonin’s function in plants has been intensively explored in recent years, although its role in the regulatory mechanism of floral aroma synthesis is completely unknown.

Floral volatiles have piqued human interest, and this appeal has spread to various parts of human life. Floral scent is widely used for perfumes, cosmetics, flavorings, and therapeutic applications. Recently, several compounds of terpenes, benzenoids/phenylpropanoids, derivatives of fatty acids have been extensively used in aforementioned cosmetics and pharmaceuticals industry (Dudareva et al., 2006). Moreover, floral volatiles play crucial roles in maintaining the ecological linkage between flowers and a diverse range of visitors, such as pollinators, florivores and pathogens, hence playing a key role in plant reproduction and evolutionary variety (Dudareva et al., 2013; Nagegowda and Gupta, 2020). The complex biochemical pathway underlying the synthesis of floral aroma is led by various internal and external stimuli, thus allowing the controlled emission of volatiles (Aharoni et al., 2005; Dudareva and Pichersky, 2008; Abbas et al., 2017). The presence of volatiles in ornamental plants plays a decisive role in pollinator attraction and customers’ aesthetic preferences (Raguso, 2009). The quality of flower scent is determined by complex lipophilic molecules with low molecular weight that are generated by terpenoid, phenylpropanoid/benzenoid, and fatty acid biosynthetic pathways (Pichersky et al., 2006; Muhlemann et al., 2014).

White garland or ginger lily (*Hedychium coronarium*) is a perennial flowering plant that belongs to the family Zingiberaceae. It is an economically significant crop that has been extensively cultivated for ornamental, medicinal and aromatic oil production purposes. *H. coronarium* has long strap-like leaves and large spikes of fragrant white flowers that can grow up to 25 cm long. *H. coronarium* flowers release a huge number of volatile chemicals, including monoterpenes, sesquiterpenes and benzenoids (Fan et al., 2003; Ke et al., 2019; Abbas et al., 2021a,b; Zhou et al., 2021). Several structural genes encoding terpene synthases and benzoic/salicylic acid methyltransferase have previously been characterized in the *H. coronarium* volatile biosynthesis pathway. However, less is known about the transcriptional regulation of floral aroma synthesis and emission.

The gas chromatography–mass spectrometry (GC–MS) technique has been widely used in metabolic profiling studies of plants. Recently, the GC–MS approach has been used to identify and quantify the volatile profiles of numerous flowering plants, including *Lilium* ‘Siberia’ (Abbas et al., 2019, 2020a,b, 2021c,d), *H. coronarium* (Fan et al., 2007; Zhou et al., 2021), *Silene latifolia* (Dötterl et al., 2005), *Rosa damascene* (Rusanov et al., 2011), *Osmanthus fragrans* (Cai et al., 2014), *Panax ginseng* (Lee et al., 2017), *Lantana canescens* Kunth (Pino et al., 2011), and *Luculia pinceana* (Li et al., 2016). However, integrative transcriptome and metabolome analyses have been used as a unique tool for elucidating the mechanisms that regulate metabolic networks in plants (Fukushima et al., 2014). First, high-throughput sequencing and non-targeted metabolome analysis were used to examine genes that regulate metabolic pathways and related chemicals. The findings were then analyzed to elucidate gene metabolite correlations and identify genes with unknown functions. This technique has been used to investigate the coordinated regulation of genes and metabolites involved in phenylpropanoid metabolism in *Arabidopsis thaliana* (Tohge et al., 2005), *Populus × canescens* (Behnke et al., 2010) and *Medicago truncatula* (Farag et al., 2008). Furthermore, comparative transcriptome and metabolome analyses have been performed in *M. truncatula* (Suzuki et al., 2002), *Papaver somniferum* (Zulak et al., 2007), and *Catharanthus roseus* (Rischer et al., 2006) to study alkaloid and triterpene metabolism.

In the current study, integrated metabolomes and transcriptomes obtained through headspace solid phase microextraction (HS–SPME) GC–MS and RNA sequencing analyses of flowers after exogenous melatonin application were employed. To gain a comprehensive picture of the main metabolic pathways and associated genes, we compared data...
from the same tissues using various bioinformatics analyses. We first revealed putative regulatory role of melatonin in floral aroma enhancement. This study will shed light on the novel regulatory function of melatonin in floral aroma synthesis and the gene regulation network of floral volatile biosynthesis pathways in *H. coronarium*.

**MATERIALS AND METHODS**

**Plant Materials and Growth Conditions**

*Hedychium coronarium* was grown in a growth chamber under the following conditions: 24 ± 2°C, 70–80% and a 12 h/12 h day/night photoperiod. For tissue-specific expression patterns, plant parts, including flowers, leaves and rhizomes, were isolated, immediately frozen in liquid nitrogen and stored at −80°C. The flower development process was separated into three stages: bud stage, full bloom, and senescence.

**Melatonin Treatments**

The stems of the flowers were cut into 40 cm lengths and immersed in sterilized water containing 50, 100, 500, and 1,000 µM melatonin (MT) for the hormone treatment. The melatonin stock solution was prepared following the instructions suggested by the manufacturers. Briefly, melatonin (0.2323 g) powder was dissolved in 2 mL methanol and diluted in sterilized water at the abovementioned concentrations. Detached flowers were then placed in different glass beakers that contained different melatonin solutions (50, 100, 500, and 1,000 µM). The control flowers were placed in an equal volume of sterilized water without melatonin under the same conditions as described above. After analyzing the volatile contents, the flower samples were frozen in liquid nitrogen and stored at −80°C for RNA sequence analysis. Four to five independent trials were performed with each experimental variant.

**Headspace Volatile Collection and Gas Chromatography–Mass Spectrometry Analysis**

The collection of headspace floral volatiles and GC–MS analysis were carried out as previously described (Yue et al., 2014; Ke et al., 2021). In short, entire flowers from each stage were placed in a 500 mL glass bottle, and 1.728 µg (microgram) ethyl caprate was added as an internal standard. The glass bottle was stilled for 30 min, and then a polydimethylsiloxane (50/30 µm divinylbenzene/carboxen) fiber (Supelco) was injected into the glass bottle to trap volatiles for 30 min. Thereafter, the adsorbed volatile compounds were analyzed using a GC–MS system with an Agilent 7890A GC and Agilent 5975C MSD as previously explained (Yue et al., 2015, 2021).

