The diurnal emission of floral scent in *Oncidium* hybrid orchid is controlled by *CIRCADIAN CLOCK ASSOCIATED 1 (CCA1)* through the direct regulation on *terpene synthase*

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**Abstract**

**Background:** To adapt to the periodic fluctuation of environmental factors, plants are subtle to monitor the natural variation for the growth and development. The daily activities and physiological functions in coordination with the natural variation are regulated by circadian clock genes. The circadian emission of floral scents is one of the rhythmic physiological activities controlled by circadian clock genes. Here, we study the molecular mechanism of circadian emission pattern of ocimene and linalool compounds in *Oncidium* Sharry Baby (*Onc.* SB) orchid.

**Results:** GC-Mass analysis revealed that *Onc* SB periodically emitted ocimene and linalool during 6 to 14 o'clock daily. *Terpene synthase*, one of the key gene in the terpenoid biosynthetic pathway is expressed in coordination with scent emission. The promoter structure of *terpene synthase* revealed a circadian binding sequence (CBS), 5'-AGATTT TT-3' for *CIRCADIAN CLOCK ASSOCIATED1* (CCA1) transcription factor. EMSA data confirms the binding affinity of CCA1. Transactivation assay further verified that TPS expression is regulated by CCA1. It suggests that the emission of floral scents is controlled by CCA1.

**Conclusions:** The work validates that the mechanism of circadian emission of floral scents in *Onc.* Sharry Baby is controlled by the oscillator gene, *CCA1*(CIRCADIAN CLOCK ASSOCIATED 1) under light condition. CCA1 transcription factor up-regulates *terpene synthase* (TPS) by binding on CBS motif, 5'-AGATTTTT-3' of promoter region to affect the circadian emission of floral scents in *Onc.* SB.

**Keywords:** Floral scent, Circadian rhythm, *Oncidium* Sharry Baby, CIRCADIAN CLOCK ASSOCIATED 1 (CCA1), CBS binding site (CBS)

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**Background**

Plants live in environments that oscillate with a period of approximately twenty-four hours. To adapt to the periodic environmental fluctuations such as light and temperature, they constantly monitor changes in the surrounding environment, and response to biological processes by a circadian rhythmic pattern. The circadian clock is an intrinsic and entrainable timekeeping system.
mechanism, conferring plants to be able to buffer against both subtle and extreme changes in environment [1, 2]. Therefore, it contributes to timing multiple biological processes among various environmental factors. The role of circadian clock encompasses to almost every aspect of growth and developments. Generally, the detection on day-length changes is the most effective way to regulate gene expression. In addition to regulating daily activities, circadian clocks are also involved in the seasonal regulation of physiological function such as flowering, photoperiodism [3]. The circadian clock in plants is conducted by several circadian oscillator genes, which are clock components capable of generating the multiple negative regulatory feedback loops. Coordinated regulation of circadian oscillators and circadian-regulated output genes at various steps to establish and maintain circadian rhythms [4–6]. Recent progresses in genomic, biochemical and bioinformatics research have provided great understanding in the molecular architecture of the circadian clock in Arabidopsis [7, 8]. Most clock components possess transcriptional activity, and nearly 80% of transcriptomic genes in rice, poplar and Arabidopsis are regulated by internal circadian clock [9]. As a central circadian rhythm regulator, CCA1 occupied more than 1000 genomic regions identified in the seedling stage based on the CHIP sequencing data [10]. Among the targets of CCA1, although many of the target genes are repressed, some are activated, such as LHCB1.1 (CAB2) [11], this indicates the CCA1 is the master clock regulator containing broad function.

