Dynamics of Toona ciliata transcriptome in response to a polyphagous herbivore, Hypsipyla robusta Moore

Huiyun Song
South China Agricultural University
Yue Li
South China Agricultural University
Zhi Wang
South China Agricultural University
Zhihao Duan
South China Agricultural University
Yueyang Wang
South China Agricultural University
Endian Yang
South China Agricultural University
Wenmai Mao
South China Agricultural University
Qingmin Que
South China Agricultural University
Xiaoyang Chen
South China Agricultural University
Pei Li (lipei-meinv@163.com)
South China Agricultural University

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Abstract

Background

Toona ciliata is a traditional woody plant that can be used as a medicinal material in China. The extracts of roots, stems, leaves, and flowers all have a wide range of bioactivities. However, *T. ciliata* has been facing an unresolved pest problem caused by *Hypsipyla robusta* Moore (HRM), which seriously affects its growth and development.

Results

In this study, transcriptome sequencing of young stems was eaten by HRM for 0, 3, 12, and 21h were performed. A large number of differentially expressed genes (DGEs) were identified including jointly up-regulated genes (263) and down-regulated genes (378). JA synthesis and signaling transduction, terpene biosynthesis, and MAPKs signaling pathway were analyzed in depth and found that some key genes *TcOPR3, TcJAR1, TcJAZs*, and *TcTPS9* possessed anti-insect potential. Moreover, MYBs and ERFs transcription factor family were significantly strengthened that may participate in induced defense mechanism in *T. ciliata*.

Conclusions

The novel transcriptome and DGE profiling provide an important resource for functional genomics during HRM stress in *T. ciliata*. These data not only provide insights into the molecular mechanisms in resistance of *T. ciliata* to HRM but also helps to explore the new biocontrol strategies against insects in eco-friendly woody plant.

Background

Toona ciliata is a wild and endangered plant under the national level II key protection, which belongs to Meliaceae family [1]. It is called "Chinese Mahogany" because the wood has beautiful texture, straight dry shape, russet, soft and corrosion-resistant [2]. It is often used as a high-end furniture material for export and has high economic value [3]. The extracts from the leaves, stems, roots and flowers of *T. ciliata* have antibacterial, anti-tumor, anti-glycation, and cytotoxic functions, that also be used as medicine to treat gastric ulcers [4–7]. However, the *Hypsipyla robusta* Moore (HRM) adult worms like to lay their eggs on the *T. ciliata* leaves, so the undignified stems and apical buds are susceptible to the gnawing of the *H. robusta* larvae, causing the generation of multiple shoots, resulting in numerous lateral branches and consequently in poorly formed trees [8]. Reduced tree height and poor form caused by repeated attack greatly diminish the quantity and value of timber. Insect pests of *T. ciliata* are not only a regional problem, but also a global problem. Looking at the main areas of *T. ciliata* overseas, such as Australia and Brazil, it is also threatened by *H. robusta*[9]. Considerable research has been conducted on *H. robusta*, and many

prevention methods have been proposed, but none of them have achieved successful commercially [8, 10]. Due to the inaccessibility of larvae, heavy rains, and high temperatures in tropical areas, chemical applications are considered impractical in terms of environment and economy [8]. The number of natural enemies found is very small, indicating that the natural population cannot achieve the required level of biological control [10].

When suffering from pests, plants will activate a series of effective defense mechanisms [11]. For example, the physical and chemical barriers and defensive substances (its existence does not depend on the environment) against insects, are called constitutive defenses [12]. In addition, after plants are invaded by insect pests, a large number of defensive compounds are synthesized to activate defense related genes expression to produce self-defense response, which is similar to immune response and called inductive defense [13]. Studies have shown that by releasing volatile substances, such as terpenoids, aromatic compounds and green leaf volatiles, plants attract predatory or parasitic natural enemies of insect pests and play an indirect defense role [14]. The products of a single maize sesquiterpene synthase form a volatile defense signal that attracts natural enemies of maize herbivores [15]. Inducible defense will also cause the body to produce a series of complex signal networks triggered by hormones and so on [16]. Jasmonic acid (JA), salicylic acid (SA) and ethylene (ET) are important barriers in plant-induced defense responses. These three signal pathways are transmitted to the nucleus and initiate a series of defense responses. The JA pathway is induced by insects chewing mouthparts during feeding and mechanical damage, and the ET signaling pathway works in concert with JA. The SA pathway is mainly induced by insect feeding with piercing and sucking mouthparts. At the same time, abscisic acid (ABA), auxins, and phytokinins (CKs) play important regulatory roles [17, 18]. Each signal pathway has a synergistic or resistance effect, providing a mechanism for plants and the defense mechanism of layer plasticity [19]. When the brown planthopper (*Nilaparvata lugens*) attacked rice, it would induce the JA and ET pathways to coordinate and negatively regulate defenses [20]. Therefore, cultivating insect-resistant species of *T. ciliata* by means of gene editing has become an effective means to completely solve the problem.

Our team found that there is no insect-resistant *T. ciliata* provenance in nature, so it's important to have a deep dig and understanding of the induced defense mechanism. RNA-Sequencing (RNA-seq) is a new generation of high-throughput sequencing technology developed in recent years [21]. Through high-throughput sequencing, almost all transcript sequence information and expression level of a specific cell or tissue in a certain state can be obtained comprehensively and quickly [22]. RNA-seq has the advantages of high throughput, high resolution, low cost, high accuracy, and applicable to any species [23]. It has been widely used to explore the defense mechanism of plants against pests and has made great progress. In *Eucalyptus*, it was found that 698 and 1,115 significantly differentially expressed genes from the resistant and susceptible interactions, respectively. In addition, terpenoids profiles were significantly different under *Leptocybe invasa* stress by comparing the transcriptome [21]. RNA-seq of corn revealed that the JA defense signaling pathway is the main defense pathway of the Asian corn borer against herbivorous insects [24]. By analyzing the transcriptome of apple (*Malus ieversii*) under *Agrilus mali* stress, different expressed genes were mainly involved in signal transduction pathway of plant

hormones and in the synthesis of compounds such as terpenes, quinones, flavonoids, and JA [25]. Meanwhile, Giovino *et al*. [26] studying the transcriptome of *Phoenix canariensis* induced by feeding by *Rhynchophorus ferrugineus* and found JA and SA pathways were induced to participate in the defense against insects. The Illumina HiSeq 2000 sequencing platform was used to sequence the transcripts of cotton buds fed by *Anthonomus grandis* larvae and a total of 443 differentially expressed genes were identified, and 432 were *Arabidopsis* orthologous genes, including ET and JA signal genes [27]. However, as far as we know, the response mechanism on transcriptional level of *T. ciliata* against *H. robusta* is not yet reported.

In this study, RNA-seq was performed on the tender stems of *T. ciliata* that were gnawed at 0, 3, 12, and 21 hours by *H. robusta*. Then dig out the relevant genes and pathways of endogenous insect resistance to clarify the molecular mechanism in respond to *H. robusta*. At present, the organic combination of conventional breeding and plant molecular biology technology is an important way for the genetic improvement of *T. ciliata*. This study provides an insect-resistant gene library for breeding of insect-resistant varieties, which is also important for the prevention and control of *H. robusta*.

Results

A preliminary study on the response pattern of *T. ciliata* to the stress of HRM

The response of plants to insect pests will change dynamically with time. Therefore, Q-PCR were used to quantify the expression of *TcMYB* at different times of HRM stress and gene pattern of *T. ciliata* were initially understand in response to HRM stress (Fig. 1B). The results showed that the relative expression level of *TcMYB* gradually increased from 3-12h, reaching the maximum value at 12h. And it was in a declining-increasing-declining-increasing fluctuation state at 12-36h, but the relative expression levels were all lower than 12h. Therefore,0h (TC0), 3 h (initial response stage, TC3), 12 h (strongest response stage, TC12), and 21 h (response decline stage and followed the time rhythm, TC21) samples were selected for RNA-seq sequencing, to further explore the response pattern of *T. ciliata* against HRM.

RNA-seq sequencing, assembly and annotation

The young stems of *T. ciliata* feed by HRM for 0, 3, 12, and 21 h were selected as the materials (Fig. 1A), and every sample has three biological replicates. The quality evaluation of RNA-seq results showed that the 12 samples had 7.82G, 7.28G, 7.50G, 7.91G, 8.01G, 6.99G, 7.89G, 7.47G, 6.62G, 6.51G, 7.65G, and 6.67G clean bases, the GC content of each sample is distributed between 43.20%-44.67%, Q20 is greater than 98%, Q30 is greater than 94%, and Error rate is less than 0.025%, which indicates that the quality of sequencing data is reliable (Table 1). Principal component analysis (PCA) was performed among the four samples on gene expression and results showed no abnormal values (Fig. 1C). In addition, there was a variable difference in the expression betweenTC0, TC3, TC12, and TC21. However, perhaps due to the short time interval between TC3 and TC12 samples caused there is not large difference, but it's acceptable for subsequent analysis.

Since no genomic database in *T. ciliata* was available, the transcriptome sequencing and assembly was carried out firstly. A review of the transcriptome database of young stems of *T. ciliata* feed by HRM shows as Table 2. About 84,227 unigenes and 76.19 million bases were generated. When the assembled unigenes are sorted by length from largest to smallest, and the length of the transcript is accumulated to half of the total length, the length of the corresponding transcript is N50 length. N50 length is 1616bp, indicating that the assembly quality is great. TransRate score (0.14238) and BUSCO score (72%) illustrate the integrity of the transcriptome assembly is very good. In addition, the number of assembled unigenes gradually decreases as the length of unigenes becomes longer, among which 42459 unigenes shorter than 500bp, 20394 unigenes between 501-1000bp, and 1060 unigenes longer than 4500bp (Fig. S2A).

Then, all unigenes sequences were aligned with six public databases: NCBI non-redundant protein (Nr) database, Cluster of Orthologous Groups of proteins (COG), Gene Ontology (GO), Swiss-Prot, Protein family (Pfam), and Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Finally, 41568 unigenes obtained annotation information, accounting for 49.35% of all unigenes (Table 3). In Nr database, the top three species most similar to *T. ciliata* belong to *Citrus*, including *Citrus clementina* (28.68%), *Citrus sinensis* (22.47%), and *Citrus unshiu* (7.08%), which is similar to *Toona sinensis* that was closer to the genus *Citrus* [28]. Followed by *Vitis vinifera* (2.45%), *Quercus suber* (2.41%), *Theobroma cacao* (2.15%) *Populus trichocarpa* (1.89%), *Hevea brasiliensis* (1.31%) and *Durio zibethinus* (1.22%), and other species accounted for 30.34% (Fig. 1D). In addition, based on BLASTX search against the Nr database with an E-value cut-off of $1.0e^{-5}$, 39370 unigenes (46.74% of all unigenes) returned a significant BLAST hit (Table 3). Among them, 62.55% were annotated to unigenes had very strong homology with the top matching sequence ($0<E\text{-vale}<1e^{-30}$), 10.60% shows strong homology ($1e^{-30}<E\text{-vale}<1e^{-20}$) and the homology of the remaining unigenes is weak ($1e^{-20}<E\text{-vale}<1e^{-3}$) (Fig. 1E).

