Genome-wide identification and expression pattern of SnRK gene family under several hormone treatments and its role in floral scent emission in *Hedychium coronarium*

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ABSTRACT

The SnRK (Snf1-Related protein Kinase) gene family plays crucial roles in various plant signaling pathways and stress-adaptive responses including biotic and abiotic stresses via activating protein phosphorylation pathways. However, there is no information available on the role of the SnRK gene family in *Hedychium coronarium*. *H. coronarium* is an important crop widely cultivated as an ornamental plant, herb, spice, or condiment. In this study, 60 *HcSnRK* genes were identified from the *H. coronarium* genomic and transcriptome data. Phylogenetic and gene structure analysis showed that the *HcSnRK* genes were divided into three groups (*HcSnRK1*, *HcSnRK2* and *HcSnRK3*) and among them *HcSnRK3* subfamily was further subdivided into two clades according to the number of introns. Chromosome localization analysis showed that *HcSnRK* genes were unevenly mapped onto all chromosomes, and the *Ka/Ks* ratio of 24 paralogues includes four tandems and 20 segmental duplications indicated that the *HcSnRK* gene family underwent a purifying selection. *Cis*-regulatory elements analysis suggested that the *HcSnRK* genes respond to multiple hormones and other stresses. The responsiveness of *HcSnRK* genes to several hormones was analyzed by quantitative real-time PCR. Based on the different transcriptome data, two candidates *HcSnRK* genes (*HcSnRK2.2* and *HcSnRK2.9*) were screened out for further characterization. The subcellular localization experiment revealed that both genes were located in the nucleus and cytoplasm. Moreover, virus-induced gene silencing (VIGS) of *HcSnRK2.2* and *HcSnRK2.9* significantly reduced the floral volatile contents by suppressing the expression of terpene synthase genes (*HcTPS1*, *HcTPS3*, and *HcTPS5*), indicating that *HcSnRK2.2* and *HcSnRK2.9* genes play an important role in the regulatory mechanism of floral aroma. These results will provide novel insights into the functional dissection of *H. coronarium SnRK* gene family.

Subjects Agricultural Science, Bioinformatics, Genomics, Molecular Biology, Plant Science

Keywords SnRK, *Hedychium coronarium*, Hormones, Stress responses, Floral scent

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INTRODUCTION

Floral scent is an important plant secondary metabolite that plays key roles in different developmental processes. Floral scent also plays a crucial role in plant communication both above and below-ground and stress signaling (Dudareva et al., 2006; Das et al., 2013; Muhlemann, Klempien & Dudareva, 2014; Abbas et al., 2017). The main role of floral scent is to protect the plant against external stimuli (biotic and abiotic stresses) and attract pollinators (Dudareva et al., 2006; Das et al., 2013). According to biosynthetic pathway, terpenoids, benzenoids/phenylpropanoid, and derivatives of fatty acids are the main classes of volatile organic compounds (VOCs) emitted from the plant surface. Among them, terpenoids constitute the largest class of plant secondary metabolites playing several functions throughout plant life and the expression profile of terpene synthase genes determines the involvement of terpenoid metabolites in different ecological and physiological functions in response to external stimuli. Protein kinases are considered as central components in defense mechanisms, which activate several protein phosphorylation pathways to regulate the expression of downstream genes related to stress response (Tena, Boudsocq & Sheen, 2011). In recent years, protein kinases such as mitogen-activated protein kinase (MAPK) (Xu & Zhang, 2015), calcium-dependent protein kinases (CDPK) (Baba, Rigó & Ayaydin, 2018), glycogen synthase kinase 3 (GSK3) (Beurel, Grieco & Jope, 2015), and sucrose non-fermenting 1 (SNF1) related protein kinases (SnRK) (Hrabak et al., 2003; Wang et al., 2015; Colina et al., 2019; Wang et al., 2019; Zhang et al., 2020) have been extensively studied. Among them, SnRK proteins play essential roles throughout plant life.

SnRK protein kinases contain a similar Ser/Thr kinase domain, conserved UBA, and the KA1 domains in SnRK1. Meanwhile, the osmotic stress-activated domain I was found in SnRK2, and a unique NAF domain in the SnRK3 subfamily (Coello, Hey & Halford, 2011). Furthermore, some SnRK2 protein kinases have an acidic amino acid–base sequence called domain II that can participate in abscisic acid (ABA)mediated responses to abiotic stresses (Yoshida et al., 2006). The SnRK1 subfamily is evolved in early eukaryotes, before the divergence of fungi, animals, and plants (Halford & Hey, 2009). Thus, SnRK1 protein kinases in plants are highly homologous to SNF1 genes in yeast and AMP-activated protein kinases in mammals, which is mainly involved in carbon and nitrogen response metabolism and energy-sensing (Coello, Hey & Halford, 2011). In Arabidopsis thaliana, AKIN10 and IDD8 constitute a sugar metabolic pathway that mediates flowering time under low-sugar conditions (Jeong et al., 2015). Unlike the SnRK1 subfamily, SnRK2 and SnRK3 are unique in plants and are considered to be evolved from the SnRK1 family via gene duplication during plant evolution, playing a key role in the stress, calcium and ABA signaling pathway with epigenetic and metabolic responses (Halford & Hey, 2009). SnRK2 is the most widely studied subfamily and mainly focused on the participation of SnRK2 protein kinases in ABA-dependent and ABA-independent abiotic stress. In A. thaliana, 8/10 AtSnRK2 (Boudsocq, Barbier-Brygoo & Laurière, 2004; Boudsocq et al., 2007) and in Oryza sativa, all 10 stress/ABA-activated Serine/threonine-protein kinase 1 (OsSAPK1 to OsSAPK10) (Kobayashi et al., 2004) can be activated by hyperosmotic and saline stress. Among them, AtSnRK2.2/2.3/2.6 and OsSAPK8/9/10 were strongly activated.
by ABA and act as core positive regulators to regulate ABA-dependent stress responses \citep{Fujita2009}. The SnRK2 subfamily also plays a key role in the regulation of gene expression via activating basic region-leucine zipper (bZIP) transcription factors connected to an epigenetic mechanism that controls the activation or repression of a gene \citep{Baena-González2008}. The SnRK3 subfamily commonly called calcineurin B-like interacting protein kinases (CIPK) with a self-inhibitory NAF domain that can interact with calcineurin B-like (CBL) protein \citep{Cheong2007}. The CBL-CIPK protein complex constitutes a precise calcium signaling system, which plays a vital role in the process of achieving information regarding integration and physiological coordination to resist various stresses in plants \citep{Tang2020, Tripathi2009}. In \textit{Arabidopsis}, \textsc{AtCIPK1} can interact with \textsc{CBL1} to participate in the ABA-independent signaling pathway and interact with \textsc{CBL9} in response to ABA-dependent pathways \citep{D’Angelo2006}. Furthermore, numerous shreds of evidence indicate that the SnRK family is widely involved in almost all hormone signaling pathways. For example, SnRK1 phosphorylates FUSCA3 (FUS3) and MYC transcription factors regulate ABA, ethylene, gibberellin synthesis and jasmonic acid signaling \citep{Gazzarrini2004, Lumba2012, Im2014, Chan2017}. SnRK2.8 phosphorylation NONEXPRESSER OF PATHOGENESIS-RELATED GENES1 (NPR1) respond to the systemic immunity in SA-independent systemic signals \citep{Lee2015}. In short, hormone signals are important for the metabolism of floral aromas and SnRK proteins are deeply involved in the hormone signaling pathway \citep{Mai2011, Ma2018, Ke2019}. However, either SnRK protein kinases respond to plant hormone signals or participates in the regulation of floral fragrance in \textit{H. coronarium} is still unknown.