In the transcriptional level, CCA1 plays an important role in either repressor or activator in circadian rhythm, depending on which cis-element it has interacted with. As the further reports, CCA1 represses gene expressions when it interacts with EE (Evening Element; -AAA TATCT-), and the peak of the gene expression in circadian rhythm highlights during 10–14 o’clock [12, 13]. In contrast, it works as an activator to induce the gene expressions of light-harvesting chlorophyll a/b protein: LHCB3.1, when it interacts with CBS element (-AAMAATCT-), and the peak of gene expression was at dawn [14]. It was also reported that when the EE element was point-mutated to the CBS element on the promoter of hydrogen peroxide catalase 3 (CAT3), the peak of circadian rhythm was shifted from 10–14 to 0–4 o’clock, suggesting that the circadian rhythm was affected by the cis-element which CCA1 interacted with [15].

Floral scent in plants is one of the most important floral characteristics to attract pollinators. The past studies on floral scent were generally assumed that floral scent is genetic adaption under the long-term pollinator-mediated natural selection, but recent evidence showed floral scent emissions are also influenced by environmental elements like light and temperature [16, 17]. To attract species-specific pollinators, floral scent emission usually oscillates with the circadian rhythm in parallel with insect activity [18–22]. For example, petunia emits fragrance mostly at night and less during the day, indicating a nocturnal rhythmic pattern [23, 24]. On the contrary, the floral emission of moth orchids is conducted with a diurnal rhythmic pattern [25]. It suggests that the rhythmic emission of floral scent is under the genetic adaption between plants and pollinators.

In this work, we used the Oncidium hybrid orchid, Oncidium Sharry Baby to study the molecular mechanism of its diurnal rhythmicity of floral scent emission. The synchronization between its terpenoid emission and gene expression of terpene synthase is an interesting biological process. Terpene synthase is one of the two key enzymes for synthesizing floral scent compounds, ocimene and linalool, in terpenoid biosynthetic pathway in Onc. SB. Ocimene and linalool are acyclic monoterpene, produced by terpene synthase by different backbone rearrangement from the precursor geranyl diphosphate (GDP). Our survey on the promoter structure of terpene synthase disclosed the DNA motif of CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) binding site (CBS). CCA1 is a key circadian clock component responsible for regulating the circadian rhythmic expression [3]. Our current study demonstrates that CCA1 appears to upregulate the expression of terpene synthase. Our finding elucidates the influence of the diel rhythm on scent emission in Oncidium Sharry Baby orchid.

Results

Floral scent is emitted by diel rhythmic pattern synchronizing with terpene synthase expression in Oncidium Sharry Baby

As our investigation, the floral volatile compounds were known as ocimene and linalool (Supplemental Fig. 1). Both of them were belong to monoterpene, converted from geranyl diphosphate. In this work, we monitor its emission pattern on the whole day. Data revealed that the diel oscillation displayed a peak around 10 o’clock. The emission starts from 6 o’clock and decreases from 14 o’clock until 22 o’clock. The fragrant scents are dominant in the morning time, and vanish in the night time (Fig. 1). It suggests that the floral scent emission pattern is a diel control and regulated by oscillated genes.

Diel rhythmic expression of terpene synthase is light-dependent, and abolished in constant light (LL) and constant dark (DD) environment