Expression analysis of unigenes gene

Transcript abundance can directly reflect the expression level of a gene. The higher the transcript abundance, the higher the gene expression level [29]. In RNA-seq analysis, the expression level of genes is estimated by counting the number of Reads located in the genomic region or gene exon region. Transcripts Per Million reads (TPM) is the number of reads from a transcript per million reads. The homogenization process of TPM makes the total expression level in different samples consistent, so that the expression level can be compared more intuitively [30]. The distribution of unigene expression level in 12 samples of *T. ciliata* is shown in Fig. S2B, the most concentrated area of each sample is when $\log_{10}TPM=-0.5$. The number of unigenes with $Log_{10}TPM>2$ and $Log_{10}TPM<-1$ is relatively small, showing polarization. The unigene expression level is divided into 5 expression levels according to the TPM interval, which includes 0-1, 1-3, 3-15, 15-60 and greater than 60 (high expression level) (Table 4). About 60% of all samples, unigene's TPM value is concentrated in 0-1, which is required to maintain the normal growth of plants. The number of genes with TC21, TC12 and TC3 TPM greater than 60 is larger than that of TC0, indicating that there are more genes in *T. ciliata* that are induced to express against the HRM (Table 4).

DEGs analysis under HRM stress

In order to identify significant DEGs of *T. ciliata* in different time periods under the stress of HRM, Firstly, compare TC3, TC12, and TC21 with TC0, and obtain DEGs (up regulated and down regulated) of TC0 vs TC3, TC0 vs TC12, and TC0 vs TC21, respectively. Then compare the up-regulation or down-regulation of TC0 vs TC3, TC0 vs TC12 and TC0 vs TC21, and finally obtain the DEGs that are jointly up-regulated or jointly down-regulated were distinguished. The conditions for screening DEGs are $|\log_2(\text{foldchange})| > 1$ and *p*-adjust <0.05. The results showed that there were 263 and 378 genes that were jointly up-regulated and down-regulated, respectively (Fig. 2A, B). After 3 hours of stress, the number of genes that were up-regulated (763) and down-regulated (1009) were higher than the number of genes that were up-regulated (763) and down-regulated (1009) after 12 hours of stress. And the number of up-regulated (1787) and down-regulated (2717) genes rose to the maximum after 21 hours of stress (Fig. 2C). It shows that different and more complex biological events occurred in *T. ciliata* at 3h and 21h.

For exploring the biological changes of *T. ciliata* during the stress of HRM, all DEGs were analyzed by GO enrichment (Fig. 2D). Most DEGs are mainly enriched in oxidoreductase activity, dioxygenase activity, protein disulfide oxidoreductase activity, etc., which suggest *T. ciliata* may activate xanthine oxidoreductase through MAP kinase-dependent pathways [31] in order to protect itself. Meanwhile, the rhythm-related pathways are detected due to the difference in sampling time, such as entrainment of circadian clock, positive regulation of circadian rhythm, circadian rhythm, etc. [32]. In addition, all DEGs were subjected to KEGG enrichment analysis (Fig. 2E). Among them, the five most enriched metabolic pathways are plant hormone signal transduction, phenylpropanoid biosynthesis, MAPK signing pathway, Glycine, serine and threonine metabolism, and α-linolenic acid metabolism.

Analysis of putative insect-resistant genes in JA biosynthesis and signal transduction

JA is an important signal in many developmental processes such as seed germination, root and whole plant growth, stamen development, and senescence [33] and JA regulates the induction of direct and indirect defenses against herbivores. More importantly, α-linolenic acid metabolism and plant hormone signal transduction pathways closely related to JA are significantly enriched under the HRM attack in *T. ciliata*. Therefore, the genes in JA biosynthesis and signal transduction pathways were excavated and analyzed.

The starting material for JA synthesis is α-linolenic acid located in the chloroplast, which is catalyzed by the key enzyme 13-lipoxygenas (LOX) to produce 13-HPOT. Then in allene oxide synthase (AOS) and allene oxide cyclase (AOC) are continuously catalyzed to form JA precursor cis-(+)-12-oxophytodienoic acid (cis- (+) -OPDA) [33]. Five *Tc-LOX* genes, two *TcAOS,* and one *TcAOC* genes were identified in the *T. ciliata* RNA-seq library. The expression levels of *Tc-LOX1/2/3/5* up-regulated 3-4 times when suffering 3h HRM stress, especially *Tc-LOX2* expression levels up-regulated continuously and up to maximum multiple (5) at 21h (Fig. 3). Phylogenetic analysis showed that *Tc-LOX1/2/3* belonged to 13-LOX subfamily;

TcLOX4/5 belong to the 9-LOX subfamily, suggesting 13-LOX subfamily genes are main force to resist HRM attacks in *T. ciliata* (Fig. 4A).

The OPDA produced was transferred to peroxisome, then reduced by oxophytodienoate reductase 3 (OPR3) and three β-oxidized, finally (+)-7-*iso*-JA was produced [33]. A *TcOPR3* gene was identified and expression level up-regulated 7-fold at 3h (Fig. 3). Then JA is transported to the cytoplasm to form the active form of jasmonic acid-isoleucine (JA-Ile) under the catalysis of jasmonoyl amino acid conjugate synthase (JAR1) [33]. Although the expression level of *TcJAR1* increased at 3h, it declined linearly in the subsequent period, indicating that its performance may be a stress response without the ability to resist insects (Fig. 3).

The jasmonate ZIM□domain (JAZ) is a zinc finger protein, including Jas domain and ZIM domain and plays the role of "suppressor" in JA pathway [34]. In the absence of stress, JAZ protein interacts with NINJA to recruit inhibitor topless (TPL), which makes JAZ protein to inhibit the transcriptional activation of JA response gene by interacting with MYC2[35] (Fig. 3). When JA accumulation, JA-Ile is formed and transported to the nucleus that facilitates the interaction of JAZ with CORONATINE INSENSITIVE1 (COI1). Moreover, COI1 is the F-box component of a Skip-cullin-F-box-(SCF)-type E3 ubiquitin ligase required for all JA-dependent responses tested so far [36]. Ubiquitination of the JAZ protein would lead to its proteasomal degradation and release TFs to modulate the expression of JA-responsive genes, thereby regulating defenses and growth [36]. In RNA-seq library, four *TcJAZs* genes were identified and the expression level of *TcJAZ1/2/34* up-regulated by 13, 10, 73 and 20 times at 3h, respectively. However, TcCOI1expression level only changed slightly. The phylogenetic analysis of 13 AtJAZ genes in *Arabidopsis* and four *TcJAZs s*howed that *TcJAZ1*/2 belong to clade1 and *TcJAZ3/4* belong to clade2. Among them, *TcJAZ1* and *AtJAZ5/6*, *TcJAZ2* and *AtJAZ1/2*, and *TcJAZ3/4* and *AtJAZ10* are closely related, respectively, indicating these genes have similar function (Fig. 4B). In addition, the expression levels of TFs regulated by JA, including ERF, WRKY, MYC, MYB, and NAC were significantly up-regulated. In conclusion, JA synthesis and signal transduction pathway were activated under HRM attack in *T. ciliata*. Q-PCR analysis showed that all tested genes were induced to express under HRM stress, that is similar to RNA-seq data. Especially the relative expression levels of *TcOPR3, TcJAR1*, *TcJAZ1,* and *TcJAZ3* reached about 100 and it is worthy to further study (Fig. 4C).

Analysis of putative insect-resistant genes in terpene biosynthesis

After gnawed by insects, plants will be induced to produce volatile compounds with anti-insect functions, such as terpenes. Therefore, the author focused on digging out the terpene bio-related genes after HRM stress in *T. ciliata*. In plants, sesquiterpenes are usually synthesized in the cytoplasm through the MVA pathway, which provides precursors for terpenoids in the cytoplasm or mitochondria. The MVA pathway is regulated by six catalytic enzymes, including acetyl-CoA acetyltransferase (AACT) gene, hydroxymethylglutaryl-CoA synthase (HMGS) gene, Hydroxymethyl glutaryl-CoA reductase (HMGR) gene, phosphomevalonate kinase (PMK) gene, mevalonate diphosphate decarboxylase (MDC) gene and mevalonate kinase (MK) gene[29, 37]. In the *T. ciliata* RNA-seq library, seven genes on the MVA pathway

were identified, including *TcACCT* gene, two *TcHMGS* genes, *TcHMGR* gene, *TcMK* gene and two *TcPMK* genes, but *TcMDC* gene was not found (Fig. 5). It is predicted that above genes don't contain signal peptides and located in the cytoplasm (Fig. 5). RNA-seq data showed that the expression levels of *TcACCT, TcHMGR,* and *TcMK* gene were low and didn't change significantly. However, the expression levels of two *TcHMGS* and two *TcPMK* genes continued to up-regulate (Fig. 5).

Monoterpenes and triterpenes are usually synthesized through MEP pathway in plastids. Six genes on the MEP pathway have been identified, including five 1-deoxy-D-xylulose 5-phosphate synthase (DXS), 1-Deoxy-D-xylulose 5-phosphate reductoisomerase (DXR), two 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase (MCT), 4-(cytidine 5-diphospho)-2-C-methyl-D-erythritol kinase (CMK) and three 4-hydroxy-3-methylbut-2-enyl-diphosphate synthase (HDS) and three 4-hydroxy-3-methylbut-2-enyl diphosphate reductase (HDR) (Fig. 5). By subcellular location prediction found above genes all located in plastids, which is consistent with the location of MEP pathway enzymes in *Arabidopsis* [38]. The first catalytic enzyme of the MEP pathway is DXS, which is also considered a rate-limiting enzyme. Studies have shown that the DXS gene family is generally divided into three different phylogenetic branches [39]. The expression of DXS genes in different evolutionary branches varies with development, tissue types and environmental conditions. Phylogenetic analysis showed that *TcDXS3/*5 clustered to clade 1 that mainly used as a reference gene [39] (Fig. 6A). Obviously, *TcDXS5* wasn't a reference gene but involved in insect-resistance (Fig. 5). Other genes expression level in the MVA pathway, such as *TcDXR*, *TcHDS* and *TcHDR*, up-regulated at TC3 and TC12. However, the expression levels of *TcMCT* and *TcCMK* are significantly down-regulated, and there are large fluctuations, which may reflect the reduced demand of the main metabolism for metabolic flow [29].