\textit{H. coronarium} is a perennial herb, commonly known as “White Butterfly Flower” or “Butterfly Ginger”. \textit{H. coronarium} is popular due to its elegant shape and refreshing fragrance of flower and widely cultivated in tropical and subtropical regions \citep{Chen2013, Yue2014}. The blooming of flower results in a strong refreshing scent which is mainly composed of monoterpenes, sesquiterpenes, and some benzenoids \citep{Lan2013, Yue2015, Chen2019, Ke2019}. Some studies also reported that hormone signaling plays an important role in floral scent formation \citep{Schmelz2003, Dudareva2013, Cna'ani2015}. SnRK gene family plays an essential role in plants, however, its function in floral scent formation is completely missing. Our previous research showed that auxin and auxin signaling components can alter the amount of floral volatile compounds \citep{Ke2019}. In the present study, a total of 60 \textsc{Hc SnRK} genes were identified and analyzed in \textit{H. coronarium} genome. The expression patterns of \textsc{HcSnRK} gene family in response to several hormones (ABA, auxin, jasmonic acid and ethylene) and their corresponding hormone inhibitors were measured by qRT-PCR. Furthermore, based on the expression profile, two \textsc{HcSnRK} genes (\textsc{HcSnRK2.2} and \textsc{HcSnRK2.9}) were screened out and their involvement in the metabolism of floral fragrance was demonstrated by virus-induced gene silencing (VIGS). Also, the subcellular localization of these genes was performed. These results will provide the theoretical basis for better understanding the function of \textit{SnRK} genes in hormone signaling and the regulatory metabolism of floral scent formation in \textit{H. coronarium}. 
MATERIALS AND METHODS

Plant growth environment and hormone treatments
The plant materials were grown in a greenhouse under natural light (South China Agricultural University, Guangzhou, China). Plant material was immediately frozen in liquid nitrogen and stored at \(-80^\circ\text{C}\) for RNA isolation and further experimentation.

Plants used for hormone treatment were cut into about 40 cm with a wedge-shape and placed in a 500 mL beaker filled with ultrapure water. The concentration of hormones used for treatments was as followed: 400 \(\mu\text{M}\) for ABA, 100 \(\mu\text{M}\) for nordihydroguaiaretic acid (NDGA), auxin (IAA), methyl jasmonate (MeJA) and acetylsalicylic acid (ASA), 1.5 mM for 2-(4-chlorophenoxy)-isobutyric acid (PCIB), 10 \(\mu\text{L/L}\) for ethylene (ET), and 4 \(\mu\text{L/L}\) for 1-methylcyclopropene (1-MCP).

RNA extraction, cDNA synthesis and qRT-PCR analysis
Total RNA was extracted using the HiPure plant RNA mini kit (Magen, China) according to the manufacturer’s suggestions. One microgram of total RNA was reverse transcribed using PrimeScript\textsuperscript{TM} RT reagent Kit with gDNA Eraser (TaKaRa, Japan) according to the manufacturer’s instructions. The qRT-PCR experiment was executed in an ABI 7500 Fast Real-Time PCR System (Applied Biosystems, Waltham, MA, USA) by using iTaq\textsuperscript{TM} Universal SYBR Green Supermix (BIO-RAD, Hercules, CA, USA) with a 20 \(\mu\text{L}\) sample volume according to the manufacturer’s protocols. The reaction system was as followed: 95 \(^\circ\text{C}\) for 1 min, then 40 cycles of 95 \(^\circ\text{C}\) for 15 s, 55 \(^\circ\text{C}\) for 30 s, and 72 \(^\circ\text{C}\) for 30 s. The relative expression level of each gene was calculated by the standard \(2^{-\Delta\Delta Ct}\) method (Livak & Schmittgen, 2001).

Sequence retrieval and genome-wide identification of \textit{HcSnRK} genes
\textit{Arabidopsis} protein sequences were obtained from the Phytozome database (http://www.phytozome.net/) (Lamesch et al., 2012), genome data of rice was downloaded from the Rice Annotation Project (RAP) (https://rapdb.dna.affrc.go.jp/) (Sakai et al., 2013), and genomic data (Supplementary raw data) of \textit{H. coronarium} was obtained from the Beijing Novogene Bioinformatics Technology Corporation (China).

The local BLASTP search (E-value-5) was performed using the 39 and 48 SnRK protein sequences of \textit{Arabidopsis} and rice according to the Hidden Markov Models profile from the Pfam database (http://pfam.xfam.org/) (Finn et al., 2016). The candidate HcSnRK protein sequences were sent to the NCBI Conserved Domain Database (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) (Marchler-Bauer et al., 2011), SMART database (http://smart.embl-heidelberg.de/) (Letunic, Doerks & Bork, 2012) and Pfam database (Finn et al., 2016) for domain search. Based on the information from the above three databases, we manually select HcSnRK genes with conserved functional domains. Furthermore, molecular weight (MW) and isoelectric point (pI) of confirmed 60 HcSnRK protein sequences was calculated by ExPASy online software (http://www.expasy.ch/tools/pi_tool.html).
Multiple sequence alignment and phylogenetic analysis of HcSnRKs

The ClustalX software (Thompson et al., 1997) was used for multiple sequence alignment of 60 HcSnRK protein and MEGA 7 software (Kumar et al., 2018) was used to construct a phylogenetic tree using the neighbor-joining method (Saitou & Nei, 1987) with 1,000 replicates of bootstrap values. The DNAMAN software was used to show multiple sequence alignment of 60 HcSnRK genes.