**RNA-Seq**

Total RNA extraction and other experimental details were performed by Novogene (UK Sequencing Center, Cambridge, United Kingdom). The experimental details were derived from Novogene's data. Quality control and RNA-seq were performed by Novogene, and high-quality samples were ensured. The library was built from total RNA, and RNA sequencing was performed by Illumina (San Diego, CA, United States) platforms using the SBS mechanism (sequencing by synthesis). Then, a bioinformatics analysis was carried out. The treatments were analyzed and compared to each other. The detailed methodology provided by Novogene is included in the Supplementary Material. For RNA sequencing, the cDNA library was generated using an Illumina HiSeq® RNA Sample Prep Kit (Illumina, United States) following the manufacturer’s protocols. The size and concentration of the library were evaluated using Qubit 2.0 and Agilent 2100. Through the HiSeq 2500 sequencing machine, paired-end (150 PE) Illumina high-throughput sequencing was performed.

**Gene Functional Annotation and Expression Level Analysis**

From the raw reads, the low-quality reads and adopters were filtered. The clean reads were de novo assembled into contigs with an optimized k-mer length = 25 and group pair distance = 300 using the Trinity program 2. The unigene functions were predicted via BLAST against the NCBI non-redundant protein (Nr), NCBI nucleotide sequences (Nt), and Swiss-Prot databases (E-value of 10−5). The resulting datasets were validated to the Protein family (Pfam) database (Finn et al., 2016) with HMMER (E-value 10−18). Unigene sequences were aligned against the Gene Ontology (GO) (Ashburner et al., 2000), Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa et al., 2004), and euKaryotic Orthologous Groups (Koonin et al., 2004) databases. The expression levels of genes in the samples were measured via the FPKM procedure. Based on gene read count data, DEGseq software was employed to identify differentially expressed genes (DEGs). The following criteria were established for screening DEGs: | log2 (fold change)| ≥ 1 and false discovery rate (FDR) < 0.05.
Quantitative Real-Time PCR and Expression Validation

To validate the RNA-seq data, quantitative real-time PCR (qRT–PCR) was performed as described previously (Abbas et al., 2019, 2020a,b). Isolation of total RNA was performed using a HiPure Plant RNA Mini Kit (Magen) following the manufacturer’s guidelines. The concentration of RNA was calculated with a spectrophotometer. Conversion of total RNA into cDNA was carried out using the PrimeScript RT Reagent Kit (TaKaRa) according to the manufacturer's suggestions. Approximately 1 µg of total RNA was reverse transcribed by a PrimeScriptTM RT reagent kit according to the manufacturer's protocol. qRT–PCR analysis was performed using the ABI 7500 Fast Real-Time PCR System (Applied Biosystems, United States) with a 96-well plate. The reaction volume (20 µL) contained 10 µL pink SYBR Supermix, 0.4 µL of each forward and reverse primer, 7.2 µL of ddH2O and 2 µL of cDNA. GAPDH was selected as an internal reference control, and the assay was performed in three technical and three biological replicates. The relative expression level of genes was measured according to the 2−∆∆CT method (Livak and Schmittgen, 2001).

Data Analysis

SPSS 19.0 (SPSS Inc., Chicago, IL, United States) was used for the statistical analyses and analysis of variance (ANOVA). A correlation analysis was based on the Spearman correlation coefficient using R 4.1.1. The PLS-DA was implemented with the “mixOmics” and “RVAideMemoire” packages of R (4.1.1). The regression coefficient network was constructed using Cytoscape version 3.6.2 (Cline et al., 2007). Identification of transpose elements (TE) was performed using the Protein-based RepeatMasking1 with a e-value of 0.00001. The mean FPKM of different TE were calculated from the transcriptome data.

RESULTS

Effect of Melatonin on Floral Scent Emission in H. coronarium Flowers

To evaluate the differences in volatile organic compounds (VOCs) between treated and untreated flowers during different developmental stages, an HS–SPME–GC–MS approach was employed. Hedychium coronarium flowers were treated with 100 µM melatonin solution. The data showed that compared to the control flowers (non-treated flowers), melatonin-treated flowers exhibited a significantly high level of floral scent emission (Figure 1). Under the melatonin treatment, the emission of floral volatile contents was increased by 47.3% during the half bloom stage of the flower compared to flowers not treated with melatonin. Similarly, the emission level of volatile contents was increased by 52.36 and 30.86% during the full bloom stage and senescence stage, respectively, compared to the control flowers.

Volatile organic compounds and their relative quantities emitted during flower development are summarized in Table 1.

Identification of the Important Volatile Organic Compounds Influenced by Melatonin Treatment

During the flower developmental process, 47 VOCs were identified, including 16 monoterpenes, 10 sesquiterpenes, 15 benzenoids/phenylpropanoids, 2 fatty acid derivatives, and 4 other categories (Table 1 and Figure 1A). The relative quantities and kinds of VOCs in the flowers showed substantial differences. Monoterpenes and sesquiterpenes were the main floral volatile compounds of H. coronarium, which is in line with our previous findings. The total volatile contents of monoterpenes were increased by 46.91, 48.3, and 22.94% during the half bloom, full bloom and senescence stages, respectively, compared to the control. During the half bloom, full bloom and senescence stages, the total volatile contents of sesquiterpenes increased by 41.61, 49.51, and 63.64%, respectively. Similarly, the total volatile contents of benzenoids/phenylpropanoids increased up to 53.75, 53.97, and 83.97% during the half bloom, full bloom and senescence stages, respectively. Interestingly, the contents of fatty acid derivatives increased by 100% at the full bloom stage of the flowers after melatonin treatment but did not change during the bud and senescence stages.