The conversion between IPP and DMAPP is a reversible reaction. The reaction is catalyzed by isopentenyl pyrophosphate isomerase (IPI) to dynamically adjust the content of IPP and DMAPP [40]. Then short-chain isopentenyl transferases (including GPPS, FPPS, and GGPPS) catalyze the head-to-tail condensation of IPP and DMAPP to isoprene GPP, FPP, and GGPP [40]. There are two *FPPS* and one *GGPPS* in the *T. ciliata* RNA-seq library. The expression levels of the three genes all significantly up-regulated at TC3, TC12 and TC21(Fig. 5), indicating that *TcFPPSs* and *TcGGPPS* are involved in insect resistance.

Terpene synthases (TPSs) are a diverse class of enzymes which catalyzes the biosynthesis of hemiterpenes (C5), monoterpenes (C10), sesquiterpenes(C15), or diterpenes (C20) using the substrates DMAPP, GPP, FPP, or GGPP, respectively [41]. Through KEEG annotation and homologous sequence search, 15 TPS genes were identified in RNA-seq library. The subcellular location prediction shows that *TcTPS2/7/10/11/12/14/15* are located in the cytosol, and *TcTPS1/3/4/5/6/8/9/13* are located in the plastid (Fig. 5). Among these gene, *TcTPS4/6/9/13/14/15* expression level significantly upregulated and is necessary to analyze in-depth. The TPS family in *Arabidopsis* is generally divided into 5 different subfamilies, including TPS-a, TPS-b, TPS-c, TPS-e/ f and TPS-g [42]. Phylogenetic analysis showed that *TcTPS14/15* clustered into the TPS-a subfamily, which mainly involved in monocotyledonous and dicotyledonous sesquiterpenes synthesis. *TcTPS6/9* clustered into the TPS-b subfamily, whose genes

participated in angiosperm monoterpene biosynthesis. *TcTPS4* clustered into the TPS -c subfamily and *TcTPS13* clustered into the TPS-e/f subfamily (Fig. 6B).

Q-PCR analysis showed that the expression level of *TcGPPS1, TcTPS9*, and *TcTPS14* was significantly up-regulated by about 10 times under HRM stress (Fig. 6D). Some studies have shown that TPS is a synthase of various terpenoids that have anti-insect effects [15]. Therefore, to further determine the functions of terpene synthase under HRM stress, TcTPS *9/13/14/15* expression level in the leaves were detected. Interestingly, *TcTPS9* was significantly induced to up-regulate 60 times and gradually increase over time (Fig. 6C), suggesting that *TcTPS9* catalyzes the synthesis of a certain terpenoid in the tender stem and leaf tissues under HRM stress, but whether this substance is important for insect resistance to *T. ciliata* requires further study.

Analysis of transcription factors responding to HRM

In order to discover the potential TFs with anti-insect function, the classification and expression level of TFs in the RNA-seq database were analyzed in depth. It was found that a total of 1120 TFs were divided into 35 families (Fig. S3). The most abundant TF family was MYB (189), followed by NAC (121), AP2/ERF (120), and bHLH (86), C2C2 (72), WRKY (69) and bZIP (50). There are 323 (28.8%) differentially expressed TFs (DETFs) members, which are divided into 31TFs families (Fig. S3). The MYB TF family contains the most DETFs, followed by AP2/ERF (36), C2C2(35), NAC (32), bHLH (32), and WRKY (24) that were found in *Medicago truncatula* against aphid [43]. Then the detailed information and expression patterns of the top 40 TFs in terms of expression difference was listed on Table 5. Among the 9 MYB family members, only TRINITY_DN4447_c0_g1 was down-regulated under the HRM stress, TRINITY_DN7191_c0_g1 and TRINITY_DN18799_c0_g1was up-regulated by 36.8 and 20.35 times respectively at 3h, which were sensitive to stress. But TRINITY_DN4331_c0_g1 and TRINITY_DN17485_c0_g1 were increased 19.36 and 13.88 times respectively at 21h. It shows that the gene response time is inconsistent or there is functional redundancy between genes. In RNA-seq library of *T. ciliata*, there are 9 DETFs of AP2/ERF family, of which TRINITY_DN4586_c0_g1 and TRINITY_DN1335_c0_g2 are down-regulated and the other members were up-regulated of which TRINITY_DN8732_c1_g1 was up-regulated the most at 3h (27.79 times). In addition, most members of the C2C2 TF family (5/7) were down-regulated, and TRINITY_DN14674_c1_g1, TRINITY_DN53864_c0_g1 were down-regulated by 44.52 and 33.92 times, respectively. Meanwhile, TRINITY_DN8705_c0_g1 up-regulated about (21.73) may have potential to resist HRM. In addition, the bHLH TF (TRINITY_DN15909_c0_g1) down-regulated by 24.77 times at 12h. The genes mentioned above play a vital role in the process of *T. ciliata* resisting pests, and it is worthy of in-depth study in the later stage.

Discussion

Wounding damage from insect herbivores quickly triggers plant defense signals [44]. The first reported damage-related peptide signal is systemin that promotes the accumulation of JA and activates the expression of genes encoding protease inhibitors with insecticidal activity [44]. In addition, other wound-

inducing peptides have been found in plants, including *Arabidopsis* [45], rice [46] and *maize* [47]. Through transcriptome data analysis, JA synthesis-related gene, such as LOX, AOS, OPR3, were significantly induced, which demonstrating JA accumulation (Fig. 3). However, there is no wound-induced polypeptides were identified in *T. ciliata*. A Ca^{2+}-binding protein kinase (CDPK) signaling pathway involved in crosstalk with mitogen-activated protein kinases (MAPKs) also leads to the formation of JA [12]. Meanwhile, in our RNA-seq library, MAPK signing pathway was significantly enriched (Fig. 2E), and this pathway genes were also significantly poorly up- or down-regulated (Table 6). Therefore, when *T. ciliata* is eaten by HRM, calcium ion homeostasis is break firstly, which acts as a second messenger in various plant signaling pathways. Then Ca^{2+} bind to protein kinases to activate MAPK signaling, thereby activating JA synthesis and signaling pathways (Fig. 7). α-linolenic acid (C18: 3) is the precursor of *trans*-jasmonic acid and *trans*-jasmonic acid is stress-related plant hormone that participates in the defense of insect herbivory and necrotizing pathogens [48]. Therefore, it is speculated that α-linolenic acid, participates in the attack of HRM through induction of JA-mediated pathways, that exists in *citrus senensis* response to '*Candidatus* Liberibacter asiaticus' [48]. *LOX*, *AOS*, and *OPR* are the key genes supporting JA biosynthesis and are studied well in other species. Studies show that feeding by *Spodoptera exigua* larvae on *Zea mays* induces expression of 9-lipoxygenases to a greater extent than 13-lipoxygenases [49]. However, in our study, *Tc-LOX1/2/3* belonging to 13-LOX subfamily are main force to resist HRM attacks in *T. ciliata* (Fig. 3, 4A), that provides a new perspective on insect resistance research. *OsAOS1* and *OsAOS2* had been proved that play a vital role in determining the resistance of rice to chewing and phloem-feeding herbivores [50]. Similar to *OsAOS1* and *OsAOS2*, *TcAOS2* expression level also up-regulated 8-fold at 3h, suggesting *TcAOS2* has great potential to resist HRM attack. In other plants, such as *Arabidopsi*s [51], tomato [52], and potato [53], OPR3 all have the function of resisting pests and which remind that *TcOPR3* is worthy of further study. JA-Ile is formed through JA rapid accumulation. On the one hand, JA-Ile will be transported to the nucleus to negatively regulate JAZ, then JAZ inhibits MYC2 while negatively regulating EIN3 and ET. downstream transcription factors will be activated to participate in insect resistance, such as MYB, ERF, NAC, etc. A mount of TFs from different TFs families have been shown to play a key role in regulating the pest stress in *Arabidopsis*, rice, wheat and many other plant species[54]For example, *TaMYB19, TaMYB29*, and *TaMYB44* are co-regulators against aphid of wheat [55] and *AtERF5, AtERF6* and *AtRAP2.2* have been implicated in resistance to Botrytis cinerea mediated by JA and ethylene in *Arabidopsis* [56]. In this study, there are 323 TFs from 31 families were identified, which responded HRM stress (Fig. S3). Among them, MYB, AP2/ERF, C2C2 and NAC families comprise a high proportion of pest stress-responsive members. NAC (NAM, ATAF1/2 and CUC) domain proteins play a role in diverse processes, including development, morphogenesis, senescence, and stress responses and studies show that *ATAF1* and *ATAF2* genes were highly induced by wounding and pathogen infection [57-59]. Meanwhile, *IbNAC1*, upregulates sporamin gene expression by binding the SWRE motif against mechanical wounding and herbivore attack in potato [60]. In Cotton, GhJAZ2 interact with GhbHLH171 and inhibit its transcriptional activity, and negative feedback regulation of JA-mediated defense response [61]. On the other hand, JA-Ile transported to other cells or tissues affects plant volatile organic compounds (VOCs) emissions and JA-mediated changes in monoterpenes may protect trees under biotic and abiotic stress [62, 63]. Spraying MeJA on Norwegian spruce (*Picea*

abies) can induce the accumulation of terpenoids in its resin canal to effectively deal with the damage of beetles [64]. In plants, over 1000 volatile organic compounds (VOCs), mainly composed of 6-carbon aldehydes, alcohols, esters and various terpenes. VOCs are used to attract pollinators and predators or repel herbivores as well as in communication between or within plants [12]. The synthetic pathways of terpenoids, MVA and MEP, in the young stems of *T. ciliata* were activated, and *TcTPS9* express highly in leaf tissues. Therefore, it is inferred that JA-Ile is transported into leaf cells to activates key insect resistance genes expression that can synthesize signal substances to attract natural enemies or communicate with peers. In maize, *TPS10* can produced the sesquiterpene that can provide a volatile signal for the indirect defense of the plant against herbivore attack [15]. Rice fed on by fall armyworm will induce volatiles and the corresponding volatile biosynthetic genes potentially involved in indirect defense [65]. HMGR is a synthesis rate-limiting enzyme of MVA pathway. In this study, HMGRs were activated to promote the synthesis of terpenoids (Fig. 5). Also, *HMGS* and *HMGR* were highly expressed in tomato, which produce many secondary metabolites that act against pathogens and pests [66]. Moreover, phenylpropanes derivatives, such as flavonoids, coumarins, lignin and phenolamides, will rapidly accumulate to higher levels as components of an induced defense arsenal against herbivore attack in wild tobacco *Nicotiana attenuata* [67]. At the same time, biosynthesis pathway gene were induced expression significantly at TC3(Table 6), especially TRINITY_DN8222_c0_g4 (757 folds) and TRINITY_DN37385_c0_g1 (1250 folds), this suggests phenylpropane biosynthesis pathway may participates in the insect-resistance mechanism by producing phenylpropane derivatives in *T. ciliata*, which needs further experiments to prove.