Conserved motifs identification and gene structure analysis of HcSnRK

The exon-intron structure of the HcSnRK genes was performed using the Gene Structure Display Server (http://gsds.gao-lab.org/) (Hu et al., 2015) online program. The conserved motifs of HcSnRK protein sequences were identified by Multiple Expectation Maximization for Motif Elicitation (MEME) online software (http://meme-suite.org/tools/meme) (Bailey et al., 2009) with the following parameters: zero or one occurrence per sequence, 20 motifs should MEME find.

Ka and Ks calculation and selection mode analysis

The ratio of non-synonymous substitutions (Ka) and synonymous substitutions (Ks) were used to analyze the selection modes of HcSnRK genes. Protein sequences without stop codon of HcSnRK were aligned by MEGA 7. The Ka and Ks values were calculated by DnaSP v5 software (Librado & Rozas, 2009) with following parameters: assign coding regions, from start to end; assign genetic code, nuclear universal.

Cis-elements analysis HcSnRK genes

The upstream sequences 2000 bp of each HcSnRK gene were submitted to PlantCARE Database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) (Lescot et al., 2002) to predict the function of HcSnRK genes. Six hormone-related cis-elements including ABA-responsive, auxin-responsive, jasmonic acid-responsive, ethylene-responsive, salicylic acid-responsive, and gibberellin-responsive were identified. Four cis-acting elements involved in plant stress responses, such as defense and stress responses, low-temperature responses, drought responses, and wound responses were analyzed.

Subcellular localization of HcSnRK2.2 and HcSnRK2.9

HcSnRK2.2 and HcSnRK2.9 were fused into the vector pEAQ-HT-GFP using the Age I enzyme followed by transformation into Agrobacterium tumefaciens (strain EHA105). The infection solution (OD600 = 0.6) activated by MES solution (10 mM MgCl2, 10 mM MES and 100 µM acetosyringone, pH = 5.6) was injected into the Nicotiana benthamiana leaves as described previously (Ke et al., 2019). Two to three days later the leaves were visualized using Leica DM RXA2 upright fluorescent microscope with 40 × 0.75 numerical aperture objective as explained previously study (Yue, Yu & Fan, 2014; Abbas et al., 2019). The primers are listed in Table S5.

Headspace floral volatiles analysis

The whole flower was placed in a closed 250 mL glass bottle supplemented with an internal standard. Polydimethylsiloxane (PDMS) fiber was inserted into the bottle for 15 min.
to adsorb volatiles for 15 min followed by insertion into the gas chromatography-mass spectrometry (GC-MS) system as explained previously (Ke et al., 2019; Yue, Yu & Fan, 2014).

**Virus-induced gene silencing (VIGS)**
The barley stripe mosaic virus (BSMV) system was successfully applied in *H. coronarium*. The BSMV-VIGS system which consists of pCaBS-α, pCaBS-β, and pCaBS-γ vectors was kindly provided by Professor Dawei Li (China Agricultural University). Linearization of pCaBSγ vector using *Apa* I endonuclease and about 300 bp specific base sequence of *HcSnRK2.2* and *HcSnRK2.9* were fused to the vector according to the protocol system (Yuan et al., 2011) and optimized by extending the connection time to 2 min. The solution contains a mixture of an equal proportion of pCaBS-α, pCaBS-β, and pCaBS-γ/*HcSnRK2.2*/HcSnRK2.9 with a final OD<sub>600</sub> of 0.5 to 0.6. The flowers at Stage S1 were dipped into the solution followed by vacuum infiltration at -0.8 atmosphere standard for 10 min. After vacuum infiltration, the flowers were immediately washed with sterilized water and were placed in an incubator with following conditions: 12 h day/night period at 16 °C for 5 days (Ke et al., 2019). The floral volatile contents were measured as described above, and the experiment was repeated three to four times.

**RESULTS**

**Identification of *HcSnRK* gene family in *H. coronarium***
Base on the BLAST and Hidden Markov Model search, a total of 60 candidate genes were identified in *H. coronarium* genome. According to subfamily and chromosomal localization of genes, *HcSnRK* genes were named *HcSnRK1.1 ~ HcSnRK1.4, HcSnRK2.1 ~ HcSnRK2.13,* and *HcSnRK3.1 ~ HcSnRK3.43,* respectively. The physical parameters of these genes are summarized in Table 1. The amino acid (aa) length ranged from 326 aa (*HcSnRK2.8*) to 526 aa (*HcSnRK3.14*), and the length of *HcSnRK2* subfamily is shorter than the other two subfamilies. The average length of *HcSnRK1, HcSnRK2,* and *HcSnRK3* subfamily were 500, 352, and 451 aa, respectively. Meanwhile, protein molecular weight varies greatly from 36.87 kDa (*HcSnRK2.8*) to 58.80 kDa (*HcSnRK3.14*), and the isoelectric point from 4.81 (*HcSnRK2.11*) to 9.48 (*HcSnRK3.12*).

**Phylogeny and multiple sequence alignment of *HcSnRK* gene family***
The evolutionary relationships of the *SnRK* genes in *H. coronarium, A. thaliana,* and *O. sativa* was revealed by constructing the phylogenetic tree based on multiple sequence alignment of amino acids. The full-length protein sequence of 39 AtSnRK, 48 OsSnRK, and 60 *HcSnRK* genes were used to construct a phylogenetic tree using MEGA 7 and by choosing the neighbor-joining method (Fig. 1). The results showed that 60 *HcSnRK* genes were divided into three groups as expected. Alike *Arabidopsis* and rice, the member of *HcSnRK3* family were the highest (43) followed by *HcSnRK2* (13) and *HcSnRK1* (4), respectively.