Moreover, some VOCs were development-specific and released during the specific flower development stage (Figure 1B). For example, the emission of D-limonene, methyl salicylate and δ-elemene was only observed during the half bloom stage of the flower, while 2-methylypropionamide oxime, propanoic acid, phenylmethyl ester and butanoic acid, phenylmethyl ester were only emitted during the senescence stage of the flower. Similarly, methyl anthranilate was released during the full bloom and senescence stage of the H. coronarium flowers. Furthermore, a correlation network analysis of total and major differentially accumulated metabolites (DMs) that produced floral volatile content between the treated and untreated flowers was performed (Supplementary Figure 1 and Figure 1C). The analysis showed that among differentially accumulated metabolites (DMs), monoterpenes were the foremost compounds interlinked with other floral volatiles (Figure 1C). Among DMs, 2-methylpropionamide oxime was connected with the maximum number (17) of nodes, followed by α-thujene (14), eucalyptol (12), D-limonene (11), linalool (9), propanoic acid, phenylmethyl ester (9), and β-pinene (8). Overall, the results showed that several compounds presented a significant response to melatonin treatments.

Identification of the Important Volatile Organic Compounds Influenced by Melatonin Treatment

Partial least squares-discriminant analysis (PLS-DA) is an effective and highly reliable approach for the discriminative variable selection and descriptive and predictive modeling. This technique is useful to clarify the distinction between groups of observations and aids in elucidating which variables transmit class-defining information. The PLS-DA results showed that metabolite profiles in three flower development stages were substantially modified (Figure 2). As shown in Figure 2A, there was a significant difference between the samples before and after melatonin treatment. The PLS-DA score plot model discriminated the flower development stages,
FIGURE 1 | Content and type of volatile compounds identified by HS–SPME–GC–MS in *H. coronarium*. (A) Changes in the total floral volatile contents of *H. coronarium* flowers between melatonin-treated and control flowers. (B) Heatmap of volatile compounds identified between control and treated flowers. (C) Spearman correlation of identified volatile compounds detected by HS–SPME–GC–MS based on significance (p < 0.05). The gradient color coding of the edges and the line thickness denote the level of correlation (0.6–1). Data are shown as the ± SEM value of five repeats.
| Compound name                      | Code     | Chemical formula | RI1 | RI2 | MS | CK-HB | 100MT-HB | CK-FB | 100MT-FB | CK-FS | 100MT-FS |
|-----------------------------------|----------|------------------|-----|-----|----|-------|---------|-------|----------|-------|----------|
| 2-propenyl)±-propenyl)±-Humulene S4 | C15H24  | 1466 1453 97 0.04 | ±   |     |    | ±     | ±      | ±     | ±        | ±     | ±        |
| α-thujene                        | C10H16  | 925 923 90 0.06 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| α-pinene                         | C10H16  | 932 937 95 0.17 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| β-thujene                        | C10H16  | 972 966 91 0.23 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| β-pinene                         | C10H16  | 977 979 95 0.68 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| β-myrcene                        | C10H16  | 989 990 91 1.06 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| α-terpinene                      | C10H16  | 1017 1017 96 0.07 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| O-cymene                         | C10H16  | 1026 1030 83 0.02 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| D-limonene                       | C10H16  | 1029 1029 94 0.18 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| Eucalyptol                       | C10H18O | 1032 1033 97 6.31 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| (2)-β-ocimene                    | C10H16  | 1048 1040 97 13.2 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| Cyclopentene, 3-isopropenyl-5,5- | dimethyl- | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| γ-terpinene                      | C10H16  | 1059 1058 96 0.05 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| Terpinolene                      | C10H16  | 1086 1085 96 0.1  | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| Methyl benzoate                  | C8H8O2  | 1094 1095 95 3.2 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| Linalool                          | C10H18O | 1099 1102 97 12.45 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| (E,E)-cosmeine                   | C10H14  | 1124 1130 96 0.03 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| Allo-cimcine                      | C10H14  | 1131 1131 97 0.5 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| Benzyl nitrile                    | C8H7N   | 1140 1150 96 0.18 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| Methyl salicylate                | C8H8O3  | 1193 1191 91 0.09 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| α-terpineol                      | C10H18O | 1196 1199 87 0.02 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| Geraniol                          | C10H18O | 1251 1256 86 0.01 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| Propanoic acid, phenylmethyl ester | B5      | C8H12O2 1258 91 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| Indole                            | C8H7N   | 1292 1290 97 0.17 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| Benzoic acid, 2-methoxy-, methyl ester | B6    | C9H10O3 1333 1336 83 0.01 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| γ-limonene                        | C15H24  | 1340 1339 90 0.01 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| Methyl anthranilate               | C8H9NO2 | 1344 1346 96 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| Butanoic acid, phenylmethyl ester | B8      | C8H11O4 1347 94 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| Eugenol                           | C10H12O2 | 1354 1356 98 0.02 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| Methyl cinnamate                  | C10H10O2 | 1387 1397 95 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| Caryophyllene                     | C15H24  | 1429 1420 99 0.38 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| 1-Butanol, 3-methyl-, benzoate    | C12H16O2 | 1442 1441 83 0.32 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| Trans-isoeugenol                  | C10H12O2 | 1450 1448 98 0.08 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| (E)-β-farnesene                   | C15H24  | 1454 1456 94 0.3 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| Humulene                          | C15H24  | 1466 1453 97 0.04 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| Alloaromadendrene                 | C15H24  | 1470 1461 99 0.02 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| Germacrene D                      | C15H24  | 1482 1477 91 0.01 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| Jasmine lactone                   | C10H16O2 | 1492 96 0.1 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| Benzene, 1,2-dimethoxy-4-(1-propanyl)- | C11H14O2 | 1494 1492 96 0.02 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| Benzyl tiglate                    | C12H14O2 | 1500 1498 94 0.05 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| α-farnesene                       | C15H24  | 1506 1524 97 0.78 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| α-amorphene                       | C15H24  | 1521 1519 97 0.03 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |
| δ-cadinene                       | C9      | 1524 1525 96 0.04 | ±   | ±   | ±   | ±     | ±      | ±     | ±        | ±     | ±        |