Both geranyl diphosphate synthase (GDP) (gene accession number: MH171283.1) and terpene synthase (TPS) (gene accession number: MH171282.1) constitute the
two-step enzymes of the biosynthetic pathway to produce monoterpene compounds. The GC–MS data suggested that the final product was linalool and ocimene (supplementary Fig. 2). However, because the reaction condition was not similar with the nature circumstance, the ratio of both terpene compounds was also different. While we check the expression pattern of both GDPS and TPS, GDPS is highly expressed whole the day with a peak at 2–10 o’clock (0.5〜1.5 relative expression level; Fig. 2a), while TPS is much less expressed (0.01〜0.07 relative expression level), but it shows an enormous peak around 10:00 o’clock in parallel with the emission pattern of floral scent (Fig. 2b). In conclusion, TPS is a bottle-neck enzyme in the floral scent biosynthesis. However, the TPS expression pattern is closely coincident with the emission pattern of ocimene/linalool, suggesting TPS could determine the diel rhythmicity of floral scent biosynthesis (Fig. 2b). In order to study the regulatory function of CCA1 on TPS transcription. A full OncSBCCA1 cDNA sequence was further cloned based on the CCA1 contig sequences existing in the available transcriptomic database (Gene Bank accession number GIQW00000000). cDNA sequence containing 1,869 bp ORF and with the deduced protein molecular weight of 67.9 kDa was completed. Its cDNA sequence showed a higher homology to Phalaenopsis equestries (moth orchid) (Fig. 5). Notably, only one conspicuous MYB motif is present in each cDNA sequence, suggesting it is a single MYB-domain type of OncSBCCA1 transcription factor (Fig. 5).
Furthermore, the cDNA CDS fragment was subcloned in pET28a expression vector and was transformed to *E. coli* BL21(DE3) for recombinant protein production. The recombinant protein was purified through High Affinity Ni-Charged Resin column following the general protocol. Analysis on SDS-PAGE profile displayed a 68.74 kDa His-tag fused protein band (data not shown), indicating the overexpressed recombinant protein is coincident with the predicted molecular weight of CCA1. Furthermore, Electrophoresis mobility shift assay (EMSA) was performed to identify the binding activity between CCA1 protein and CBS cis-acting motif of *TPS* promoter region. The DNA probe, was synthesize as -AAAAATCT-, and added to react with *OncSBCCA1* recombinant protein as

Fig. 2  Gene expression levels of *geranyl diphosphate synthase* (a), and *terpene synthase* (b) in *Oncidium Sharry Baby*. Data revealed that **GDPS** is highly expressed whole the day, while **TPS** is expressed relatively less with an enormous peak at 10 o’clock. Results represent mean ± SEM from three biological replicates.
described in Materials and Methods. The significant fluorescence signals shown on 10% native PAGE data clearly appear at the top of gel, and the signal intensity increases in accordance with the gradually increasing concentration of CCA1 (Fig. 6, Supplemental Fig. 3). This data confirmed the specific affinity between CCA1 protein factor and CBS cis-acting sequence. EMSA data demonstrate the binding activity of CCA1 on TPS promoter. Moreover, it strongly suggests that CCA1 can directly regulate TPS gene expression.

To further demonstrate the regulatory interactions between CCA1 and CBS, transactivation assay was
performed. The result showed that when CCA1 interacted with CBS, the fluorescence accumulation was significantly higher than those one in which CCA1 interacted with mutated CBS, suggesting that CCA1 upregulated TPS expression by binding on CBS motif of promoter (Fig. 7).

**Circadian rhythmicity of CCA1 is responsible for regulating TPS transcription**

CCA1 has been known as a core oscillator gene in circadian network [10]. Although our data has confirmed the binding activity on CBS morning element of OncSBTPS (Fig. 6, Supplemental Fig. 3), the expression pattern of OncSBCCA1 in Onc. Sharry Baby is still unclear. To verify the circadian expression of OncSBTPS is coordinately regulated by CCA1, the expression pattern of OncSBCCA1 in flower organ was monitored by qRT-PCR. As shown in Fig. 8a, the CCA1 expression is active from 6:00 to 10:00 o’clock. It indicated a diel expression type, and in coincidence with OncSBTPS expression pattern (Fig. 2b). Further, we conducted a 72-h time-course experiment to monitor the expression by using 2-week-old floral plants grown under 12L/12L (Fig. 8b) and 12D/12D respectively (Fig. 8c). The expression level of CCA1 clearly exhibited a rhythm, indicating CCA1 expression pattern is in accordance with expression pattern of OncSBTPS and emission pattern of the floral scents.