To explore the gene structure of the *HcSnRK* gene family, multiple sequence alignment was performed using DNAMAN 8.0 software. As shown in Fig. 2, ATP binding site and
Table 1  The characteristics of the HcSnRK gene family in *H. coronarium*.

| Gene Name | Gene ID | Position | CDS (bp) | Amino Acids | Exons | pI | MW (kDa) |
|-----------|---------|----------|----------|-------------|-------|----|----------|
| HcSnRK1.1 | Hc42.68 | Chr6:60322670-60328745(−) | 1,497 | 498 | 11 | 8.43 | 56.70 |
| HcSnRK1.2 | Hc154.85 | Chr14:13692396-13697801(−) | 1,533 | 510 | 12 | 8.71 | 58.24 |
| HcSnRK1.3 | Hc25.126 | Chr0:1124760-1129850(−) | 1,497 | 498 | 11 | 8.63 | 56.16 |
| HcSnRK1.4 | Hc1223.4 | Chr0:18406-24187(+) | 1,482 | 493 | 12 | 7.08 | 40.51 |
| HcSnRK2.1 | Hc479.26 | Chr5:3118456-3123025(+) | 1,092 | 363 | 9 | 4.86 | 41.10 |
| HcSnRK2.2 | Hc163.43 | Chr5:35295876-35298554(+) | 1,098 | 365 | 9 | 5.48 | 41.76 |
| HcSnRK2.3 | Hc39.21 | Chr5:45454971-45460547(+) | 1,098 | 365 | 9 | 4.95 | 41.27 |
| HcSnRK2.4 | Hc782.12 | Chr8:7883604-7886707(−) | 1,125 | 374 | 10 | 6.00 | 43.00 |
| HcSnRK2.5 | Hc54.19 | Chr8:40661379-40668342(−) | 1,002 | 333 | 9 | 5.35 | 38.48 |
| HcSnRK2.6 | Hc843.18 | Chr11:43521897-43523363(+) | 1,020 | 339 | 9 | 5.67 | 38.47 |
| HcSnRK2.7 | Hc275.28 | Chr13:2181732-2184478(+) | 1,032 | 343 | 10 | 5.35 | 38.73 |
| HcSnRK2.8 | Hc326.23 | Chr14:223114-225278(−) | 981 | 326 | 9 | 5.26 | 36.87 |
| HcSnRK2.9 | Hc326.60 | Chr14:473891-477474(+) | 1,020 | 339 | 9 | 5.43 | 38.51 |
| HcSnRK2.10 | Hc57.125 | Chr1:53838578-53842095(+) | 1,377 | 458 | 12 | 8.58 | 51.54 |
| HcSnRK2.11 | Hc25.205 | Chr0:1124788-11478680(+2) | 1,323 | 440 | 2 | 8.37 | 49.78 |
| HcSnRK2.12 | Hc115.24 | Chr3:6979416-6980852(−) | 1,437 | 478 | 1 | 9.06 | 53.69 |
| HcSnRK2.13 | Hc171.23 | Chr3:1192883-11930468(+) | 1,236 | 411 | 1 | 9.34 | 44.60 |
| HcSnRK3.1 | Hc438.56 | Chr1:11474898-11476860(+2) | 1,323 | 440 | 2 | 8.37 | 49.78 |
| HcSnRK3.2 | Hc438.54 | Chr1:11605358-11605298(+) | 1,314 | 437 | 1 | 8.96 | 48.32 |
| HcSnRK3.3 | Hc108.24 | Chr1:28981635-28984540(+) | 1,317 | 438 | 2 | 9.32 | 50.06 |
| HcSnRK3.4 | Hc253.154 | Chr1:53838578-53842095(+) | 1,377 | 458 | 12 | 8.58 | 51.54 |
| HcSnRK3.5 | Hc504.12 | Chr2:35890951-35893118(+) | 1,545 | 514 | 3 | 8.64 | 57.18 |
| HcSnRK3.6 | Hc219.55 | Chr2:61929342-61931157(+2) | 1,338 | 445 | 1 | 7.31 | 49.19 |
| HcSnRK3.7 | Hc115.24 | Chr3:6979416-6980852(−) | 1,437 | 478 | 1 | 9.06 | 53.69 |
| HcSnRK3.8 | Hc171.23 | Chr3:1192883-11930468(+) | 1,236 | 411 | 1 | 9.34 | 44.60 |
| HcSnRK3.9 | Hc256.98 | Chr4:8831260-8833000(−2) | 1,305 | 434 | 1 | 9.48 | 48.76 |
| HcSnRK3.10 | Hc280.50 | Chr4:44679903-44683582(+2) | 1,332 | 443 | 3 | 8.75 | 50.59 |
| HcSnRK3.11 | Hc484.68 | Chr5:184915-186806(−) | 1,581 | 526 | 1 | 9.03 | 58.80 |
| HcSnRK3.12 | Hc484.67 | Chr5:189058-191912(−) | 1,401 | 466 | 3 | 8.45 | 52.34 |
| HcSnRK3.13 | Hc316.81 | Chr5:44515665-44521645(−) | 1,305 | 434 | 13 | 6.41 | 48.87 |
| HcSnRK3.14 | Hc32.31 | Chr6:8904624-8906421(+) | 1,356 | 451 | 1 | 8.80 | 50.82 |
| HcSnRK3.15 | Hc32.36 | Chr6:51928707-51942543(+) | 1,326 | 441 | 14 | 8.67 | 50.28 |
| HcSnRK3.16 | Hc3.374 | Chr7:3703792-3708273(−) | 1,365 | 454 | 14 | 5.66 | 51.08 |
| HcSnRK3.17 | Hc971.16 | Chr7:76394897-7645951(+) | 1,446 | 481 | 13 | 7.87 | 55.94 |
| HcSnRK3.18 | Hc33.46 | Chr7:8729879-8733633(−) | 1,311 | 436 | 2 | 9.06 | 49.17 |
| HcSnRK3.19 | Hc247.6 | Chr8:47314082-47316537(−) | 1,353 | 450 | 3 | 8.68 | 49.12 |
| HcSnRK3.20 | Hc102.99 | Chr9:1148877-1153212(+) | 1,323 | 440 | 16 | 6.82 | 49.96 |
| HcSnRK3.21 | Hc369.81 | Chr9:2688438-2692404(+) | 1,389 | 462 | 13 | 5.95 | 51.98 |