(Continued)
with five samples exhibiting satisfactory distance. Furthermore, this finding reflects that all stages have substantially different metabolite compositions. **Figure 2B** shows that component 2 was the most important variable and positively correlated with most of the monoterpenes and benzenoids/phenylpropanoid compounds. These results showed that the production of

### TABLE 1 (Continued)

| Compound name     | Code   | Chemical formula | RI1 | RI2 | MS  | CK-HB | 100MT-HB | CK-FB | 100MT-FB | CK-FS | 100MT-FS |
|-------------------|--------|------------------|-----|-----|-----|-------|----------|-------|----------|-------|----------|
| Nerolidol         | S10    | C15H26O          | 1563| 1562| 90  | –     | 0.02 ± 0.01| –     | –        | –     | –        |
| Methyl jasmonate  | F2     | C13H2O3          | 1681| 1638| 96  | 0.05 ± 0.03 a| 0.03 ± 0.01 bc| 0.02 ± 0.01 ab| 0.03 ± 0.01 ab| 0.01 ± 0 c| 0.01 ± 0 c |
| Benzyl benzoate   | B15    | C14H12O2         | 1780| 1760| 96  | 0.01 ± 0.01 a| 0.02 ± 0.01 a| 0.02 ± 0 a   | 0.03 ± 0.01 a| 0.02 ± 0.01 a| 0.02 ± 0.02 a|

1. RT, real time.
2. LRI calc, the calculated linear retention indices.
3. LRI Nist, linear retention indices in the literature; Column phase type, DB-5MS.
4. MS, mass spectrum comparison using NIST libraries. Figures in the table are means and standard error. 
a, b, c, d, means within a row refer to the significant difference ($p < 0.05$).
Different letters indicate significant differences among means according to ANOVA analysis ($p < 0.05$).

**FIGURE 2** | PLS-DA correlation analysis of volatile compounds detected by HS–SPME–GC–MS. (A) Plot indicating the correlation analysis of melatonin-treated samples and control samples during three flower development stages. (B) Plot showing the loading plot of the PLS-DA analysis of volatile compounds measured by HS–SPME–GC–MS. (C) Variables capable of discriminating volatile compounds identified between melatonin-treated and control flowers are shown, as ordered by VIP score. VIP scores $\geq 1$ (above the red line) identified key variables for predicting Y responses (relapse).
most volatile compounds was positively correlated with the melatonin treatment.

**Figure 2C** shows the data for variable importance in projection (VIP), which is a weighted sum of squares of the PLS-DA loadings based on the amount of explained Y variation in each dimension. The results showed that 15 volatile compounds, including 6 monoterpene (linalool, β-myrcene, (Z)-β-ocimene, allo-ocimene, α-pinene and geraniol), 4 sesquiterpenes (germacrene D, α-farnesene, nerolidol, and δ-cadinene), 4 benzenoids/phenylpropanoids (benzoic acid, methyl cinnamate, eugenol and 1-butanol, 3-methyl-, and benzoate) and one other compound (butanal, 3-methyl-), and oxime) with VIP > 1, were identified by PLS-DA and were substantially influenced by the melatonin treatments. Briefly, the results showed that these 15 compounds were greatly influenced by the melatonin treatment and played key roles during floral aroma production in *H. coronarium*.

**Transcriptome Analysis of H. coronarium Flowers Under the Melatonin Treatment**

We performed a transcriptome analysis on *H. coronarium* treated with exogenous melatonin to investigate the molecular mechanism of exogenous melatonin in ginger plants. Information regarding the sequencing and assembly is provided in **Supplementary Table 1**. RNA-seq data (PRJNA777930) resulted in 6.04–7.52 GB clean bases with an error ratio of less than 0.02%, a Q20 value higher than 98.2%, a Q30 value greater than 94.44%, and a GC percentage ranging from 46.04 to 50.13% (**Supplementary Table 1**). The total mapped reads ranged from 94.14 to 95.67%, which included more than 83.68% unique mapped reads (**Supplementary Table 2**). Briefly, the data suggested that sequences were of high quality and met the requirements for further analysis.

**Analysis of Differentially Expressed Genes in H. coronarium Flowers Under the Melatonin Treatment**

In this study, a total of 37,937 differentially expressed genes were found, and the length of the genes varied from 81 bp to 20,934 bp (**Figure 3A**). The DEGs for different comparisons were identified by DESeq based on the FPKM (fragments per kilobase per million mapped reads) values, and DEGs with log2 (fold change) ≥ 1 and FDR < 0.05 were considered significantly different. A total of 1,188, 1,337, 1,58, 4,451, and 3,921 genes were upregulated and 184, 173, 1,330, 1,623, 3,917, and 2,200 genes were downregulated in the half stage ck (CK-HS) vs. half stage treatment (100MT-HS), full bloom ck (CK-FB) vs. full bloom treatment (100MT-FB), fade stage ck (CK-FS) vs. fade stage treatment (100MT-FS), 100MT-HS vs. 100MT-FB, 100MT-HS vs. 100MT-FS, and 100MT-FB vs. 100MT-FS comparisons, respectively (**Figure 3B**). Overall, all six abovementioned comparisons had 1,372, 1,510, 1,488, 2,161, 8,368, and 6,121 DEGs, respectively. In short, these results indicate that there was a significant difference in the gene expression levels after treatment.

A Venn diagram analysis showed that in the CK-HS vs. 100MT-HS and CK-FB vs. 100MT-FB comparisons, the number of upregulated and downregulated genes was significantly higher than the number of downregulated genes, while the opposite trend was observed in CK-FS vs. 100MT-FS. The number of downregulated genes was higher than that of upregulated genes (**Supplementary Figure 2**). The number of upregulated and downregulated DEGs in each comparison and their overlapping relationship were evaluated (**Figures 3B–D**). Overall, 1,376 genes (1,188 genes were upregulated and 184 were downregulated compared to CK-HS) were significantly changed between CK-HS and 100MT-HS. A total of 1,510 genes (1,337 genes were upregulated and 173 were downregulated compared to CK-FB) were differentially expressed between CK-FB and 100MT-FB. Similarly, 1,488 genes (158 genes were upregulated and 1,330 were downregulated compared to CK-FB) were substantially modified between CK-FS and 100MT-FS. Overall, the number of DEGs overlapping in the CK-HS vs. 100MT-HS, CK-FB vs. 100MT-FB, and CK-FS vs. 100MT-FS comparisons was 119, 113, and 63, respectively. Furthermore, transpose element analysis was performed to identify the significantly influenced transpose super-families under melatonin treatment compared to control. Results showed that 57 type of transpose elements were identified from the transcriptome data which showed significant up and downregulated expression under MT treatment (**Supplementary Figure 3**). Among different transpose super-families, DNA, LINE and LTR super-families were abundant and significantly influenced by MT treatment.