**Discussion**

To ensure the fine control of metabolic processes associated with the physiological functions, plants have developed an endogenous system for a precise measurement of photoperiod, represented by circadian rhythms, synchronized with the prevailing environmental conditions. The system is the regulatory mechanism of gene expression. For example, the temporal regulation of plant scent emission is correlated with the expression pattern of biosynthetic genes in the metabolic pathway of scent production [26–28]. The scent emission is closely tied with the oscillation of expression pattern of metabolic genes. In the scent biology, several model plants, such as rose, snapdragon, tobacco, and petunia have been comprehensively studied for the oscillatory emission patterns. In this regard, Petunia hybrida has been perhaps the most complete model in scent emission [29]. The analysis of gene expression within the floral volatiles

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**Fig. 4** The primary structure analysis showing cis-acting elements in TPS promoter region (~ 1375 bp length). The putative CCA1 binding site (CBS), AGATTTTT- located at -423 ~ -430 bp of TPS promoter was marked by red color.
benzenoid/ phenylpropanoid (FVBP) pathway provided key insights into the mechanism of scent regulation. Thus far, most studies of the regulatory mechanism of oscillatory emission of floral volatiles are focused on FVBP pathway. FVBP pathway is composed of a series of enzymes. For example, the oscillation of methyl benzoate compound is closely correlated with the expression patterns of the biosynthetic enzymes - BSMT1 and BSMT2 (Salicylate/benzoate carboxyl methyltransferase 1 and 2). PAL (phenylalanine lyase) mRNA expression pattern also oscillates in a similar pattern to that of benzoic acid synthesis in diurnal conditions [30]. Recently, two R2R3-type MYB transcription factors, ODORANT1 (ODO1) and EMISSION BENZENOIDS II (EOBII) were identified as regulatory components of floral scent metabolism. Both are involved in FVBP synthesis [31]. Up-regulation and down-regulation of their transcription increases and decreases the transcripts of many key enzymes in the FVBP pathway and subjective to affect scent production. Most recently, it revealed the identification of putative binding sites of clock gene, i.e., LHY (LATE ELONGATED HYPOCOTYL) in the promoter of ODO1 of P. hybrida [32]. When PhLHY was over-expressed, the floral emission of hybrida was almost lost, and many genes involved in FVBP pathway, including ODO1, EPSPS (enolpyruvylshikimate 3-phosphate synthase), CM1 (chorismate mutase 1), ADT (aroogenate dehydratase), and PAL, were down-regulated [15]. However, when PhLHY was mutated, the peak of floral scent emission and gene expression levels in FVBP pathway
were move up from dusk to afternoon. As its homologs in other plants, PhLHY peaks around dawn [26]. Further investigation reported it can bind to other genes in the FVBP pathway and controls the expression phase of these genes. These researches delivered the information that temporal expression of scent appears to be primarily regulated through manipulation of the timing of transcriptional regulators in the biosynthetic pathway [16]. In recent years, MYB transcription factors were reported to regulate the production of terpenoids [7].

Our current study firstly found that the floral scent emitted from Onc. Sharry Baby closely related with the expression of terpene synthase. Its relative less expression level influences the scent quantity. The circadian expression is controlled by the promoter structure. Analysis on promoter structure of OncSBTPS disclosed a putative CBS (CCA1 binding site), -AGATTTTT-, located at -423〜-430 bp of TPS promoter (Fig. 4). The EMSA assay identified that OncSBCCA1 transcriptional factor is able to bind on CBS sequence of TPS promoter (Fig. 6). Transactivation assay confirmed the CCA1 regulation on the TPS promoter by binding CBS motif (Fig. 7). Similar to the emission pattern of floral scent, TPS and CCA1 are expressed at peak of 6:00〜10:00 o’clock in the morning, demonstrating the circadian clock function in scent emission. Interestingly, circadian oscillation of OncSBTPS and OncSBCCA1 expression showed distinct patterns in light- dependent manners. When Onc. SB orchid plants were kept in continuous light (LL) and continuous dark, robust oscillation of OncSBTPS and OncSBCCA1 abolished in the first day (Fig. 3). The results indicated that the proper light/dark cycle is the critical condition for robust circadian oscillation of OncSBTPS and OncSBCCA1 in Onc. SB. Evidence suggested that both light and internal clock mutually interact in the rhythmic expression of clock genes.