(continued on next page)
Table 1 (continued)

| Gene Name     | Gene ID | Position         | CDS (bp) | Amino Acids | Exons | pI     | MW (kDa) |
|---------------|---------|------------------|----------|-------------|-------|--------|----------|
| HcSnRK3.25    | Hc71.72 | Chr10:906032-912303(−) | 1,134    | 377         | 14    | 5.78   | 42.95    |
| HcSnRK3.26    | Hc48.119| Chr11:9100841-9192641(−) | 1,317    | 438         | 10    | 6.65   | 49.63    |
| HcSnRK3.27    | Hc439.14| Chr11:1652738-16532095(−) | 1,386    | 461         | 15    | 8.70   | 52.46    |
| HcSnRK3.28    | Hc286.62| Chr11:46635686-46645148(−) | 1,470    | 489         | 15    | 9.31   | 55.72    |
| HcSnRK3.29    | Hc158.15| Chr12:1277125-1278952(+) | 1,449    | 482         | 2     | 6.15   | 53.97    |
| HcSnRK3.30    | Hc5.4   | Chr12:34636145-34637497(+) | 1,353    | 450         | 1     | 9.11   | 50.94    |
| HcSnRK3.31    | Hc79.41 | Chr12:43896038-43897396(+) | 1,359    | 452         | 1     | 6.74   | 50.06    |
| HcSnRK3.32    | Hc114.20| Chr14:2279142-2284718(−) | 1,323    | 440         | 2     | 8.72   | 49.65    |
| HcSnRK3.33    | Hc279.1 | Chr14:16032098-16047415(−) | 1,332    | 443         | 8     | 8.75   | 50.19    |
| HcSnRK3.34    | Hc132.10| Chr15:33199307-33201621(+) | 1,476    | 491         | 10    | 8.11   | 54.23    |
| HcSnRK3.35    | Hc74.133| Chr16:2385971-2387422(−) | 1,452    | 483         | 1     | 6.11   | 54.51    |
| HcSnRK3.36    | Hc74.7  | Chr16:36843545-36847614(+) | 1,335    | 444         | 2     | 9.01   | 50.38    |
| HcSnRK3.37    | Hc26.164| Chr17:1572756-1575773(−) | 1,308    | 435         | 2     | 9.16   | 49.33    |
| HcSnRK3.38    | Hc259.23| Chr17:6843287-6866867(−) | 1,335    | 444         | 16    | 8.70   | 49.91    |
| HcSnRK3.39    | Hc259.98| Chr17:7354852-7357698(+) | 1,392    | 463         | 13    | 7.22   | 51.51    |
| HcSnRK3.40    | Hc15.312| Chr20:2384884-2386204(−) | 1,215    | 404         | 2     | 9.37   | 44.25    |
| HcSnRK3.41    | Hc15.375| Chr29:2948868-2950520(+) | 1,446    | 481         | 1     | 8.88   | 53.95    |
| HcSnRK3.42    | Hc414.48| Chr30:563909-565482(+)   | 1,308    | 435         | 1     | 8.81   | 47.59    |
| HcSnRK3.43    | Hc444.50| Chr30:650222-651310(+)   | 1,089    | 362         | 1     | 7.01   | 40.66    |

The combined phylogenetic tree and web server Gene Structure Display Server (GSDS) analysis was performed to determine the intron/exon structure of HcSnRK genes (Fig. 3C). The data showed that the members of the same subfamily share similar features. The HcSnRK1 subfamily genes have 11 to 12 introns, while the HcSnRK2 subfamily contains 9 to 10 introns. However, the number of introns in the HcSnRK3 subfamily varies. The 27 HcSnRK3 genes contained less than 3 introns, and the 16 HcSnRK3 genes contained 8 to
Figure 1  Phylogenetic tree of SnRK genes from *H. coronarium*, Arabidopsis, and rice. Sixty *HcSnRK* genes, 39 *AtSnRK* genes, and 48 *OsSnRK* genes are clustered as SnRK1, SnRK2, and SnRK3 subgroups. The detailed information of these genes from different plants are provided in Table S4. Clustal X software was used for multiple sequence alignment and MEGA 7 software used to construct a phylogenetic tree by the neighbor-joining method.

Wang et al. (2021), *PeerJ*, DOI 10.7717/peerj.10883/fig-1

13 introns. Therefore, *HcSnRK3* genes can be divided into two subgroups, intron-rich and intron-poor subgroups, respectively, based on the number of introns. Previously, similar intron numbers and classification pattern was observed in both monocots and dicots species, such as *Arabidopsis*, rice, maize, poplar, etc. The characteristic of intron number indicates that the evolution of SnRK genes was conserved in plants.

**Chromosomal location and gene duplication analysis**

The chromosomal localization analysis showed that 60 *HcSnRK* genes were unevenly mapped onto all 17 chromosomes, including four *HcSnRK1* genes, 13 *HcSnRK2* genes and 43 *HcSnRK3* (Fig. 4), whilst nine *HcSnRK* genes were localized on an unknown chromosome which will, later on, be assigned on anyone among 17 via refining whole-genome sequencing. Six *HcSnRK* genes were located on chromosome 5, and 14, while chromosome 1 has five *HcSnRK* genes distribution. However, chromosomes 10, 13, and 15 only contain one *HcSnRK* gene. In short, all the *HcSnRK* genes were randomly distributed on all chromosomes in *H. coronarium* genome.
To identify the segmental duplication of *HcSnRK* genes, BLAST and MCScanX methods were used. Among 60 *HcSnRK* genes, 10 duplicated pairs derived from segmental duplication and 2 from tandem duplication were observed (Fig. 4). The majority of
the duplicated gene pairs were found on chromosome 11 and 14. To demonstrate and reveal the effect of selection pressure on the evolution of *HcSnRK* genes, synonymous (*K*$_s$), non-synonymous substitutions (*K*$_a$), and *K*$_a$/*K*$_s$ ratios per site between every duplicated pair were calculated by DnaSP 5.0 software. More importantly, *K*$_a$/*K*$_s$ = 1 indicates neutral selection, *K*$_a$/*K*$_s$<1 represents purifying selection, and *K*$_a$/*K*$_s$> 1 represents positive selection and accelerated evolution. The *K*$_a$/*K*$_s$ ratio of 12 duplicated gene pairs ranged from 0.095 to 0.465 (Table S2), suggesting that all duplicated gene pairs of *HcSnRK* had undergone purifying selection.