**Gene Ontology and Kyoto Encyclopedia of Genes and Genomes Annotation and Enrichment Analysis of Differentially Expressed Genes**

The function of the DEGs was investigated using GO-based enrichment annotation, which was classified into three key functional categories: biological process (BP), molecular function (MF), and cellular component (CC) (**Figures 4A–C**). Between CK-HS and 100MT-HS, 2,393 DEGs had GO annotations, most of which were classified as protein folding (GO: 0006457), extracellular region (GO: 0005576) and unfolded protein binding (GO: 0051082) (**Figure 4A**). All CK-FB vs. 100-FB DEGs were annotated to the GO database, and the majority were highly enriched in "cellular glucon metabolic process (GO: 0006073), cell wall (GO: 0005618) and heme binding (GO: 0020037) (**Figure 4B and **Supplementary Table 3**). Similarly, DEGs between CK-FS and 100MT-FS were annotated to the GO database, and they were primarily enriched in response to wounding (GO: 0009611), cell wall (GO: 0005618) and serine-type endopeptidase inhibitor activity (GO: 0004867) (**Figure 4C**). Interestingly, among the DEGs, most of the genes related to biological processes, cellular components and molecular functions were upregulated in the CK-HS vs. 100MT-HS and CK-FB vs. 100MT-FB comparisons, whereas the opposite trend was observed in CK-FS vs. 100MT-FS. Moreover, the molecular function category included the majority of the GO annotations, followed by biological process and cellular component.
Based on the pathways involved or the functions performed, DEGs were categorized using the KEGG database. The KEGG pathway database classifies biological metabolic pathways into three levels, with the first category including the second. The KEGG pathway enrichment analysis of differentially expressed genes revealed that there were three common pathways among the CK-HS vs. 100MT-HS, CK-FB vs. 100MT-FB and CK-FS vs. 100MT-FS comparisons. Among them, “MAPK signaling pathway” and “plant–pathogen interaction” were the highly significantly enriched pathways (Figures 5A–C). Moreover, three common pathways (“plant hormone signal transduction,” “fatty acid elongation,” and “isoquinoline alkaloid biosynthesis” pathways) were found between the CK-HS vs. 100MT-HS and CK-FB vs. 100MT-FB comparisons. Similarly, there were nine common pathways between the CK-FB vs. 100MT-FB and CK-FS vs. 100MT-FS comparisons. These nine common pathways included “phenylpropanoid biosynthesis,” “pentose and glucuronate interconversions,” “amino sugar and nucleotide sugar metabolism,” “starch and sucrose metabolism,” “galactose metabolism,” “cyanoamino acid metabolism,” “steroid biosynthesis,” “nitrogen metabolism,” and “ascorbate and aldarate metabolism”. The analysis of the top 20 significantly enriched KEGG pathways with DEGs is listed in Supplementary Table 4. Compared with the set of
CK-FB vs. 100MT-FB and CK-FS vs. 100MT-FS, fourteen significantly enriched unique pathways were found in the set for CK-HS vs. 100MT-HS, which were “protein processing in endoplasmic reticulum,” “brassinosteroid biosynthesis,” “glyoxylate and dicarboxylate metabolism,” “tryptophan metabolism,” “thiamine metabolism,” “lysine biosynthesis,” “linoleic acid metabolism,” “glutathione metabolism,” “carbon metabolism,” “fatty acid degradation,” “propanoate metabolism,” “glycine, serine, and threonine metabolism,” “beta-alanine metabolism,” and “tropaeone, piperidine, and pyridine alkaloid biosynthesis” pathways. Similarly, compared with the set of CK-HS vs. 100MT-HS and CK-FS vs. 100MT-FS, seven significantly enriched pathways (“fructose and mannose metabolism,” “pentose and glucuronate interconversions,” “SNARE interactions in vesicular transport,” “carbon fixation in photosynthetic organisms,” “ubiquinone and other terpenoid-quinone biosynthesis,” “isoquinoline alkaloid biosynthesis,” and “arginine and proline metabolism”) were observed, which were unique to the set of CK-FB vs. 100MT-FB. Similarly, in comparison with the set of CK-HS vs. 100MT-HS and CK-FB vs. 100MT-FB, eight significantly enriched unique pathways were identified for CK-FS vs. 100MT-FS, which were “valine, leucine, and isoleucine degradation,” “alanine, aspartate, and glutamate metabolism,” “cysteine and methionine metabolism,” “valine, leucine, and isoleucine biosynthesis,” “terpenoid backbone biosynthesis,” “phosphatidylinositol signaling system,” “biosynthesis of amino acids,” and “sulfur metabolism” pathways. The presence of unique pathways at each developmental stage with respect to the control suggests their potential relative role in the floral aroma mechanism under the melatonin treatments. Furthermore, a total of 242, 196, and 283 DEGs were found by comparing the libraries CK-HS vs. 100MT-HS, CK-FB vs. 100MT-FB, and CK-FS vs. 100MT-FS, respectively.
Hence, metabolism-related genes in To reveal the regulatory mechanism of exogenous melatonin Melatonin Treatment floral Aroma Synthesis Under the Key Scent-Related Genes Involved in melatonin treatment. among these three stages of flower development under the TFs, such as ARF, Dof, GATA, TCP, and LSD, were also observed in FB, the expression of the majority of transcription factors was interconnected with 12 nodes. Five transcription factors, AP2 (66), WRKY (48), MYB (46), bHLH (34), bZIP (19), HSF (19), and GRAS (10). The melatonin treatment increased the expression of the majority of these transcription factor genes. Between CK-HS vs. 100MT-HS and CK-FB vs. 100MT-FB, the expression of the majority of transcription factors was upregulated during the half and full bloom stages of the flowers. AP2, ARF, bHLH, bZIP, DoF, GATA, GRAS, HSF, LSD, MYB, TCP, and WRKY were edged with 13 nodes, and four (HcMYB_395.61, HcWRKY_71.45, HcAP2_99.133, and HcGATA_3.189) were interconnected with 14 nodes. Nine transcription factors (HcMYB_125.110, HcMYB_478.81, HcMYB_299.9, HcHCT_576.21, HcHCT_1075.10, HcAP2_26.297, HcAP2_432.10, HcAP2_579.37, and HcGRAS_405.14) were edged with 13 nodes, and four (HcRLKL_331.80, HcMYB_107.61) were connected with 16 nodes each. Five transcription factors, MYB (HcMYB_1223.6 and HcMYB_106.16), bHLH (HcHCT_43.153), GRAS (HcGRAS_211.12), and AP2 (HcAP2_519.11), were connected with 15 nodes. HcAP2_441.22 and HcGRAS_988.7 were interlinked with 14 nodes. Nine transcription factors (HcHCT_125.110, HcHCT_478.81, HcHCT_299.9, HcHCT_576.21, HcHCT_1075.10, HcAP2_26.297, HcAP2_432.10, HcAP2_579.37, and HcGRAS_405.14) were edged with 13 nodes, and four (HcRLKL_395.61, HcWRKY_71.45, HcAP2_99.133, and HcGATA_3.189) were interconnected with 12 nodes. In addition, HcMYB_3.94 was linked with 11 edges, and fourteen other transcription factors were connected with 10 nodes, suggesting that these are potential candidates for enhancing floral aroma production in H. coronarium flowers under the melatonin treatment.