**Conclusion**

In the present work, we address the genetic mechanism how the circadian clock controls the floral volatiles emission in Onc. SB orchid. CCA1 plays role of the core oscillator, directly regulating terpene synthase transcription, by which volatiles of ocimene and linalool were synthesized timely in floral tissues. The robust diel rhythmicity is synchronizing among core oscillator, rhythm-regulated gene expression levels, and scent emission period. The rhythmic function occurs in terpenoid metabolic pathway in Oncidium orchids was first demonstrated.

**Materials and methods**

**Plant materials and growth condition**

Oncidium orchid plants were purchased from Xu-Tung nursery Co. Thirty plants as early-blooming stage two weeks prior to experiments were grown in greenhouse at
When we investigate the circadian rhythm of gene expression levels and VOC emission of *Oncidium* orchids, plants were grown in 25 °C, constant light, or constant dark, or 12 h light/12 h dark, which switches at 6 and 18 o’clock, environment conditions. The light intensity in this study was 250–300 μmol m⁻² s⁻¹. Three independent plants with full blooming were used to measure volatile emission and circadian rhythmic assays. A single flower from an independent orchid plant was used in each sample for the study of timing emission. Total three flowers were used to measure and evaluated on average.

**Floral volatile collection and GC–MS analysis**

The solid-phase micro-extraction (SPME) technique coupled with GC–MS system was used to collect and analyze floral volatile compounds, following the method described by Lin et al. [25]. The whole floret organs were cut at 2:00, 6:00, 10:00, 14:00, 18:00 and 22:00 o’clock separately and immediately sealed in 20 ml headspace sample vials with 50 μm SPME fiber (Sigma-Aldrich, MO, USA) following injection of 100 ng of an internal standard *n*-nonane. The volatiles absorbed by the SPME fiber was directly desorbed for 5 min in the GC inlet. The GC–MS system is a Trace GC Ultra Gas Chromatograph and a Polaris Q mass spectrometer (Thermo Fisher Scientific, USA). The GC column was a 30 m × 0.25 mm × 0.25 μm column (DB-5 ms, Agilent Technologies). The GC oven temperature process was at 60 °C initially, then rose up to 220 °C at 4 °C/min and stay in 220 °C for 2 min. Afterwards, the temperature ramped to 250 °C and held for 3 min with the heating speed 20 °C/min. The temperature of inlet is 250 °C. The carrier gas is helium (99.9995%) and the flow speed was 1 mL/min. The split ratio is 10:1. The temperature of transferline and ion source of MS are 250 °C and 230 °C, respectively. The ionization energy is 70 eV and the ion scan range of MS is 50 – 400 amu. The identification of the component of floral scent is using the Kovats index, MS spectra from NIST. VOC data was collected from three floral individuals each from one of three different blooming orchid plants and calculated by average.
Gene expression analysis

Total RNA of floral tissues was extracted by using the pine tree method [33]. The real-time quantification PCR (ABI 7500, Thermo Fisher Scientific, MA, USA) was performed to assay gene expression profile following the manufacturer’s protocol (The KAPA SYBR qPCR kit, Wilmington, USA). The gene expression patterns of OncSBTPS (terpene synthase) were determined by the primer pair of 5’-CTCTGGATGTGCCTTTGGTCAGAAG-3’ and 5’-AGCTCTTCCACAGTCCCA TAATTATCATA-3’. For CCA1, the primer pair was: 5’CAAGCTTTGTGAGATATTCTCATTTGC-3’ and 5’-TCAAACCTTTCTTGCAGACGAATTTTC-3’. The internal standard used in this study was actin 7 cloned from Oncidium SB.
Electrophoresis mobility shift assay
Two primers, which were designed based on the nucleotide sequence of candidate genes in Oncidium SB transcriptome (Gene Bank accession number GIQW000000000, Contig TR3792_c0_g1_i1) were employed to amplify the CCA1 cDNAs of Onc. SB. The forward and reverse primers were as following: 5′-AGAGATGAACTCTTTTGGAGAAGATC-3′ and 5′-TCA AACTTTCCTCAGACGAAATT-3′ respectively. The full-length CCA1 CDS was cloned into the pET28a vector, transformed and expressed in E. coli BL21(DE3).