**Cis-regulatory elements analysis**

The upstream 2 kb of promoter sequences of 60 *HcSnRK* genes were submitted to the PlantCARE database, to analyze the function and regulatory mechanism of *HcSnRK* genes. The results showed that almost all *HcSnRK* genes contained hormone-responsive cis-elements; however, less than half of *HcSnRK* genes contained abiotic stresses cis-elements. Interestingly, 53 out of 60 *HcSnRK* promoters contained ABRE cis-elements, while 50 and 44 out of 60 *HcSnRK* promoters contained MeJA-responsive and ethylene responsive cis-acting regulatory elements, respectively. Meanwhile, about half of the promoters of the *HcSnRK* gene contained auxin, salicylic acid, and gibberellin responsive cis-elements. On the other hand, less than half of the promoters of *HcSnRK* genes contained low-temperature and drought-inducible cis-elements, and about one-third of promoters contained defense, stresses, and wound-responsive element (Fig. 5; Table S3). Cis-regulatory element analysis data suggested that *HcSnRK* genes might respond to multiple hormones and abiotic stresses.
Expression pattern of *HcSnRK* genes in different varieties, tissues and flower developmental stages

The analysis of differential expression pattern of *HcSnRK* genes was performed by using the transcriptome data of three different tissue (leaf, rhizome, and flower) and three flower development stages (D1; bud stage, D4; full-bloom and D6; flower senescence stage) of *H. coronarium* and three different varieties of *Hedychium* (*Yue, Yu & Fan, 2015*). The volatile compounds among different varieties vary significantly. The GC-MS analysis showed that the emission of volatile compounds from *H. coronarium* were higher as compared to *H. 'Jin'*, while volatile compounds in *H. coccineum* are very low (*Fan et al., 2007*). The expression level of HcSnRK genes is presented in a heat map and HcSnRK genes with similar expression patterns were grouped into distinct groups. Cluster I represent the group of HcSnRK genes which had the highest expression in *H. coccineum*, Cluster II in *H. 'Jin'* and cluster III had preferential expression *H. coronarium*, respectively (Fig. 6A). Previous studies showed that the number of volatile contents were higher in the flowers compared to leaf and rhizome. Moreover, the amount of floral volatiles was low at the D1 stage and peak at the full-bloom stage (D4) with flower development and declined at the D6 stage (*Ke et al., 2019; Abbas et al., 2021*). Similarly, expression of *HcSnRK* genes in different tissues and different flower development stages of *H. coronarium* were also...
grouped in three clusters. The tissue-specific expression is important for gene functioning. Cluster I showed preferential expression in leaf, Cluster II represents the group of genes that had the highest expression in flower, and cluster III represents specific expression in the rhizome, respectively (Fig. 6B). Based on different transcriptome data and preferential expression of cluster III in *H. coronarium*, flower-specific expression and highest expression at full-bloom stage of flower indicate its potential role in the flower development and in the regulation of floral scent formation processes (Fig. 6C). Based on transcriptome data, *HcSnRK2.2* and *HcSnRK2.9* were screened out for further experimental analysis.

**Expression patterns of HcSnRK genes in response to hormone treatments**

Auxin, ethylene, and ABA are the major hormones involved in the development of the flower. *H. coronarium* flowers were subjected to various hormone treatments. The results revealed that the volatile compounds of *H. coronarium* flowers were increased by 16%, 21%, 20%, and 22% under ABA, IAA, ethylene, and methyl jasmonate treatment, respectively. Meanwhile, the emission of volatile compounds decreased by 30%, 35%, 52%, and 34% under their corresponding hormone inhibitors nordihydroguaiaretic acid (NDGA), 2-(4-chlorophenoxy)-isobutyric acid (PCIB), 1-methyl cyclopropane (1-MCP) and acetylsalicylic acid (ASA), respectively (*Ke et al., 2019*). The expression level of selected *HcSnRK* genes was measured by qRT-PCR under hormone treatments (Fig. 7). The data showed that the expression level of *HcSnRK2.2* and *HcSnRK2.9* significantly
Figure 6  Expression Patterns of HcSnRK genes from different transcriptome data. (A) Heat map of HcSnRK genes expression pattern in three different varieties. (B) Expression patterns of HcSnRK genes in different tissues. (C) Expression profiles of HcSnRK genes in different flower developmental stages. Levels of upregulated expression (red) and downregulated expression (yellow) are shown on a log2 scale from the highest to lowest expression.

up-regulated by ABA treatment, while down-regulated by NGDA (Fig. 7A). Overall, 24 HcSnRK genes were significantly up or downregulated under ABA treatment and 25 genes significantly changed under NDGA treatment. Under IAA and PCIB treatments, 24 HcSnRK genes showed significant difference in their expression pattern (Fig. 7B). Notably, HcSnRK2.2, 2.4, and 2.9 genes significantly increased in IAA treatment and decreased in PCIB treatment. Furthermore, 21 HcSnRK genes significantly up or down-regulated after ethylene treatment, and 24 genes significantly changed after 1-MCP treatment (Fig. 7C). Moreover, HcSnRK2.2 and 2.9 were highly up-regulated under ethylene treatment and down-regulated after 1-MCP treatment. In addition, 24 HcSnRK genes significantly increase or decrease in methyl jasmonate treatment, and 28 genes significantly changed in acetylsalicylic acid treatment (Fig. 7D). As expected HcSnRK2.2, 2.6, and 2.9 genes significantly increased in methyl jasmonate treatment and decreased in acetylsalicylic acid treatment. In particular, the expression level of the HcSnRK2.9 gene increased by 4 times under MeJA treatment. In short, plant hormones, such as ABA, IAA, ethylene, and jasmonic acid have a crucial effect on the regulation of floral aroma and the response pattern of HcSnRK2.2 and 2.9 genes towards hormones were consistent with the changes of floral aroma contents. The results indicate that HcSnRK2.2 and 2.9 genes play an important role in regulating the metabolism of floral aroma substances via crosstalk in hormone signaling.
Figure 7  *HcSnRK* genes respond to several hormone treatments. The relative expression levels of *HcSnRK* genes in response to ABA (A), IAA (B), ET (C), MeJA (D), and corresponding inhibitor NDGA, PCIB, 1-MCP, ASS was analyzed by qRT-PCR. The expression level of the control group was set to 1, error bars represent standard deviation from three to four biological replicates. Significant differences between the control group and hormone treatment samples are indicated by an asterisk (*p* < 0.05).

Subcellular localization of *HcSnRK2.2* and *HcSnRK2.9*

The amino acid sequence of *HcSnRK2.2*/2.9 was submitted to WoLP PSORT (https://wolfpsort.hgc.jp/) to predict subcellular localization. The predicted results showed that all *HcSnRK2.2*/2.9 proteins were expressed in the nucleus and cytoplasm. To experimentally verify the subcellular localization, full-length sequences of candidate *HcSnRK2.2* and *HcSnRK2.9* were fused to a GFP reporter gene and transferred to *N. benthamiana* leaves (Fig. 8). Subcellular localization experiments results revealed that both *HcSnRK2.2* and *HcSnRK2.9* were localized in the cell nucleus and cytoplasm as predicted. Similarly, multiple SnRK proteins have been reported previously from *Arabidopsis* and rice, which were in the nucleus and cytoplasm and were involved in the regulation of several ABA responses.