In summary, our finding not only provides insights into the molecular mechanisms in resistance of *T. ciliata* to HRM but also helps to explore the new biocontrol strategies against insects in eco-friendly woody plant. At the same time, many key genes have been found in these biological pathways, including *TcOPR3, TcJAR1, TcJAZs,* and *TcTPS9,* which are worthy of follow-up in-depth study.

Conclusion

In this study, a comparison of transcriptomic profiles from young *T. ciliata* stems subjected to 0, 3, 12, and 21 h HRM stress was performed using RNA-Seq. A total of 84,227 unigenes were generated from 12 samples, and 41568 unigenes were annotated in different databases. Many differentially expressed genes (DEGs) were identified that were mainly involved in JA synthesis, signal transduction, and terpene biosynthesis. At the same time, many key genes have been found in these biological pathways, including *TcOPR3, TcJAR1, TcJAZs*, and *TcTPS9.* These genes are worthy of follow-up in-depth study. This study not only provides insights into the molecular mechanisms underlying the resistance of *T. ciliata* to HRM but also helps to explore new biocontrol strategies against insects in eco-friendly woody plants.

Materials And Methods

Plant materials and treatment

The experiment materials were healthy 3-year-old clones of *T. ciliata*, which grew in the College of forestry and landscape architecture in South China Agricultural University under natural light (SCAU, Guangzhou). The HRM were collected from Qilin North Experimental Base (SCAU) and starved in a blank culture dish for 12 hours. Then HRM were placed on the young stem of *T. cilata* (3-4cm away from the terminal bud) and each *T. ciliata* was inoculated with one HRM. When the HRM began to bite *T. ciliata*, the time was counted. The young stems bitten by HRM for 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, and 36 h were taken respectively, and each time point had three repetitions (Fig. 1A). Collected young stems were quickly freezing in liquid nitrogen, then stored at -80℃.

Identification samples of RNA sequencing

When plants are subjected to external stress, a series of dynamic changes of endogenous gene genes will occur. Meanwhile, previous studies have shown that MYB transcription factor (TFs) not only confers resistance to brown planthopper by regulating phenylalanine ammonia lyase pathway in rice [68], but also synergistically regulates the defense of phloem against English grain aphids in wheat [55]. Therefore, the relative expression level of *TcMYB* in *T. ciliata* under HRM stress was detected to preliminarily determine the expression pattern of endogenous gene in response to HRM stress. *HIS1* was selected as the reference gene [3]. The samples for RNA sequencing were determined though the relative expression level of *TcMYB*. The first sampling point was the time when the relative expression of *TcMYB* increased for the first time, the second sampling point was the time when the relative expression of *TcMYB* increased to the maximum, and the third sampling point =the second sampling point + (the second sampling point - the first sampling point). Meanwhile, 0h was the control group. RNA sequencing was performed on samples of oh and the three sampling points.

RNA extraction, library preparation, and sequencing(□□□□□)

Total RNA of *T. ciliata* young stem was extracted by TRIzol® Reagent (Plant RNA Purification Reagent for plant tissue, Invitrogen), then genomic DNA was eliminated through DNase I (TaKara). Total RNA quality was determined by 2100 Bioanalyser and quantified using the ND-2000. Finally, the high-quality RNA sample (OD260/280=1.8~2.2, OD260/230≥2.0, RIN≥8.0, 28S:18S≥1.0, >1μg) was used to construct sequencing library [69]. Library construction, and sequencing were performed at Shanghai Majorbio Bio-pharm Biotechnology Co., Ltd. according to the manufacturer's instructions (Illumina, San Diego, CA). The transcriptome libraries of *T. ciliata* young stem were prepared using Illumina TruSeqTM RNA sample preparation Kit (San Diego, CA). Firstly, poly-A tail mRNA was enriched from 5ug total RNA with oligo (DT) magnetic beads. Then the fragmentation buffer was added to randomly break the mRNA into small fragments of about 200bp [69]. Then, a single strand cDNA was synthesized by using superscript double stranded cDNA asynchronous (Invitrogen, CA) kit and adding six base random primers (Illumina) with mRNA as inversion, and then two strand synthesis was carried out to form a stable double strand structure [69]. Double stranded cDNA is a sticky end. End repair mix is added to make it a flat end, and then a base is added at the 3 'end to connect the Y-shaped connector. After PCR enrichment, 200-300 bp bands were recovered with 2% agarose gel. After quantification by tbs380 (PicoGreen), the library used

illuminahiseqxten / novaseq6000 sequencing platform for high-throughput sequencing, and the sequencing reading length was PE150 [69].

De novo assembly and annotation

The original paired end reading is determined by SeqPrep and sickle with default parameters for trimming and quality control. Then Clean data from samples (young stems of *T. ciliata*) were used to do de novo assembly with Trinity [70]. BLASTX was used to search all assembled transcripts against NCBI protein non redundant (NR), COG, and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases to identify the protein with the highest sequence similarity to a given transcript to retrieve its functional annotation and typical cut-off E-values less than 1.0×10^{-5} was set. BLAST2GO [71] the program is used to obtain GO annotations of uniquely assembled transcripts to describe biological processes, molecular functions and cellular components. Metabolic pathway was analyzed by KEGG [72].

Differential expression analysis and functional enrichment

After obtaining the number of Read Counts of genes, the samples were analyzed for gene expression differences between samples. The software identifies differentially expressed genes between samples, and then studies the function of differentially expressed genes. The software used for differential expression is DESeq2 [73, 74] and EdgeR [75], which calculates differential expression based on the read count data of the aligned genes, and the analysis is based on a negative binomial distribution model. The default screening criteria for significantly different genes are: FDR < 0.05 and $|\log_2 FC| > 1$. When a gene satisfies both conditions, the gene is regarded as a differentially expressed gene. In addition, GO and KEGG was performed to determine which DEGs were significantly enriched in GO terms and metabolic pathways with Bonferroni-corrected *P*-values ≤ 0.05 compared to the whole transcriptome background. GO functional enrichment and KEGG pathway analysis by imageGP (http://www.ehbio.com/ImageGP/).

Screening and identification of insect resistance genes

Plant hormone biosynthesis and signal transduction pathways play a vital role in the defense process induced by insect feeding after insects feeding, three types of volatile compounds of plant can be induced, including terpenes and fatty acid derivatives and phenyl/phenylpropanes (derivatives of shikimic acid), which have important anti-insect functions. Therefore, this study combined the results of differential gene function annotation and the KEGG pathway to analyze the Terpene synthesis and JA biosynthesis and signal transduction pathways. The synthetic transduction schematic diagrams of the two pathways are drawn through the reference, and the heat maps of related Gene expression are drawn by TBtools [76]. Through NCBI-Blast to query the homologous sequence to initially confirm the gene family, then through predicting the gene functional domain by NCBI-Batch-CDD for the second confirmation. Meanwhile, for larger gene families, homologous sequences are distinguished by constructing phylogenetic trees by MEGA7. Target P 1.1 Server (http://www.cbs.dtu.dk/services/TargetP/) and WoLF PSORT (https://wolfpsort.hgc.jp/) were combined to predict protein subcellular location.

Analysis of transcription factors

By analyzing the domain information contained in the transcription product, the TFs prediction and family analysis of the transcript are carried out. Then the HMMER analysis method is used to compare with the database PlantTFDB (http://planttfdb.cbi.pku.edu.cn/) to obtain the same homologous TFs information. At last, the specific information of transcription factor analysis and transcription factor family are counted.

Quantitative Real-time PCR (Q-PCR)

According to CDS sequence from RNA-seq database design Q-PCR primers by NCBI-PrimerBlast (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) (Table S1 and Fig. S1). The template cDNA of Q-PCR includes the tender stems and leaves under 0, 3, 12, and 21h SMH stress. *HIS1* were selected as reference genes of tender stem under HRM stress. The Q-PCR reaction mixture consisted of 10 μL ChamQ Universal SYBR qPCR Master Mix (Vazyme), cDNA 2 μL, each primer 0.4 μL (10 μM), 7.2 μL ddH_2O. Q-PCR reaction was performed in LightCycler480 (Roche Molecular Biochemicals, Mannheim, Germany) with an optical 96-well plate. The reaction procedure is 95°C for 30 s; 95°C for 15 s, 60°C for 20 s, 72°C for 10 s, 40 cycles; performing melting curve analysis at 65°C-95°C. Then through the peak diagram of the melting curve to determine the specificity of primers. There are three biological replicates for each sample and three technical replicates for each biological replicate. According to the Ct value obtained in the experiment, the $2^{-\Delta\Delta Ct}$ method was used to calculate the expression under different conditions.

Abbreviations

HRM
Hypsipyla robusta Moore
DGEs
Differentially expressed genes
JA
Jasmonic acid
TFs
Transcription factors
SA
Salicylic acid
ET
Ethylene
ABA
Abscisic acid
CKs
Phytokinins
RNA-seq

RNA-Sequencing
Q-PCR
Quantitative real-time PCR
TPM
Transcripts per million reads
LOX
13- lipoxygenas
AOS
Allene oxide synthase
AOC
Allene oxide cyclase
OPR3
Oxophytodienoate reductase 3
JAR1
Jasmonoyl amino acid conjugate synthase
JA-Ile
Jasmonic acid-isoleucine
JAZ
Jasmonate ZIM□domain
TPL
Topless
COI1
Coronatine insensitive1
AACT
Acetyl-CoA acetyltransferase
HMGS
Hydroxymethylglutaryl-CoA synthase
HMGR
Hydroxymethyl glutaryl-CoA reductase
PMK
Phosphomevalonate kinase
MDC
Mevalonate diphosphate decarboxylase
MK
Mevalonate kinase
DXS
1-deoxy-D-xylulose 5-phosphate synthase
DXR
1-Deoxy-D-xylulose 5-phosphate reductoisomerase
MCT

2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase
CMK
4-(cytidine 5-diphospho)-2-C-methyl-D-erythritol kinase
HDS
4-hydroxy-3-methylbut-2-enyl-diphosphate synthase
HDR
4-hydroxy-3-methylbut-2-enyl diphosphate reductase
IPI
isopentenyl pyrophosphate isomerase
TPS
Terpene synthase.

Declarations

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Authors' contributions

PL and HS conceived and designed research. HS performed the experiments and write the manuscript. HS, YL, ZW and ZD analyzed the data. YW, EY, and WM performed RT-qPCR experiment. QQ, PL and XC guided experiment and revised manuscript. All author read and approved the final manuscript.

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Availability of data and materials

The raw sequence fles used for diferential gene expression analysis are available in FASTQ format in the NCBI BioProject repository (BioProject Accession number: SAMN27407607, SAMN27407608, SAMN27407609, SAMN27407610, SAMN27407611, SAMN27407612, SAMN27407613, SAMN27407614, SAMN27407615, SAMN27407616, SAMN27407617 and SAMN27407618.