Transformed E. coli containing the pET28a-CCA1 vector was grown in 50 mL of LB broth at 37 °C overnight. After cell culture growing to reach OD595 ~ 0.5, 100 μL IPTG (0.1 M) was added to induce CCA1 expression for 4 h. Expressed proteins were harvested and purified by using a His column (Genscript, New Jersey, USA) following the manufacturer’s instructions. EMSA performance was conducted as described by Chen et al. [34]. Experiment was carried out in photophobic condition. At the beginning, the 6-FAM fluorescence dye was ligated to a 24-nucleotide of the double-strand CBS DNA, -TAT TTT TT- by T4 ligase to produce the 1 mL W5 solution (154 mM NaCl, 125 mM CaCl2 2H2O, 5 mM KCl, 2 mM MES (pH5.7), 5 mM glucose) under 4°C.

Partial promoters of pOncSBTPS(-1 ~ -441 bp) was cloned from Onc. SB genome. pOncSBTPS_mCBS, in which CBS was mutated from -AGATTTTTT- to -GGG TTTTTT-. Both were cloned into vector pGreenII0800-Luc as a reporter construct. In the reporter construct, pCaMV::Renilla luciferase was an internal standard. The full length CCA1 was driven by CaMV 35S promoter in effector vector. Two vectors were co-transfected into protoplasts. The Dual-Luciferase Reporter Assay System (Promega, WI, USA) was applied to perform the Luciferase Assay. The firefly luciferase activities were normalized by the Renilla luciferase activities.

Statistics
To investigate the Significant difference in the transactivation assay, one way ANOVA was applied. The program to calculate the statistics was Excel 2019 (Microsoft corp., Washington, U.S.A), and the Add-in was Real-statistics (https://www.real-statistics.com/). The Post Hoc test was Tukey HSD Test.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12870-022-03850-z.

Additional file 1: Supplemental Fig. 1. The GC-MS spectrum of floral scent from Oncidium SB flowers in different times. The volatile terpenes, oicinone and linalool display the peaks at RT=11.31 and 12.91 respectively. The profiles decipher floral scents are diurnally emitted highly at 1000 oclock and lower at 18:00 oclock. The GC spectrum showed that there is a circadian rhythm of floral scent emissions. Supplemental Fig. 2. The enzymatic activities assay of TPS. TPS recombinant protein was purified, then fed with the precursor GDP, and reacted at 37°C for 4hrs. The GC-MS data suggested that the final product was linalool and oicinone. The SPME and GC-MS analysis conditions was described in text. Supplemental Fig. 3. The uncropped image of Fig. 6 was attached. In order to ensure the compliance with the digital image and integrity policies, the uncropped image of Fig. 6 was attached. At two lanes on the right, we increased the concentration of CCA1 when the competitor was added. As the result showed, the fluorescence intensities of free probe decreased. To focus on the interaction between CCA1 and CBS, Figure 6 didn’t show these two lanes.

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Authors' contributions
Chao-Wei Yeh and Dr. Kai-Wun Yeh constructed the research idea. Chao-Wei Yeh and Yung-Feng Ho analyzed the floral scent compounds and key biosynthesis gene, TPS, in Oncidium orchids. Hui-Qin Zhong and Yung-Feng Ho investigated the regulatory mechanism of TPS. Dr. Zhi-Hong Tian and Dr. Kai-Wun Yeh are principal investigators and supervise the research. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on request.