Silencing of *HcSnRK2.2* and *HcSnRK2.9* genes

To verify the function of *HcSnRK2.2* and *2.9* in the regulation of floral volatile synthesis, the virus-induced gene silencing (VIGS) system was used to suppress gene expression in *H.
Figure 8  Subcellular localization of HcSnRK2.2 and HcSnRK2.9 proteins. HcSnRK2.2-GFP and HcSnRK2.9-GFP fusion vectors were transformed into N. benthamiana leaves and subcellular localization was carried out 48 h after infiltration using Leica DM RXA2 upright fluorescent microscope. The bar indicates 20 µm.

As shown in Fig. 9, the expression of HcSnRK2.2 and HcSnRK2.9 genes were significantly reduced by 66% and 58% compared to control, after silencing HcSnRK2.2 and 2.9, respectively. Moreover, the content of the main floral volatile substance, such as eucalyptol, ocimene, and linalool decreased significantly by 51%, 54%, and 48% after silencing HcSnRK2.2 gene, and decreased by 27%, 28%, and 47% after silencing HcSnRK2.9, respectively. Furthermore, the expression levels of main volatile synthesis genes significantly decreased, such as HcTPS1 which is responsible for eucalyptol synthesis, HcTPS3 for ocimene, and HcTPS5 for linalool synthesis. HcTPS1, HcTPS3 and HcTPS5 were down-regulated 68%, 66% and 51% after the suppression of HcSnRK2.2, and down-regulated 48%, 56% and 42% after silencing HcSnRK2.9. These findings indicate that HcSnRK2.2 and HcSnRK2.9 play an important key role in the regulation of floral aroma synthesis.

DISCUSSION

The SnRK gene family plays a crucial role in different physiological processes and is conserved in all eukaryotes. The SnRK1 is involved in the functioning of cell energy sensing, while SnRK2 and SnRK3 play fundamental roles in the signaling pathway and the regulation of gene expression (Halford et al., 2004; Li et al., 2010; Colina et al., 2019). The SnRK2 and SnRK3 subfamily are unique in plants and originate from the duplication of the SnRK1 subfamily (Colina et al., 2019; Halford & Hey, 2009). The expansion of the SnRK family may be partly since plants are sessile organisms and are forced to face more biotic and abiotic stresses than animals (Colina et al., 2019). In plants, the SnRK family represents an interface between stress signaling and metabolic pathway and is widely
The silencing of HcSnRK2.2 and HcSnRK2.9 genes reduces the amount of the volatile compounds in H. coronarium flowers. (A) The relative expression level of HcSnRK2.2 and HcSnRK2.9 genes after BSMV-HcSnRK2.2/2.9 silencing were analyzed by qRT-PCR. (B) The main volatile compounds measured by GC-MS after silencing of HcSnRK2.2 and HcSnRK2.9 genes. (C) The relative expression level of key volatile biosynthesis genes. Error bars represent standard deviations from three to four biological replicates and asterisks indicate statistically significant differences (p < 0.05).

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involved in ABA-dependent and ABA-independent abiotic stress. The SnRK family has been reported from a wide range of plant species, including Arabidopsis, maize (Chen et al., 2011), rice (Kanwar et al., 2014), and cotton (Cui et al., 2020), however, the SnRK family from H. coronarium has not been studied.

In the current study, 60 HcSnRK genes including 4 HcSnRK1, 13 HcSnRK2, and 43 HcSnRK3 in H. coronarium were identified. Previously, 34, 48, 39, 44, and 52 SnRK genes have been identified from Eucalyptus grandis (Wang et al., 2019), Oryza sativa (Kobayashi et al., 2004), A. thaliana (Hrabak et al., 2003), Brachypodium distachyon (Wang et al., 2015) and Glycine max (Zhu et al., 2016), respectively. Moreover, different SnRK gene subfamily encompasses various conserved domains, however, all genes included a protein kinase domain present at the N-terminal. Phylogenetic analyses showed that like Arabidopsis and rice, H. coronarium also contain a similar number of SnRK1 subfamily members (3 to 4) and SnRK2 subfamily (10 or 13). However, the number of SnRK3 subfamily genes varies from species to species, such as 26 in A. thaliana, 34 in O. sativa, 24 in E. grandis, 52 in G. max, and 43 in H. coronarium.

The different number of exon-intron also plays an important role in the evolution and function of a different gene family (Jo & Choi, 2015). The SnRK3 subfamily not only varies on the number of genes, but previous findings also showed that the SnRK3 subfamily can be subdivided into two clades according to the number of introns (Tang et al., 2016; Zhu et al., 2016). Likewise, the HcSnRK3 subfamily can be subdivided into an intron-rich and intron-poor clade. The 16 HcSnRK3 genes were grouped into an intron-rich clade (more than 8 introns) and 27 HcSnRK3 genes in the intron-poor clade (less than 3 introns). Similarly, in Arabidopsis and rice, the SnRK3 subfamily was subdivided according to the number of introns indicating that an increase or decrease in the number of introns can promote the structural evolution of the SnRK3 gene family before eudicot–monocot divergence (Zhu et al., 2016). Recent findings suggest that the SnRK3 subfamily originated in green algae, and the intron-poor group first appeared in the seed plants (Colina et al., 2019). It has been assumed that when seed plants will face great environmental pressure...
during evolution, intron-rich groups will lose intron and become intron-poor groups (Colina et al., 2019). Also, HcSnRK1 has 11 to 12 introns, HcSnRK2 subfamily has 9 or 10 introns. These results indicated that the number of introns in HcSnRK genes is similar to other plants. The conserved motif analysis revealed that gene structure and conserved motifs were similar in the same subfamily, indicating the close evolutionary relationship within the same subfamily, but different subfamilies involved in different stress response pathways.