Ethics approval and consent to participate

Toona ciliata was grown and collected in South China Agricultural University (Guangzhou, Guangdong Province) and identified by Professor Xiaoyang Chen and teacher Pei Li, but the uncertified samples were stored in the public Herbarium. All samples from these cultivars were adopted for all experiment. No

specific permits are required for sample collection in this study. We comply with the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Li P, Shang Y, Zhou W, Hu X, Mao W, Li J, Li J, Chen X. Development of an efficient regeneration system for the precious and fast-growing timber tree *Toona ciliata*. Plant Biotechnol (Tokyo). 2018; 35(1):51–58.
2. Song H, Mao W, Shang Y, Zhou W, Chen X. A regeneration system using cotyledons and cotyledonary node explants of *Toona ciliata*. J Forestry Res. 2020;(338–346).
3. Song H, Mao W, Duan Z, Que Q, Zhou W, Chen X, Li P. Selection and validation of reference genes for measuring gene expression in *Toona ciliata* under different experimental conditions by quantitative real-time PCR analysis. BMC Plant Biol. 2020;20(1):450.
4. Shi QQ, Zhang XJ, Zhang Y, Wang Q, Amin M, Li Q, Wu XW, Li XL, Zhang RH, Dai XC *et al*. Toonaolides A-X, limonoids from *Toona ciliata*: Isolation, structural elucidation, and bioactivity against NLRP3 inflammasome. Bioorg Chem. 2020; 105:104363.
5. Zhu GL, Wan LS, Peng XR, Shi QQ, Li XN, Chen JC, Zhou L, Qiu MH. Cytotoxic limonoids from the twigs and leaves of *Toona ciliata*. J Nat Prod. 2019; 82(9):2419–2429.
6. Malairajan P, Gopalakrishnan G, Narasimhan S, Veni KJ, Kavimani S. Anti-ulcer activity of crude alcoholic extract of *Toona ciliata* Roemer (heart wood). J Ethnopharmacol. 2007;110(2):348–351.
7. Gautam A, Jhade D, Ahirwar D, Sujane M, Sharma G. Pharmacognostic evaluation of toona ciliata bark. J Adv Pharm Technol. 2010;1(2):216–220.
8. Mohanadas K, Varma RV: Ecology and possible control of the mahogany shoot borer Hypsipyla robusta in Kerala, India. Hypsipyla shoot borers in meliaceae an international workshop held at kandy, 2001.
9. Cunningham SA, Floyd RB. Leaf compositional differences predict variation in Hypsipyla robusta damage to *Toona ciliata* in field trials. 2004; 34(3):642–648.
10. Newton AC, Baker P, Ramnarine S, Mesén J, Leakey R. The mahogany shoot borer: prospects for control. Forest Ecol Manag. 1993;57(1):301–328.
11. Nalam V, Louis J, Shah J. Plant defense against aphids, the pest extraordinaire. Plant Sci. 2019;279:96–107.

12. Fürstenberg-Hägg J, Zagrobelny M, Bak S. Plant defense against insect herbivores. Int J Mol Sci. 2013;14(5):10242–10297.
13. Wang S, Zhang M, Huang J, Li L, Huang K, Zhang Y, Li Y, Deng Z, Ni X, Li X. Inductive and synergistic interactions between plant allelochemical flavone and Bt toxin Cry1Ac in *Helicoverpa armigera*. Insect Sci. 2020.
14. Clavijo McCormick A, Irmisch S, Reinecke A, Boeckler GA, Veit D, Reichelt M, Hansson BS, Gershenzon J, Köllner TG, Unsicker SB. Herbivore-induced volatile emission in black poplar: regulation and role in attracting herbivore enemies. Plant Cell Environ. 2014;37(8):1909–1923.
15. Schnee C, Köllner TG, Held M, Turlings TC, Gershenzon J, Degenhardt J. The products of a single maize sesquiterpene synthase form a volatile defense signal that attracts natural enemies of maize herbivores. Proc Natl Acad Sci U S A. 2006; 103(4):1129–1134.
16. Mangwanda R, Myburg AA, Naidoo S. Transcriptome and hormone profiling reveals *Eucalyptus grandis* defence responses against *Chrysoporthe austroafricana*. BMC Genomics. 2015;16(1):319.
17. Thaler JS, Humphrey PT, Whiteman NK. Evolution of jasmonate and salicylate signal crosstalk. Trends Plant Sci. 2012; 17(5):260–270.
18. Papadopoulou GV, Van Dam NMJER. Mechanisms and ecological implications of plant-mediated interactions between belowground and aboveground insect herbivores. 2017;32(1):1–14.
19. Nguyen D, Rieu I, Mariani C, van Dam NM. How plants handle multiple stresses: hormonal interactions underlying responses to abiotic stress and insect herbivory. Plant Mol Biol. 2016;91(6):727–740.
20. Ma F, Yang X, Shi Z, Miao X. Novel crosstalk between ethylene- and jasmonic acid-pathway responses to a piercing-sucking insect in rice. New Phytol. 2020;225(1):474–487.
21. Oates CN, Külheim C, Myburg AA, Slippers B, Naidoo S. The transcriptome and terpene profile of *Eucalyptus grandis* reveals mechanisms of defense against the insect pest, *Leptocybe invasa*. Plant Cell Physiol. 2015;56(7):1418–1428.
22. Ayturk U. RNA-seq in skeletal biology. Curr Osteoporos Rep. 2019;17(4):178–185.
23. Słomnicka R, Olczak-Woltman H, Sobczak M, Bartoszewski G. Transcriptome profiling of cucumber (*Cucumis sativus* L.) early response to *Pseudomonas syringae* pv. lachrymans. Int J Mol Sci. 2021;22(8).
24. Yang F, Zhang Y, Huang Q, Yin G, Pennerman KK, Yu J, Liu Z, Li D, Guo A. Analysis of key genes of jasmonic acid mediated signal pathway for defense against insect damages by comparative transcriptome sequencing. Sci Rep. 2015;5:16500.
25. Mei C, Yang J, Yan P, Li N, Ma K, Mamat A, Han L, Dong Q, Mao K, Ma F *et al*. Full-length transcriptome and targeted metabolome analyses provide insights into defense mechanisms of *Malus sieversii* against *Agrilus mali*. PeerJ. 2020;8:e8992.
26. Giovino A, Bertolini E, Fileccia V, Al Hassan M, Labra M, Martinelli F. Transcriptome analysis of *Phoenix canariensis* Chabaud in response to *Rhynchophorus ferrugineus* Olivier attacks. Front Plant Sci. 2015;6:817.

27. Artico S, Ribeiro-Alves M, Oliveira-Neto OB, de Macedo LL, Silveira S, Grossi-de-Sa MF, Martinelli AP, Alves-Ferreira M. Transcriptome analysis of *Gossypium hirsutum* flower buds infested by cotton boll weevil (*Anthonomus grandis*) larvae. BMC Genomics. 2014; 15(1):854.
28. Ji YT, Xiu Z, Chen CH, Wang Y, Yang JX, Sui JJ, Jiang SJ, Wang P, Yue SY, Zhang QQ *et al*. Long read sequencing of *Toona sinensis* (A. Juss) Roem: A chromosome-level reference genome for the family Meliaceae. Mol Ecol Resour. 2021;21(4):1243–1255.
29. Yue Y, Yu R, Fan Y. Transcriptome profiling provides new insights into the formation of floral scent in *Hedychium coronarium*. BMC Genomics. 2015; 6(1):470.
30. Zhao S, Ye Z, Stanton R. Misuse of RPKM or TPM normalization when comparing across samples and sequencing protocols. Rna. 2020;26(8):903–909.
31. Abdulnour RE, Peng X, Finigan JH, Han EJ, Hasan EJ, Birukov KG, Reddy SP, Watkins JE, 3rd, Kayyali US, Garcia JG *et al*. Mechanical stress activates xanthine oxidoreductase through MAP kinase-dependent pathways. Am J Physiol Lung Cell Mol Physiol. 2006;291(3):L345-353.
32. Gil KE, Park CM. Thermal adaptation and plasticity of the plant circadian clock. New Phytol. 2019;221(3):1215–1229.
33. Wasternack C, Song S. Jasmonates: biosynthesis, metabolism, and signaling by proteins activating and repressing transcription. J Exp Bot. 2017; 68(6):1303–1321.
34. Ju L, Jing Y, Shi P, Liu J, Chen J, Yan J, Chu J, Chen KM, Sun J. JAZ proteins modulate seed germination through interaction with ABI5 in bread wheat and *Arabidopsis*. New Phytol. 2019; 223(1):246–260.
35. Howe GA, Yoshida Y. Evolutionary origin of JAZ proteins and jasmonate signaling. Mol Plant. 2019; 12(2):153–155.
36. Thireault C, Shyu C, Yoshida Y, St Aubin B, Campos ML, Howe GA. Repression of jasmonate signaling by a non-TIFY JAZ protein in *Arabidopsis*. Plant J. 2015;82(4):669–679.
37. Vranová E, Coman D, Gruissem W. Network analysis of the MVA and MEP pathways for isoprenoid synthesis. Annu Rev Plant Biol. 2013;64:665–700.
38. Pokhilko A, Bou-Torrent J, Pulido P, Rodríguez-Concepción M, Ebenhöh O. Mathematical modelling of the diurnal regulation of the MEP pathway in *Arabidopsis*. New Phytol. 2015;206(3):1075–1085.
39. Zhang F, Liu W, Xia J, Zeng J, Xiang L, Zhu S, Zheng Q, Xie H, Yang C, Chen M *et al*. Molecular characterization of the *1-deoxy-d-xylulose 5-phosphate synthas*e gene family in *Artemisia annua*. Front Plant Sci. 2018; 9:952.
40. Lu XM, Hu XJ, Zhao YZ, Song WB, Zhang M, Chen ZL, Chen W, Dong YB, Wang ZH, Lai JS. Map-based cloning of *zb7* encoding an IPP and DMAPP synthase in the MEP pathway of maize. Mol Plant. 2012;5(5):1100–1112.
41. Abbas F, Ke Y, Yu R, Yue Y, Amanullah S, Jahangir MM, Fan Y. Volatile terpenoids: multiple functions, biosynthesis, modulation and manipulation by genetic engineering. Planta. 2017;246(5):803–816.