Declarations

Ethics approval and consent to participate
All methods were in compliance with relevant institutional, national and international guidelines and legislation.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References

1. Bell-Pedersen D, Cassone VM, Earnest DJ, Golden SS, Hardin PE, Thomas TL, Zoran MJ. Circadian rhythms from multiple oscillators: Lessons from diverse organisms. Nat Rev Genet. 2005;6(7):544–56.
2. Yerushalmi S, Green RM. Evidence for the adaptive significance of circadian rhythms. Ecol Lett. 2009;12(9):970–81.
3. Nagel DH, Kay SA. Complexity in the wiring and regulation of plant circadian networks. Curr Biol. 2012;22(16):R648-657.
4. Pruneda-Paz JL. A functional genomics approach reveals CHAC as a component of the Arabidopsis circadian clock (vol 325, pg 1481, 2009).
5. Yakir E, Hassidim M, Melamed-Book N, Hilman D, Kron I, Green RM. Cell autonomous and cell-type specific circadian rhythms in Arabidopsis. Plant J. 2011;68(3):320–31.
6. Srivastava D, Shamim M, Kumar M, Mishra A, Maurya R, Sharma D, Pardey P, Singh KN. Role of circadian rhythm in plant system. An update from development to stress response. Environ Exp Bot. 2019;162:256–71.
7. Shim JS, Imazumi T. Circadian Clock and Photoperiodic Response in Arabidopsis. From Seasonal Flowering to Redox Homeostasis. Biochemistry-Uk. 2015;54(2):157–70.
8. Millar AJ. The Intracellular Dynamics of Circadian Clocks Reach for the Light of Ecology and Evolution. Annu Rev Plant Biol. 2016;67(6):595–618.
9. Filichkin SA, Breton G, Priest HD, Dharmawardhana P, Jaiswal P, Fox SE, Michael TP, Chory J, Kay SA, Mockler TC. Global Profiling of Rice and Poplar Transcriptomes Highlights Key Conserved Circadian-Controlled Pathways and cis-Regulatory Modules. Plos One. 2011;6(6):e16907.
10. Nagel DH, Doherty CJ, Pruneda-Paz JL, Schmitz RJ, Ecker JR, Kay SA. Genome-wide identification of CCA1 targets uncovers an expanded clock network in Arabidopsis. P Natl Acad Sci USA. 2015;112(34):E4802–10.
11. Wang ZY, Tobin EM. Constitutive expression of the CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) gene disrupts circadian rhythms and suppresses its own expression. Cell. 1998;93(7):1207–17.
12. Harmar SL, Kay SA. Positive and negative factors confer phase-specific circadian regulation of transcription in Arabidopsis. Plant Cell. 2005;17(7):1926–40.
13. Harmar SL. The Circadian System in Higher Plants. Annu Rev Plant Biol. 2009;60:357–77.
14. Wang ZY, Kenigsbuch D, Sun L, Harel E, Ong MS, Tobin EM. A Myb-related transcription factor is involved in the phytochrome regulation of an Arabidopsis Lhcb gene. Plant Cell. 1997;9(4):491–507.
15. Michael TR, McClung CR. Phase-specific circadian clock regulatory elements in Arabidopsis. Plant Physiol. 2002;130(2):627–38.
16. Sagai M, Oyama-Okubo N, Ando T, Maneshi E, Nakayama M. Effect of temperature on the floral scent emission and endogenous volatile profile of Petunia axillaris. Biosci Biotechnol Bioch. 2008;72(11):105–10.
17. Hu ZH, Zhang HX, Leng PS, Zhao J, Wang WH, Wang SD. The emission of floral scent from Lilium ‘siberia’ in response to light intensity and temperature. Acta Physiol Plant. 2013;35(5):1691–700.
18. Dudareva N, Martin D, Kish CM, Kolosnova N, Falldt J, Miller B, Bohlmann J. (E)-beta-cocinene and myrcene synthesize genes of floral scent biosynthesis in snapdragon. Function and expression of three terpene synthase genes of a new terpene synthase subfamily. Plant Cell. 2003;15(5):1227–41.