A large number of cis-elements related to hormone response were found in the promoter sequences of HcSnRK genes, suggesting that HcSnRK genes respond to multiple hormone signals and interact with other metabolic pathways. Plants have developed unique strategies to cope with the external environment. Numerous evidences indicated that the SnRK family is widely involved in the response to various biotic and abiotic stresses, including salt, high or low temperature, and drought (Tang et al., 2016; Zhu et al., 2016; Wang et al., 2019). Many cis-elements related to stresses, wounding and defense response were identified in the promoter sequences of HcSnRK genes. Previous findings indicate that hormones play essential roles in the flower development and regulation of aroma (Chandler, 2011; Iqbal et al., 2017; Ke et al., 2019). The ethylene, Auxin, ABA, and MeJA responsive cis-elements were found in the majority of the promoters of HcSnRK genes suggesting their significant functions by crosstalk with HcSnRK genes in H. coronarium flower. The above results are in line with the previous findings from tomato and H. coronarium (Audran-Delalande et al., 2012; Ke et al., 2019). In our previous research, we describe that Auxin/IAA genes are involved in the regulation of floral scent and the volatile contents of H. coronarium flower were altered under different hormone treatments (Ke et al., 2019). Moreover, ethylene and ABA are also involved in floral scent regulation and flower senescence. To verify the response of the HcSnRK genes to several hormones, the expression levels of 29 genes based on their higher abundance in flower, was performed by qRT-PCR. The results showed that 27 HcSnRK genes significantly respond to ABA treatment, while 28, 24 and 28 HcSnRK genes significantly responded to IAA, ethylene, MeJA, and their corresponding inhibitors, respectively. Similarly, HbSnRK2.5, 2.7, and 2.10 from Hevea brasiliensis were also significantly up-regulated under ABA, ethylene, and MeJA treatment. Alike, HbSnRK2.8, 2.9 up-regulated under ABA and MeJA treatment, whilst, HbSnRK2.2 down-regulated under ABA and MeJA treatment, however, HbSnRK2.6 significantly up-regulated under MeJA, down-regulated under ethylene and do not respond to ABA treatment. In the present study, HcSnRK2.4 significantly up-regulated under IAA and down-regulated under PCIB, while HcSnRK2.6 up-regulated under MeJA and down-regulated under ASA treatment. Furthermore, HcSnRK2.2 and HcSnRK2.9 showed significant differential expression under ABA, IAA, ethylene, MeJA, and their corresponding inhibitor treatments. Previous studies verified that ABA, IAA, ethylene, and MeJA have a significant effect on the regulation of floral aroma. These results implied that HcSnRK2.2 and HcSnRK2.9 maybe involved in multiple hormone metabolism pathways to regulate the metabolism of floral fragrance.

Tissue-specific expression pattern of HcSnRK genes in different tissue permits different regulation of tissue development and alternate means of metabolic regulation. The
transcriptome data of different varieties (strong, moderate, and almost no floral fragrance variety), different tissues (rhizome, leaf, and flower), and different flower development stages (bud stage, full-bloom stage, and fade stage) were used to analyze the expression pattern of HcSnRK genes. The 23 HcSnRK genes showed high expression in strong floral fragrance variety, 22 HcSnRK genes were highly expressed in the flower, whereas, 23 HcSnRK genes showed their preferential expression at the full-bloom stage of the flower. It was also observed that 15 HcSnRK genes have extremely high expression during the senescence stage indicating their possible role in the regulation of flower aging. Moreover, the expression pattern of HcSnRK2.2 and HcSnRK2.9 was similar to the emission of floral substances, suggesting that these two genes might play an important role in the regulation of floral aroma synthesis. The differential expression pattern of SnRK genes has been found in many species. In apple, MdCIPK4, 9, 15, and 32 were highly expressed in the flower and MdCIPK 29 show relatively high expression in fruit implied their different biological functions in respective tissues (Niu et al., 2018). In Brassica napus L., BnCIPK9 was tissue-specific and developmental stage-specific expressed in seed, and overexpression of BnCIPK9 reduced oil synthesis in the transgenic plant (Guo et al., 2018). The virus-induced gene silencing of HcSnRK2.2 and HcSnRK2.9 confirm their role in floral scent regulation. The silencing of HcSnRK2.2 and HcSnRK2.9 genes did not alter the flowering process, however, resulted in the emission of the low amount of floral volatile and decreases the expression pattern of key genes involved in the biosynthesis of floral scent (Fig. 9). The decrease in the emission of volatile compounds might be because of their involvement in the hormone signaling especially in ABA and ethylene signaling pathway. This is the first report regarding the role of HcSnRK genes in the regulation of the floral scent biosynthetic pathway. The function of SnRK family in ABA-dependent and independent pathway have been extensively studied, however, their role in floral scent pathway needs to be elucidated further.

**CONCLUSION**

In brief, we identified the SnRK gene family in Hedychium coronarium; analyzed expression profiles based on three different transcriptome data, and screened numerous key candidate genes for functional characterization. Through virus-induced gene silencing, we find out the functional involvement of HcSnRK2.2 and HcSnRK2.9 in floral scent formation. Our findings will bring new insights into the function of HcSnRK genes in secondary metabolism

**Abbreviations**

| Abbreviation | Description                        |
|--------------|------------------------------------|
| SnRK         | Snf1-Related protein kinase         |
| VIGS         | Virus-induced gene silencing       |
| qRT-PCR      | Quantitative real-time polymerase chain reaction |
| MAPK         | Mitogen-activated protein kinase    |
| CDPK         | Calcium-dependent protein kinases   |
| GSK3         | Glycogen synthase kinase 3          |
| SNF1         | Sucrose non-fermenting 1            |

**Wang et al. (2021), PeerJ, DOI 10.7717/peerj.10883**
| Abbreviation | Description |
|--------------|-------------|
| ABA          | Abscisic acid |
| SAPK         | Stress/ABA-activated protein kinase |
| bZIP         | Basic region-leucine zipper |
| CIPK         | Calcineurin B-like interacting protein kinases |
| NDGA         | Nordihydroguaiaretic acid |
| IAA          | Indole-3-Acetic Acid |
| PCIB         | 2-(4-chlorophenoxy)-isobutyric acid |
| ET           | Ethylene |
| 1-MCP        | 1-Methylcyclopropene |
| MeJA         | Methyl jasmonate |
| ASA          | Acetylsalicylic acid |
| GSDS         | Gene Structure Display Server |
| MEME         | Multiple Expectation Maximization for Motif Elicitation |
| Ka           | Non-synonymous substitutions |
| Ks           | Synonymous substitutions |
| PDMS         | Polymethylsiloxane |
| GC-MS        | Gas chromatography-mass spectrometry |
| BSMV         | Barley stripe mosaic virus |
| GFP          | Green fluorescence protein |

## ADDITIONAL INFORMATION AND DECLARATIONS

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### Competing Interests

The authors declare there are no competing interests.
Author Contributions

• Chutian Wang conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
• Farhat Abbas performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
• Yiwei Zhou analyzed the data, prepared figures and/or tables, and approved the final draft.
• Yanguo Ke performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
• Xinyue Li, Yuechong Yue and Yunyi Yu performed the experiments, prepared figures and/or tables, and approved the final draft.
• Rangcai Yu and Yanping Fan conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

Data Availability
The following information was supplied regarding data availability:

Raw data are available in the Supplemental Files.
Sequences available at GenBank: MW393703–MW393762.

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