42. Yu Z, Zhao C, Zhang G, Teixeira da Silva JA, Duan J. Genome-wide identification and expression profile of TPS gene family in dendrobium officinale and the role of *DoTPS10* in linalool biosynthesis. Int J Mol Sci. 2020;21(15).
43. Jacques S, Sperschneider J, Garg G, Thatcher LF, Gao LL, Kamphuis LG, Singh KB. A functional genomics approach to dissect spotted alfalfa aphid resistance in *Medicago truncatula*. Sci Rep. 2020;10(1):22159.
44. Chen CY, Mao YB. Research advances in plant-insect molecular interaction. F1000Res. 2020; 9.
45. Huffaker A, Pearce G, Ryan CA. An endogenous peptide signal in *Arabidopsis* activates components of the innate immune response. Proc Natl Acad Sci U S A. 2006;103(26):10098–10103.
46. Shinya T, Yasuda S, Hyodo K, Tani R, Hojo Y, Fujiwara Y, Hiruma K, Ishizaki T, Fujita Y, Saijo Y *et al*. Integration of danger peptide signals with herbivore-associated molecular pattern signaling amplifies anti-herbivore defense responses in rice. Plant J. 2018;94(4):626–637.
47. Huffaker A, Dafoe NJ, Schmelz EA. ZmPep1, an ortholog of *Arabidopsis* elicitor peptide 1, regulates maize innate immunity and enhances disease resistance. Plant Physiol. 2011;155(3):1325–1338.
48. Killiny N, Nehela Y. Metabolomic response to huanglongbing: role of carboxylic compounds in citrus sinensis response to '*Candidatus* Liberibacter asiaticus' and its vector, *Diaphorina citri*. Mol Plant Microbe Interact. 2017;30(8):666–678.
49. Woldemariam MG, Ahern K, Jander G, Tzin V. A role for 9-lipoxygenases in maize defense against insect herbivory. Plant Signal Behav. 2018;13(1):e1422462.
50. Zeng J, Zhang T, Huangfu J, Li R, Lou Y. Both allene oxide synthases genes are involved in the biosynthesis of herbivore-induced jasmonic acid and herbivore resistance in rice. Plants (Basel). 2021;10(3).
51. Body MJA, Dave DF, Coffman CM, Paret TY, Koo AJ, Cocroft RB, Appel HM. Use of yellow fluorescent protein fluorescence to track *OPR3* expression in *Arabidopsis thaliana* responses to insect herbivory. Front Plant Sci. 2019;10:1586.
52. Bosch M, Wright LP, Gershenzon J, Wasternack C, Hause B, Schaller A, Stintzi A. Jasmonic acid and its precursor 12-oxophytodienoic acid control different aspects of constitutive and induced herbivore defenses in tomato. Plant Physiol. 2014;166(1):396–410.
53. Schoenherr AP, Rizzo E, Jackson N, Manosalva P, Gomez SK. Mycorrhiza-induced resistance in potato involves priming of defense responses against cabbage looper (Noctuidae: Lepidoptera). Environ Entomol. 2019; 48(2):370–381.
54. Naidoo S, Külheim C, Zwart L, Mangwanda R, Oates CN, Visser EA, Wilken FE, Mamni TB, Myburg AA. Uncovering the defence responses of *Eucalyptus* to pests and pathogens in the genomics age. Tree Physiol. 2014;34(9):931–943.
55. Zhai Y, Li P, Mei Y, Chen M, Chen X, Xu H, Zhou X, Dong H, Zhang C, Jiang W. Three MYB genes co-regulate the phloem-based defence against English grain aphid in wheat. J Exp Bot. 2017;68(15):4153–4169.

56. He J, Bouwmeester HJ, Dicke M, Kappers IF. Genome-wide analysis reveals transcription factors regulated by spider-mite feeding in cucumber (*Cucumis sativus*). Plants (Basel). 2020;9(8).
57. Ooka H, Satoh K, Doi K, Nagata T, Otomo Y, Murakami K, Matsubara K, Osato N, Kawai J, Carninci P *et al*. Comprehensive analysis of NAC family genes in *Oryza sativa* and *Arabidopsis thaliana*. DNA Res. 2003; 10(6):239–247.
58. Wang X, Basnayake BM, Zhang H, Li G, Li W, Virk N, Mengiste T, Song F. The Arabidopsis ATAF1, a NAC transcription factor, is a negative regulator of defense responses against necrotrophic fungal and bacterial pathogens. Mol Plant Microbe Interact. 2009;22(10):1227–1238.
59. Delessert C, Kazan K, Wilson IW, Van Der Straeten D, Manners J, Dennis ES, Dolferus R. The transcription factor ATAF2 represses the expression of pathogenesis-related genes in *Arabidopsis*. Plant J. 2005;43(5):745–757.
60. Chen SP, Lin IW, Chen X, Huang YH, Chang SC, Lo HS, Lu HH, Yeh KW. Sweet potato NAC transcription factor, IbNAC1, upregulates sporamin gene expression by binding the SWRE motif against mechanical wounding and herbivore attack. Plant J. 2016;86(3):234–248.
61. He X, Zhu L, Wassan GM, Wang Y, Miao Y, Shaban M, Hu H, Sun H, Zhang X. GhJAZ2 attenuates cotton resistance to biotic stresses via the inhibition of the transcriptional activity of GhbHLH171. Mol Plant Pathol. 2018;19(4):896–908.
62. Mujiono K, Tohi T, Sobhy IS, Hojo Y, Ho NT, Shinya T, Galis I. Ethylene functions as a suppressor of volatile production in rice. J Exp Bot. 2020;71(20):6491–6511.
63. Filella I, Peñuelas J, Llusià J. Dynamics of the enhanced emissions of monoterpenes and methyl salicylate, and decreased uptake of formaldehyde, by *Quercus ilex* leaves after application of jasmonic acid. New Phytol. 2006;169(1):135–144.
64. Mageroy MH, Wilkinson SW, Tengs T, Cross H, Almvik M, Pétriacq P, Vivian-Smith A, Zhao T, Fossdal CG, Krokene P. Molecular underpinnings of methyl jasmonate-induced resistance in *Norway spruce*. Plant Cell Environ. 2020;43(8):1827–1843.
65. Yuan JS, Köllner TG, Wiggins G, Grant J, Degenhardt J, Chen F. Molecular and genomic basis of volatile-mediated indirect defense against insects in rice. Plant J. 2008;55(3):491–503.
66. Liao P, Wang H, Hemmerlin A, Nagegowda DA, Bach TJ, Wang M, Chye ML. Past achievements, current status and future perspectives of studies on 3-hydroxy-3-methylglutaryl-CoA synthase (HMGS) in the mevalonate (MVA) pathway. Plant Cell Rep. 2014;33(7):1005–1022.
67. Gaquerel E, Gulati J, Baldwin IT. Revealing insect herbivory-induced phenolamide metabolism: from single genes to metabolic network plasticity analysis. Plant J. 2014;79(4):679–692.
68. He J, Liu Y, Yuan D, Duan M, Liu Y, Shen Z, Yang C, Qiu Z, Liu D, Wen P *et al.* An R2R3 MYB transcription factor confers brown planthopper resistance by regulating the phenylalanine ammonia-lyase pathway in rice. Proc Natl Acad Sci U S A. 2020;117(1):271–277.
69. Jung B, Park J, Kim N, Li T, Kim S, Bartley LE, Kim J, Kim I, Kang Y, Yun K *et al.* Cooperative interactions between seed-borne bacterial and air-borne fungal pathogens on rice. Nat Commun. 2018;9(1):31.

70. Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q *et al*. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat Biotechnol. 2011;29(7):644–652.
71. Conesa A, Götz S, García-Gómez JM, Terol J, Talón M, Robles M. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics. 2005;21(18):3674–3676.
72. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 2000;28(1):27–30.
73. Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics. 2011;12:323.
74. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15(12):550.
75. Robinson MD, McCarthy DJ, Smyth GK. EdgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics. 2010;26(1):139–140.
76. Chen C, Chen H, Zhang Y, Thomas HR, Frank MH, He Y, Xia R. TBtools: an integrative toolkit developed for interactive analyses of big biological data. Mol Plant. 2020;13(8):1194–1202.

Tables

Table 1 Quality control data of transcriptome database

Sample	Raw reads [a]	Raw bases [b]	Clean reads [c]	Clean bases [d]	Error rate (%) [e]	Q20 (%) [f]	Q30 (%) [g]	GC content (%) [h]
TC0_1	54,065,236	8.16G	53,542,548	7.82G	0.0244	98.29	94.65	44.67
TC0_2	50,371,992	7.61G	49,863,082	7.28G	0.0245	98.25	94.56	44.35
TC0_3	52,394,376	7.91G	51,811,406	7.50G	0.0244	98.31	94.69	44.14
TC3_1	55,408,546	8.37G	54,827,552	7.91G	0.0244	98.29	94.66	43.76
TC3_2	56,207,800	8.49G	55,608,686	8.01G	0.0244	98.32	94.70	44.19
TC3_3	48,830,750	7.37G	48,260,260	6.99G	0.0247	98.18	94.41	43.98
TC12_1	55,028,124	8.31G	54,389,768	7.89G	0.0244	98.30	94.70	44.12
TC12_2	52,064,004	7.86G	51,517,728	7.47G	0.0243	98.32	94.77	44.17
TC12_3	46,074,364	6.96G	45,555,408	6.62G	0.0244	98.30	94.71	43.89
TC21_1	45,533,974	6.88G	44,964,542	6.51G	0.0246	98.19	94.46	43.44
TC21_2	53,992,626	8.15G	53,362,776	7.65G	0.0243	98.34	94.82	43.20
TC21_3	46,905,764	7.08G	46,307,772	6.67G	0.0247	98.17	94.39	43.51

[a] Count the raw sequence data, and count the number of sequences in each sample in units of four lines

[b] The number of Raw reads is multiplied by the length and converted to G as the unit

[c] Count the number of sequencing data after filtering

[d] The number of Clean reads is multiplied by the length and converted to G as the unit

[e] Sequencing error rate

[f, g] Calculate the percentage of bases with Phred values greater than 20 and 30 in the total bases, respectively

[h] Calculate the percentage of the sum of the number of bases G and C to the total number of bases

Table 2 Summary of the *T. ciliata* transcriptome

Type	Unigene
Total number	84,227
Total base	76,189,813
Largest length (bp)	13,367
Smallest length (bp)	201
Average length (bp)	904.58
N50 length (bp)	1,616
E90N50 length (bp)	2,801
Mean mapped percent (%)	65.955
GC percent (%)	38.53
Trans Rate score [a]	0.14238
BUSCO score [b]	72.0%

[a] Use TransRate to evaluate the assembly results

[b] Use BUSCO to evaluate the integrity of the assembly

Table3 Function annotation statistics of unigenes in public databases

Database	Annotated unigenes	Percent [a]
NR	39,370	46.74%
COG	33,578	39.87%
GO	33,024	39.33%
Swiss-Prot	29,937	35.54%
Pfam	27,270	32.38%
KEGG	19,021	22.58%
Total annotated	41,568	49.35%
Total unigenes	84,277	

[a] Percent of unigenes annotated to the database

Table 4 The number of unigenes in different expression levels

TPM interval [a]	0-1	1-3	3-15	15-60	>60
TC0_1	54,630(64.86%)	9,765(11.59%)	10,486(12.45%)	6,903(8.20%)	2,443(2.90%)
TC0_2	51,315(60.92%)	11,651(13.83%)	10,794(12.82%)	7,690(9.13%)	2,777(3.30%)
TC0_3	49,957(59.31%)	12,196(14.48%)	11,183(13.28%)	7,829(9.30%)	3,062(3.64%)
TC3_1	49,030(58.21%)	12,931(15.35%)	10,755(12.77%)	8,075(9.59%)	3,436(4.08%)
TC3_2	53,625(63.67%)	10,013(11.89%)	10,034(11.91%)	7,433(8.82%)	3,122(3.71%)
TC3_3	52,304(62.10%)	11,446(13.59%)	9,921(11.78%)	7,313(8.68%)	3,243(3.85%)
TC12_1	52,943(62.86%)	10,730(12.74%)	9,971(11.84%)	7,598(9.02%)	2,985(3.54%)
TC12_2	54,612(64.84%)	9,753(11.58%)	9,327(11.07%)	7,342(8.72%)	3,193(3.79%)
TC12_3	50,795(60.31%)	11,840(14.06%)	10,666(12.66%)	7,577(9.00%)	3,349(3.98%)
TC21_1	47,088(55.91%)	14,256(16.93%)	11,480(13.63%)	8,200(9.74%)	3,203(3.80%)
TC21_2	49,903(59.25%)	12,464(14.80%)	10,965(13.02%)	7,827(9.29%)	3,068(3.64%)
TC21_3	52,308(62.10%)	11,779(13.98%)	10,083(11.97%)	7,085(8.41%)	2,972(3.53%)

[a] Grouping according to Transcripts Per Million reads (TPM)

Table 5 Expression patterns of different TFs families with large differences in expression levels.