TABLE 2 | Transcription factors identified in the significant DEGs.

| Transcription factors | 100MT-HS vs. CK-HS | 100MT-FB vs. CK-FB | 100MT-FS vs. CK-FS |
|-----------------------|-------------------|-------------------|-------------------|
|                       | Up    | Down | Up    | Down | Up    | Down |
| AP2                   | 17    | 1    | 33    | 0    | 2     | 13   |
| ARF                   | 1     | 0    | 0     | 0    | 1     | 1    |
| bHLH                  | 10    | 2    | 11    | 3    | 2     | 6    |
| bZIP                  | 3     | 0    | 5     | 1    | 0     | 10   |
| DoF                   | 1     | 0    | 1     | 1    | 1     | 2    |
| GATA                  | 0     | 0    | 4     | 0    | 0     | 1    |
| GRAS                  | 4     | 0    | 6     | 0    | 0     | 0    |
| HSF                   | 13    | 0    | 0     | 0    | 0     | 6    |
| LSD                   | 0     | 0    | 1     | 0    | 0     | 0    |
| MYB                   | 12    | 0    | 18    | 2    | 4     | 10   |
| TCP                   | 1     | 0    | 0     | 0    | 0     | 0    |
| WRKY                  | 15    | 0    | 20    | 0    | 1     | 12   |

Transcription Factors Involved in Floral Scent Regulation

Several previous studies revealed that transcription factors (TFs) can regulate the mechanism of floral scent biosynthesis. Numerous TF families were identified among the significant DEGs using the plant TFDB database (Table 2). Under the melatonin treatment, 80, 106 and 72 differentially expressed transcription factors (TFs) were identified in CK-HS vs. 100MT-HS, CK-FB vs. 100MT-FB, and CK-FS vs. 100MT-FS, respectively. The transcription factors with the most copies were AP2 (66), WRKY (48), MYB (46), bHLH (34), bZIP (19), HSF (19), and GRAS (10). The melatonin treatment increased the expression of the majority of these transcription factor genes. Between CK-HS vs. 100MT-HS and CK-FB vs. 100MT-FB, the expression of the majority of transcription factors was upregulated and downregulated in CK-FS vs. 100MT-FS. Other TFs, such as ARF, DoF, GATA, TCP, and LSD, were also observed among these three stages of flower development under the melatonin treatment.

Key Scent-Related Genes Involved in Floral Aroma Synthesis Under the Melatonin Treatment

To reveal the regulatory mechanism of exogenous melatonin on scent metabolism-related genes in H. coronarium flowers, a cluster analysis of differentially expressed genes was employed to compare the expression levels of genes linked with floral scent production in three stages. A total of 126 differentially expressed genes were related to the floral scent mechanism, and their expression levels were revealed via a heatmap (Figure 6). Between CK-HS and 100-HS, 42 genes involved in the floral mechanism were significantly upregulated. A total of 65 genes were significantly upregulated in CK-FB vs. 100-FB. Interestingly, eight genes, including 6 transcription factors, were significantly upregulated in the fade stage compared to the corresponding control. Furthermore, 22, 29, and 6 transcription factors (MYB and bHLH) were upregulated in CK-HS vs. 100-HS, CK-FB vs. 100-FB, and CK-FS vs. 100-FS, respectively. The HcTPS, HcBSMT, HcP450, HcHAT, HcGGPS, HcDXS, HcPAL, and HcHCT genes were mainly included among the upregulated genes.