19. Dudareva N, Negre F, Nagegowda DA, Orlova I. Plant volatiles: Recent advances and future perspectives. Crit Rev Plant Sci. 2006;25(5):417–40.
20. Dudareva N, Klepniek A, Muhlemann JK, Kaplan I. Biosynthesis, function and metabolic engineering of plant volatile organic compounds. New Phytol. 2013;198(1):16–32.
21. van der Niet T, Johnson SD. Phylogenetic evidence for pollinator-driven diversification of angiosperms. Trends Ecol Evol. 2012;27(6):353–61.
22. Fenske MP, Imazumi T. Circadian Rhythms in Floral Scent Emission. Front Plant Sci. 2016;7:462.
23. Simkin AJ, Underwood BA, Auldridge MD, Loucas HM, Shibuya K, Schmelz E, Clark DG, Klee HJ. Circadian regulation of the PhCDD1 carotenoid cleavage dioxygenase controls emission of beta-ionone, a fragrance volatile of petunia flowers. Plant Physiol. 2004;136(3):3504–14.
24. Underwood BA, Tieman DM, Shibuya K, Dexter RJ, Loucas HM, Simkin AJ, Sims CA, Schmelz EA, Klee HJ, Clark DG. Ethylene-regulated floral volatile synthesis in petunia corollas. Plant Physiol. 2005;138(1):255–66.
25. Lin CY, Chen YH, Chang TC, Chen YJ, Cheng SS, Chang ST. Characteristic Aromatic-Active Compounds of Floral Scent in Fluo from Barringtonia racemosa and Their Dynamic Emission Rates. J Agr Food Chem. 2013;61(51):12531–8.
26. Fenske MP, Hazelton KDH, Hempton AK, Shim JS, Yamamoto BM, Riffell JA, Imazumi T. Circadian clock gene LATE ELONGATED HYPOCOTYL directly regulates the timing of floral scent emission in Petunia. P Natl Acad Sci USA. 2015;112(31):9775–80.
27. Fenske MP, Nguyen LP, Horn EK, Riffell JA, Imazumi T. Circadian clocks of both plants and pollinators influence flower seeking behavior of the pollinator hawkmoth Manduca sexta. Sci Rep-Uk. 2018;8:2842.
28. Bloch C, Bar-Shai N, Cytter Y, Green R. Time is honey: circadian clocks of bees and flowers and how their interactions may influence ecological communities. Philos T R Soc B. 2017;372(1734):20160256.
29. Cna’ani A, Muhlemann JK, Ravid J, Maschi T, Klepniek A, Nguyen TH, Dudareva N, Pichersky E, Vainstein A. Petunia x hybrida floral scent protein synthesis and metabolic engineering of plant volatile organic compounds. New Phytol. 2015;205(3):462.
30. Ding K, Pei T, Bai Z, Jia Y, Ma P, Liang Z. SmMYB36, a Novel R2R3-MYB Transcription Factor, Enhances Tanshinone Accumulation and Decreases Phenolic Acid Content in Salvia miltiorrhiza Hairy Roots. Sci Rep. 2017;7(1):15104.
31. Spitzer-Rimon B, Marbevka E, Barkai O, Marton I, Edelbaum O, Masci T, Prathapani NK, Shklarman E, Vainstein A. EOBII, a Gene Encoding a Flower-Specific Regulator of Phenylpropanoid Volatiles’ Biosynthesis in Petunia. Plant Cell. 2010;22(6):1961–76.
32. Van Moerkercke A, Haring MA, Schuurink RC. The transcription factor EMISSION OF BENZENOID 8 activates the MYB ODORANT1 promoter at a MYB binding site specific for fragrant petunias. Plant J. 2011;65(5):917–28.
33. Chang S, Puryear J, Cairney J. A simple and efficient method for isolating RNA from pine trees. Plant Mol Biol Report. 1993;11(2):113–6.
34. Chen SP, Kuo CH, Lu HH, Lo HS, Yeh KW. The Sweet Potato NAC-Domain Transcription Factor IbrNAC1 Is Dynamically Coordinated by the Activator IbrHLH5 and the Repressor IbrbHLH4 to Reprogram the Defense Mechanism against Wounding. Plos Genetics. 2016;12(10):e1006397.
35. Sherf BA, Navarro SL, Hannah RR, Wood KV. Dual-Luciferase Reporter Assay: An Advanced Co-Reporter Technology Integrating Firefly and Renilla Luciferase Assays. Promega Notes Magazine. 1996;57(02).

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