Gene ID	Description	Fold Changes [a]		
		TC3 vs TC0	TC12 vs TC0	TC2l vs TC0
	MYB			
TRINITY_DN5568_co_gl	transcription factor MYB41	9.18	6.89	2.81
TRINITY_DN4331_co_gl	MYB family transcription factor PHL5	8.88	2.50	19.36
TRINITY_DN17485_co_g1	PREDICTED. transcription factorMYB29-Hikc	10.35	7.95	13.88
TRINITY_DN10894_co gl	hypothctical protcin ClCLE_v1OO1607lmg	9.48	6.73	7.86
TRINIY_DN19687_co gl	transcription factor MYB14-likc	11.71	9.81	12.62
TRINITY_DN11922_co gl	transcription factor SRM1-like	11.57	12.19	6.19
TRINITY_DN18799_co gl	Transcription factor MYBI5	20.35	4.18	14.45
TRINITY_DN7191_co_gl	hypothctical protcin ClSIN_lg024860mg	36.80	4.60	23.27
TRINITY_DN4447_co_gl	transcription factor MYB83	-2.45	-2.84	-12.18
	AP2/ERF			
TRINITY_DN10503_co_gl	ethylene-responsive transcription factor ERFO71	10.37	7.51	15.00
TRINITY_DN4586_cO_gl	protcin LHY isoform Xl	-17.31	-19.50	-14.17
TRINITY_DN13914_c0_g1	PREDICTED: ethylene-responsive transcription factor CRF4	5.93	5.53	3.85
TRINITY_DN7131_oo_gl	PREDICTED: ethylene-responsive transcription factorERF039	6.81	3.25	2.31
TRINITY_DN2444_co_gi	cthylcnc response factor protcin 8	9.47	11.95	6.23
TRINITY_DN1335_co_g2	PREDICTED: dchydration-responsive clemcnt-binding protcin 3	-2.29	-3.29	-9.48
TRINITY_DN6538_co_gl	cthylcnc-responsive transcription factor 12	4.80	4.39	3.30
TRINIY_DN11264_c0 gl	AP2 domain-containing protcin	3.22	3.12	16.94
TRINITY_DN8732_cl_gl	hypothcticalprotcin ClSIN_1g030002mg	27.79	6.94	8.92
	C2C2			
TRINITY_DN1600_co_gl	hypothetical protcin ClSIN_1g041881mg	6.73	26.87	7.38
TRINITY_DN33653_co gl	zine fingcr protcin hangover	-2.32	-6.60	-2.75

TRINITY_DN9163_co_gl	B-box zinc finger protcin 19	-2.61	-2.70	-2.64
TRINITY_DN53864_c0_gl	cyclic dof factor 3	-9.58	-33.92	-7.78
TRINITY_DN8729_co_gl	cyclic dof factor 2	-4.30	-3.51	4.62
TRINITY_DN35051_el_gl	GATAtranscription factor 7	2.39	4.41	20.37
TRINITY_DN14674_el_g1	hypothetical protcin cUMw_095520	-3.23	-5.39	-44.52
	NAC			
TRINITY_DN10898_co_g1	NAC domain-containing protcin 2	5.65	4.27	6.38
TRINITY_DN8921_co_g2	NAC domain-containing protcin 83	10.44	2.16	3.11
TRINITY_DN8705_co_gl	NACdomain-containing protein 72	9.32	21.73	13.70
TRINITY_DN2855_co_gl	protcin ATAF2like	7.81	4.48	3.97
	C3H			
TRINITY_DN11736_c0 gl	hypothcticalprotcin CISIN_lg019627mg	5.42	3.91	3.58
TRINITY_DN9229_cl_gl	zinc finger CCCHl domain-containing protein 14	-2.42	-2.27	-15.10
TRINITY_DN9229_el_g2	zinc finger CCCH domain-containing protein 14	-2.54	-2.32	-9.93
	WRKY			
TRNITY_DN22750_co_gl	probable WRKY transcription factor 40	16.93	8.10	7.19
TRINITY_DN8189_co_g1	probable WRKY transcription factor 65	5.85	3.29	3.57
	LOB			
TRINITY_DN1947_co_gl	LOB domsin-containing protcin 41	12.74	12.59	19.77
TRINITY_DN277_co gl	LOB domsin-containing protcin l	2.09	4.15	8.61
	bHLH			
TRINITY_DN1590_c0_gl	hypothetical protein CUMW_130160	-5.04	-3.95	-24.77
	bZIP			
TRINITY_DN5147_o0_gl	basic leucine zipper 43	-2.25	-3.73	-5.35
	EIN			
TRINITY_DN2246_co_gl	protcin ETIYLENE INSENSITIVE3	4.45	4.61	2.71
	TCP			
TRINITY_DN30038 _co gl	hypotheticalprotein CICLE_v10024246mg, partial	-2.75	-3.54	-5.25

[a] Fold changes in gene expression between different samples

Table 6 Expression patterns of other pathway under HRM stress, include MAPK signaling, Phenylpropanoid biosynthesis, and Plant hormone signal transduction.

Gene ID	Description	Fold changes [a]		
		TC3 vs TC0	TC12 vs TC0	TC21 vs TC0
	MAPK signaling			
TRINITY_DN12395_c0_g1	Mitogen-activated protein kinase kinase kinase 17	4.34	7.16	6.73
TRINITY_DN2246_c0_g1	Protein ETHYLENE INSENSITIVE 3 (EIN3)	4.45	4.61	2.71
TRINITY_DN2444_c0_g1	Ethylene-responsive transcription factor 1B	9.47	11.95	6.23
TRINITY_DN8642_c0_g4	Mitogen-activated protein kinase homolog MMK2	-1.54	-2.79	-3.4
TRINITY_DN4074_c0_g1	MYC2	29.87	35.48	26.76
TRINITY_DN6448_c0_g1	Ethylene-responsive transcription factor 1B	45.66	67.11	34.2
TRINITY_DN6448_c0_g2	Ethylene-responsive transcription factor 1B	123	543.29	1309.75
	Phenylpropanoid biosynthesis			
TRINITY_DN8222_c0_g2	Feruloyl CoA ortho-hydroxylase F6H1-2	196.55	32.36	71.55
TRINITY_DN8222_c0_g4	Feruloyl CoA ortho-hydroxylase F6H1-2	757.67	114.58	171.58
TRINITY_DN8253_c0_g1	Feruloyl CoA ortho-hydroxylase F6H1-2	3.05	4.05	3.67
TRINITY_DN37385_c0_g1	Feruloyl CoA ortho-hydroxylase F6H1-2	1250.96	352	353.71
TRINITY_DN3871_c0_g1	Feruloyl CoA ortho-hydroxylase F6H1-3	124.78	30.78	36.72
TRINITY_DN23631_c0_g1	Peroxidase 64	-7.61	-3.19	-17.66
TRINITY_DN31330_c0_g2	Peroxidase 47	-1.27	-3	-11.29
TRINITY_DN48274_c0_g2	Peroxidase 17	-1.89	-3.27	-2.9
	Plant hormone signal transduction			
TRINITY_DN17305_c0_g1	Protein ABSCISIC ACID-INSENSITIVE 5	-44.42	-8.08	-4.85
TRINITY_DN9180_c0_g1	Indole-3-acetic acid-amido synthetase GH3.6	-3.22	-5.44	-17.18