Network Analysis of Floral Volatile Compounds and Differentially Expressed Genes in Response to Melatonin Treatment

We performed a correlation test between the core volatile compounds and significant DEGs (floral scent-related genes and transcription factors) identified between the control flowers and melatonin-treated flowers to better understand the regulatory networks of floral volatile compounds (Figure 7 and Supplementary Table 5). The role of key transcription factors in floral scent metabolism was revealed based on a Spearman correlation analysis and further analyzed via cystoscopy. A gene-to-metabolite correlation analysis revealed that the MYB/bHLH transcription factors were the main regulators of floral volatile compounds. MYB (HcMYB_1021.25) was found to be the most significant transcription factor, and it was connected with 18 edges. Similarly, bHLHs (HcHCT_331.80) and MYBs (HcMYB_107.61) were connected with 16 nodes each. Five transcription factors, MYB (HcMYB_1223.6 and HcMYB_106.16), bHLH (HcHCT_43.153), GRAS (HcGRAS_211.12), and AP2 (HcAP2_519.11), were connected with 15 nodes. HcAP2_441.22 and HcGRAS_988.7 were interlinked with 14 nodes. Nine transcription factors (HcMYB_125.110, HcMYB_478.81, HcMYB_299.9, HcHCT_576.21, HcHCT_1075.10, HcAP2_26.297, HcAP2_432.10, HcAP2_579.37, and HcGRAS_405.14) were edged with 13 nodes, and four (HcHCT_395.61, HcWRKY_71.45, HcAP2_99.133, and HcGATA_3.189) were interconnected with 12 nodes. In addition, HcMYB_3.94 was linked with 11 edges, and fourteen other transcription factors were connected with 10 nodes, suggesting that these are potential candidates for enhancing floral aroma production in H. coronarium flowers under the melatonin treatment.

Validation of Scent-Related Genes via Quantitative Real-Time PCR

Quantitative RT–PCR was used to examine the expression profiles of selected genes involved in floral scent production (i.e., TPS, DXS, GGPS, HCT, HAT, BSMT, and PAL) or encoding MYB/bHLH TFs that were chosen from the RNA-seq data (Figure 8). The relative gene expression values determined by qRT–PCR were found to be strongly persistent with the FPKM determined by RNA-seq. HcPAL (HcPAL_488.9), showed a similar expression pattern with HcHCT (HcHCT_93.4) and HcGGPS (HcGGPS_206.57), with significantly higher expression during the HS and FS stages and lower expression during the FB stage compared with the control. Similarly, the transcript level of HcDXS (HcDXS_47.166 and HcDXS_597.1) was similar to that of HcBSMT (HcBSMT_68.69). Overall, the majority of genes were upregulated during the half and full bloom stages of the flowers but downregulated during the fade stage of the flowers with
Heatmap of the expression levels of scent-related biosynthesis genes and MYB transcription factors identified in DEGs. Blue indicates low gene expression, white indicates no expression pattern, and red indicates high gene expression.

respect to their control flowers. These results suggest that RNA-seq data are reliable and reproducible by independent methods, such as qRT-PCR.

DISCUSSION

Plant melatonin acts as an antioxidant and is involved in a variety of physiological processes, including seed germination, rooting, growth, osmoregulation, photosynthesis, protection against external stimuli, and gene expression regulation for a variety of physiological processes. *Hedychiun coronarium* J. Koenig, commonly known as ginger lily, is an important ornamental/medicinal plant that is widely grown in tropical and semitropical regions of the world. Terpenoids (monoterpenes and sesquiterpenes) and benzenoids/phenylpropanoids are the main volatile components of *H. coronarium* flowers, and they are released in abundant amounts during the blooming period, which is consistent with previous reports (Yue et al., 2014, 2021; Ke et al., 2019; Zhou et al., 2021). However, the molecular regulatory mechanism of melatonin underlying floral aroma production remains unknown.

Melatonin Enhanced Floral Aroma Production in *H. coronarium* Flowers

Several previous studies also showed that melatonin regulates several plant processes, including plant growth and development and senescence (Hu et al., 2020). Previously, RNA sequencing data revealed that the expression of numerous genes was influenced by melatonin regulation, causing changes in physiological activities. Exogenous melatonin application can boost endogenous melatonin contents and enhance its effects on a variety of physiological processes that lead to pleiotropic effects because of the signaling role of melatonin in plants (Byeon and Back, 2014; Fan et al., 2018; Xu et al., 2019). In the
current study, the influence of melatonin on *H. coronarium* flowers was evaluated using the HS–SPME–GC–MS method, and its effect on individual compounds was evaluated. We observed that melatonin application substantially enhanced floral scent production in *H. coronarium* relative to that of the control flowers. Under the melatonin treatment, the amount of certain volatile compounds increased during the half bloom, full bloom and fade stages of the flowers compared to the control group. Similarly, in *Camellia sinensis*, exogenous melatonin and gibberellin application significantly affected terpenoid synthesis and hormone signal transduction pathways (Di et al., 2019). The effect of melatonin varied from species to species and based on the study objectives. In tomato, melatonin promotes anthocyanin accumulation and fruit ripening after harvesting (Hardeland, 2016; Sun et al., 2016). Similarly, melatonin induced significant changes in compounds during developmental processes, and it played a pleiotropic orchestrating role in lignin formation in tea plants (Li et al., 2020). In grape fruits, exogenous melatonin and...
ABA significantly increases flavonol accumulation by upregulating the expression of flavonoid biosynthesis genes (Yang et al., 2020). These results indicate that melatonin significantly triggered the mechanism of floral aroma production.

**Melatonin Regulates the Complex Regulatory Network of Floral Scent Production**

Floral aroma production and emission is a complex and dynamic mechanism that involves the coordination of several genes that are regulated by various transcription factors (Pichersky and Gershenzon, 2002; Gershenzon and Dudareva, 2007; Nagegowda and Gupta, 2020). By analyzing the integrated metabolome and transcriptome data, we can better understand the mRNA abundance and gene profiles of *H. coronarium* flowers treated with melatonin. Our findings showed that the gene expression trends of qRT–PCR strongly supported the RNA-seq data. Under the melatonin treatment, several DEGs were significantly upregulated, suggesting that these genes can play crucial roles in the floral aroma mechanism. Among the differentially expressed genes, key scent-related enzyme genes, such as TPS, DXS, BSMT, HAT, GGPS, PAL, and HCT, were significantly upregulated. Previous studies showed that the enzyme genes TPS, DXS, BSMT, HAT, GGPS, and PAL are the key genes in the terpenoid and benzenoid/phenylpropanoid pathways and produce numerous important secondary metabolites (Pichersky and Gershenzon, 2002; Aharoni et al., 2003; Phillips et al., 2007; Abbas et al., 2019). Similarly, in *C. sinensis*, exogenous melatonin application

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**FIGURE 8 | (A–S)** Quantitative RT–PCR analysis of selected genes involved in scent-related pathways. GAPDH was used as an endogenous control. Data are presented as the mean ± SEM (*n* = 3), relative expression levels of target genes were calculated via the 2^(-∆∆Ct) method.
substantially influenced the terpenoid synthesis pathway by triggering the expression level of related genes (Di et al., 2019). Furthermore, the gene-to-metabolite analysis showed that these key enzyme genes were linked to numerous nodes of certain volatile synthesis. The RNA-seq and qRT–PCR results showed that the expression of the aforementioned key enzymes was significantly influenced by the melatonin treatment (Figure 8).

In Arabidopsis and strawberry, the overexpression of key enzyme genes in terpenoid pathways significantly enhanced the terpenoid volatile compounds, and downregulation resulted in the opposite trend (Aharoni et al., 2003, 2004; Tholl, 2006). In *Vitis vinifera* cv. Kyoho, melatonin and ABA application triggered flavonoid biosynthesis by influencing the expression level of structural flavonoid biosynthesis genes (Yang et al., 2020). Our findings are in line with previous reports that melatonin significantly influenced the expression level of certain genes involved in several biological processes (Arnao and Hernández-Ruiz, 2019; Cecon et al., 2019). Hence, we can hypothesize that melatonin is involved in the regulation of certain floral volatile compounds by influencing the expression of structural volatile synthesis genes, which can be validated via further in-depth molecular intervention.

**Melatonin Could Regulate MYB/bHLH Transcription Factors Involved in the Floral Aroma Mechanism**

Floral scent is produced in various parts of the plant and abundantly released, particularly from flowering parts. Several previous studies revealed that transcription factors play crucial roles in floral aroma biosynthesis (Zvi et al., 2012; Liu et al., 2017; Sasaki, 2018). Evidence has shown that the MYB/bHLH transcription factor regulates floral aroma metabolism by binding to the promoter region of enzyme genes (terpene synthases) (Deluc et al., 2006; Bedon et al., 2010; Colquhoun et al., 2010; Amarr et al., 2017). Among the significant differentially expressed genes, 12, 18, and 4 HcMYB transcription factor genes were significantly upregulated in the CK-HS vs. 100MT-HS, CK-FB vs. 100MT-FB, and CK-FS vs. 100MT-FS comparisons, respectively, whereas 2 and 10 HcMYB transcription factors were downregulated in the CK-FB vs. 100MT-FB and CK-FS comparisons, respectively. Furthermore, the Spearman correlation analysis showed that HcMYB/bHLH were among the main transcription factors interlinked with several nodes of major floral volatile components, indicating that these genes are candidate genes that activate floral aroma biosynthesis under the melatonin treatment. Similar findings were obtained in *C. sinensis*, where MYB transcription factors regulate lignin biosynthesis under the melatonin treatment (Han et al., 2021). Interestingly, in gene-to-metabolite correlation analysis, we observed that HcMYB_1021.25 (HcMYB248) was among the key HcMYB transcription factors connected with 18 nodes. Our previous study regarding the genome-wide analysis of HcMYB revealed that HcMYB248 was the key transcription factor controlling floral aroma biosynthesis by binding to the promoter region of structural synthesis genes, and its suppression resulted in reduced floral volatile contents by downregulating the expression of structural volatile synthesis genes (Abbas et al., 2021a). In petunia, ODO1, EOB1, and EOBII regulate the benzenoid biosynthetic pathways by regulating related gene expression (Verdonk et al., 2005; Van Moerkercke et al., 2011;
In the current study, we observed that exogenous melatonin application significantly enhanced the floral volatile contents in *Hedychium coronarium* flowers. We used metabolomics and transcriptomics approaches to analyze the phytochemical and transcriptional changes that occur during the flower developmental process of *Hedychium coronarium* flowers after melatonin treatments. The data showed that the production of certain volatile organic compounds was significantly increased, and hundreds of differentially expressed genes were identified and further analyzed. A total of 76 DEGs were significantly upregulated and involved in the complex floral aroma biosynthesis mechanism. The expression of key genes associated with the floral volatile mechanism was verified by RT-qPCR. A gene-to-metabolite correlation analysis using Cytoscape was performed to identify the key regulators of floral volatile compounds, which showed that the transcription factors HcMYB/bHLH were mainly interlinked with several nodes and considered potential candidates for the regulation of floral volatile compounds in *Hedychium coronarium* flowers. In short, although the current study has some limitations, our results provide valuable insights into the regulatory mechanism of melatonin in floral aroma biosynthesis in *H. coronarium* flowers.

**CONCLUSION**

**DATA AVAILABILITY STATEMENT**

The datasets presented in this study can be found in online repositories. The names of the repository and accession number can be found below: https://www.ncbi.nlm.nih.gov/sra/PRJNA777930.

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**AUTHOR CONTRIBUTIONS**

FA, YK, YF, and RY conceived and designed the concept, and revised and finalized the manuscript. FA, YK, and JH performed the experiments and did the formal analysis. FA and YZ analyzed the data and drafted the manuscript. All authors endorsed the final version of the manuscript.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2021.808899/full#supplementary-material

**Supplementary Figure 1 |** Correlation analysis of floral volatile compounds between the treated and untreated flowers.

**Supplementary Figure 2 |** Volcano plot of the DEGs identified in CK-HS vs. 100MT-HS, CK-FB vs. 100MT-FB, and CK-FS-100MT-FS.

**Supplementary Figure 3 |** Transpose super-families significantly influenced under melatonin treatments.

**Supplementary Table 1 |** Analysis of the sequencing data statistics.

**Supplementary Table 2 |** Statistics for the sample and reference genome comparisons.

**Supplementary Table 3 |** List of the top biological processes observed in the GO classification.

**Supplementary Table 4 |** List of significantly enriched pathways of DEGs among CK-HS vs. 100MT-HS, CK-FB vs. 100MT-FB and CK-FS vs. 100MT-FS.

**Supplementary Table 5 |** List of primers used in the assay.
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