TRINITY_DN768_c0_g2	Auxin-responsive protein IAA29	4.56	3.46	8.41

[a] Fold changes in gene expression between different samples

Figures

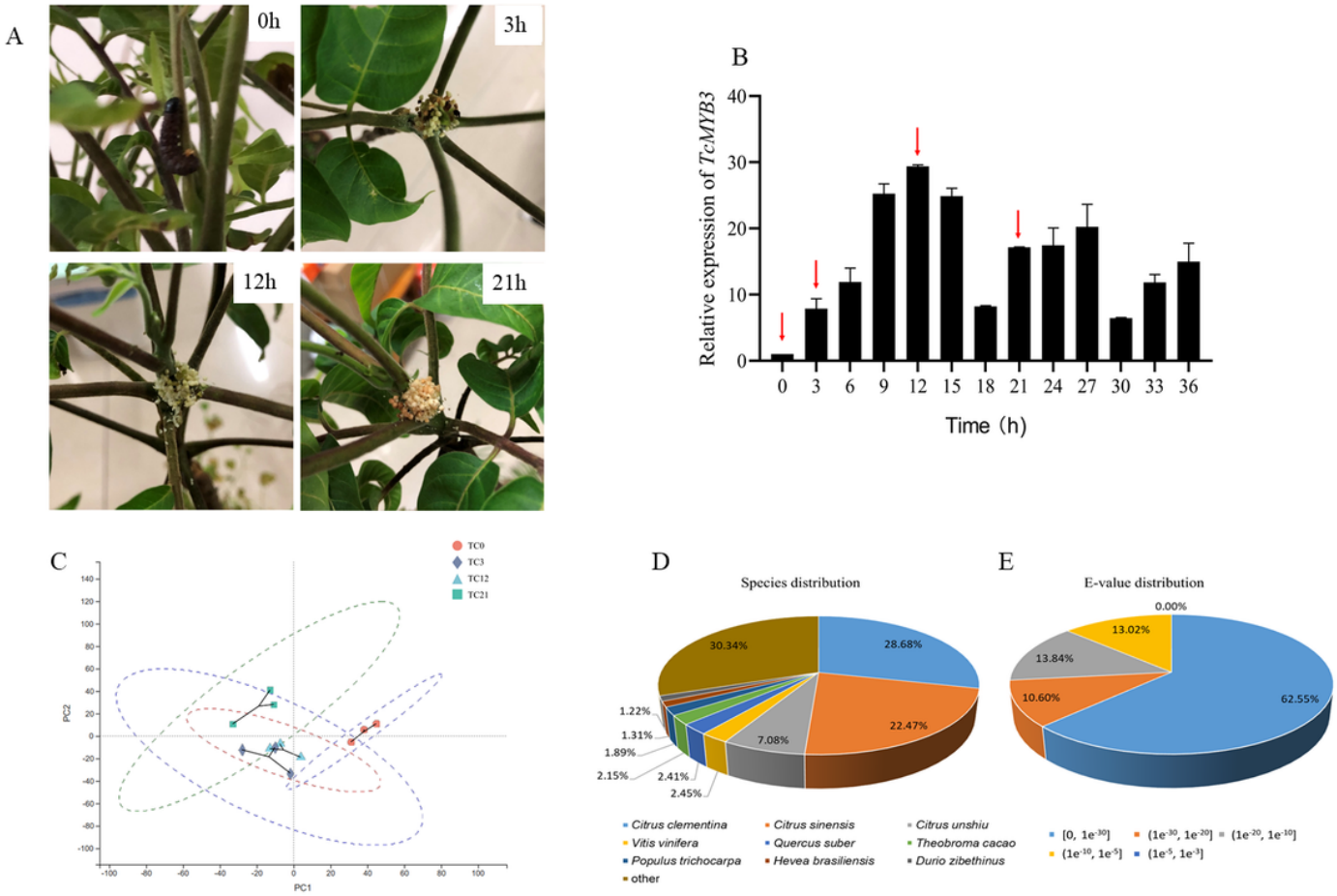

Figure 1

(A) The picture of *T. ciliate* was fed 0, 3, 12, 21h by SHM.(B) The relative expression of *TcMYB3* in different times of SHM feeding. (C) PCA analysis of transcriptome among four samples. (D) species distribution of the top 10 and (E) E-value after a single gene BLAST. The cut-off values for BLAST search set at 1.0e−5

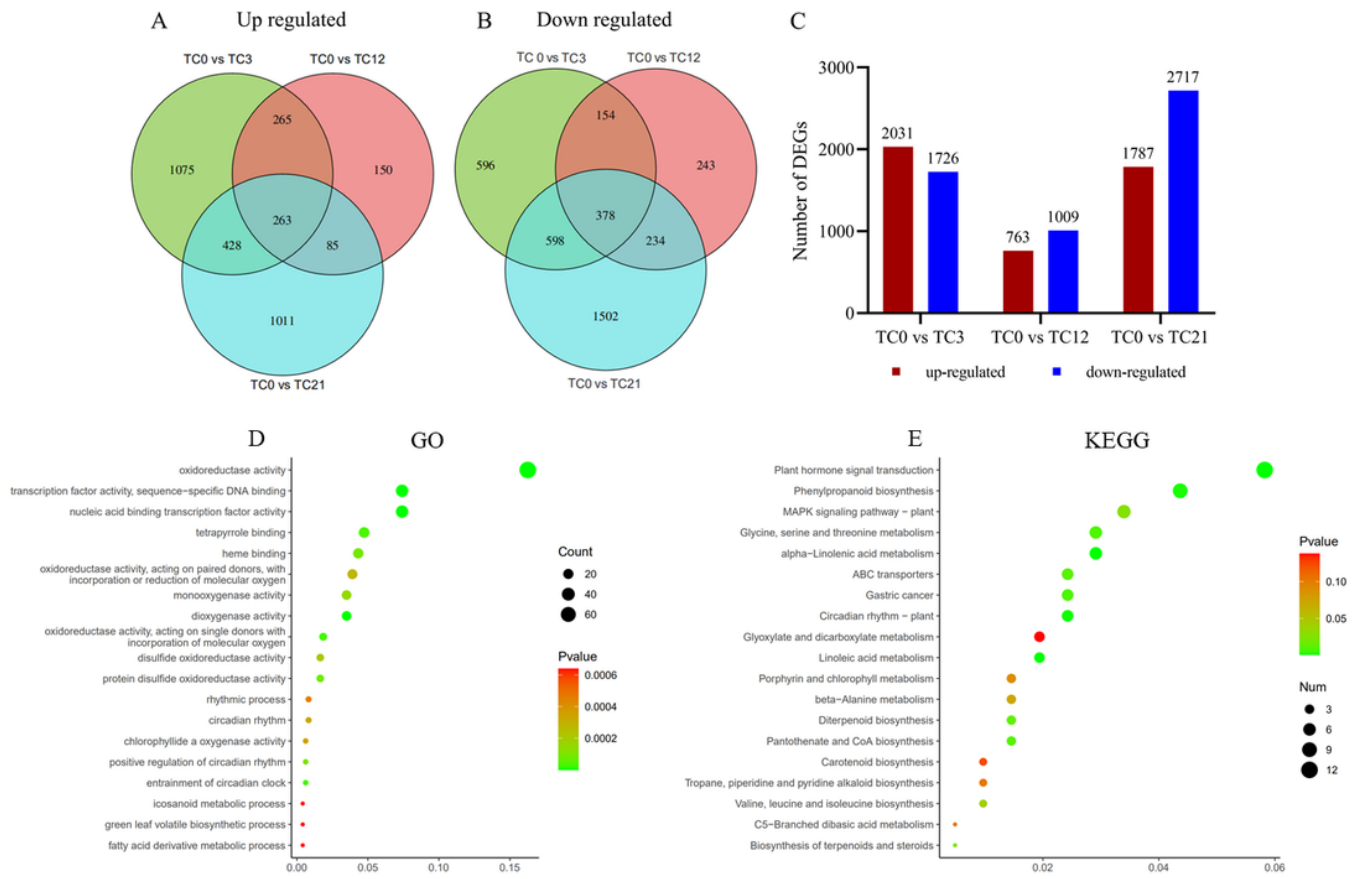

Figure 2

Gene expression comparisons. A: Venn diagram of number of up regulated DEGs. B: Venn diagram of number of down regulated DEGs. C: Changes in gene expression profile. The numbers of up-regulated and down-regulated genes between TC0 and TC3, TC0 and TC12, TC0 and TC21 are summarized. Results from the GO (D) and KEGG (E) pathway enrichment analysis for DEGs. Color indicates the P-value. The 19 most enriched pathways are listed (left).

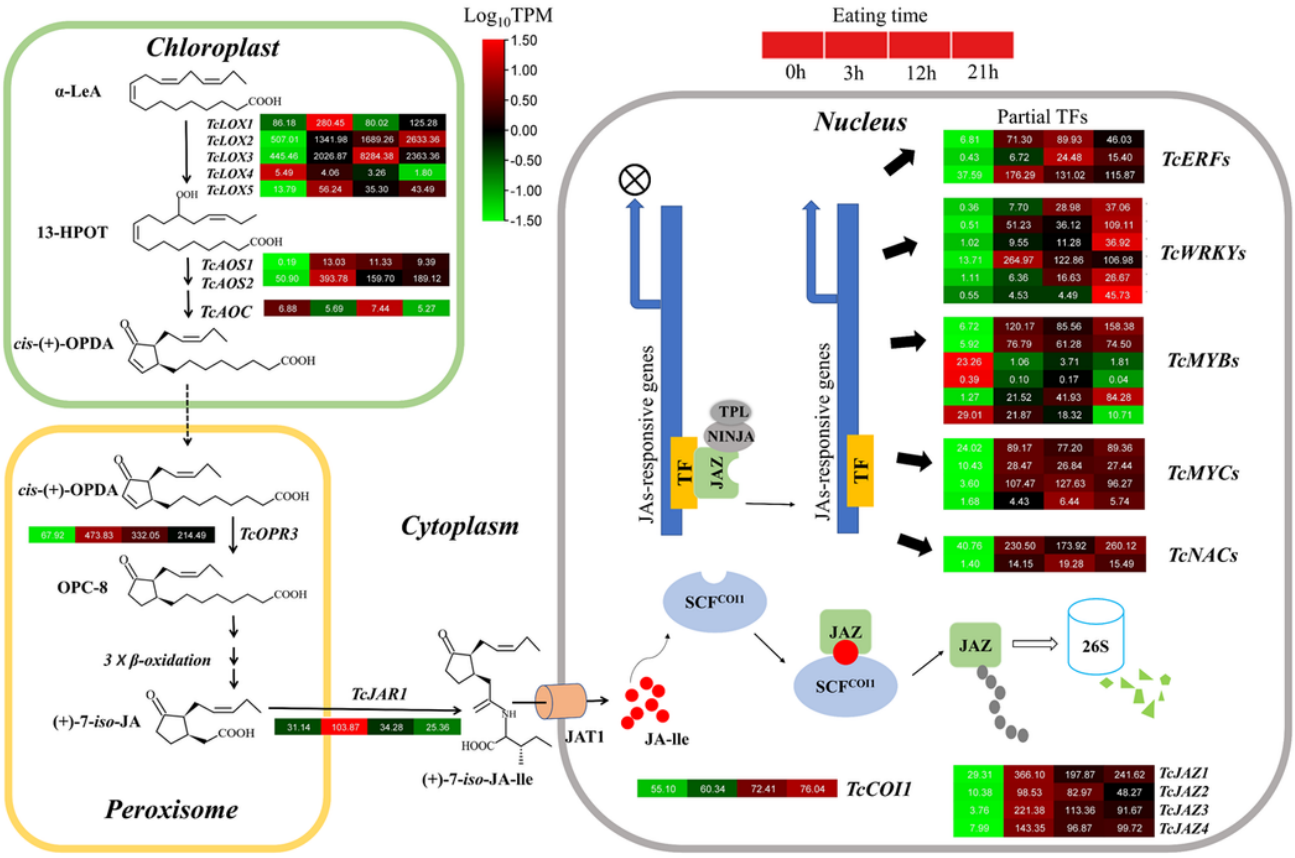

Figure 3

Gene expression pattern diagram of JA metabolism and signal transduction pathway. In the absence of abiotic stimuli or low levels of JA-Ile, transcription factors are inhibited by the JAZ protein, thereby preventing their activation of the promoters of jasmonic acid-responsive genes. Abiotic stresses elevate JA synthesis, which is readily epimerized to JA-Ile. The latter is then transported to the nucleus by the JAT1 transporter. JA-Ile facilitates the interaction of JAZ with the F-box protein COI1 within the SCF complex, leading to the proteasomal degradation of JAZ. The names of enzymes in each catalytic stage are represented by abbreviations and italics in bold. Color scales indicate gene expression levels(Log10TPM) at different time stages of HRM. Each horizontal band represents a gene, and those with more than one homologous gene are ranked in Arabic order. Gene ID of this pathway all gene are listed Table S2.

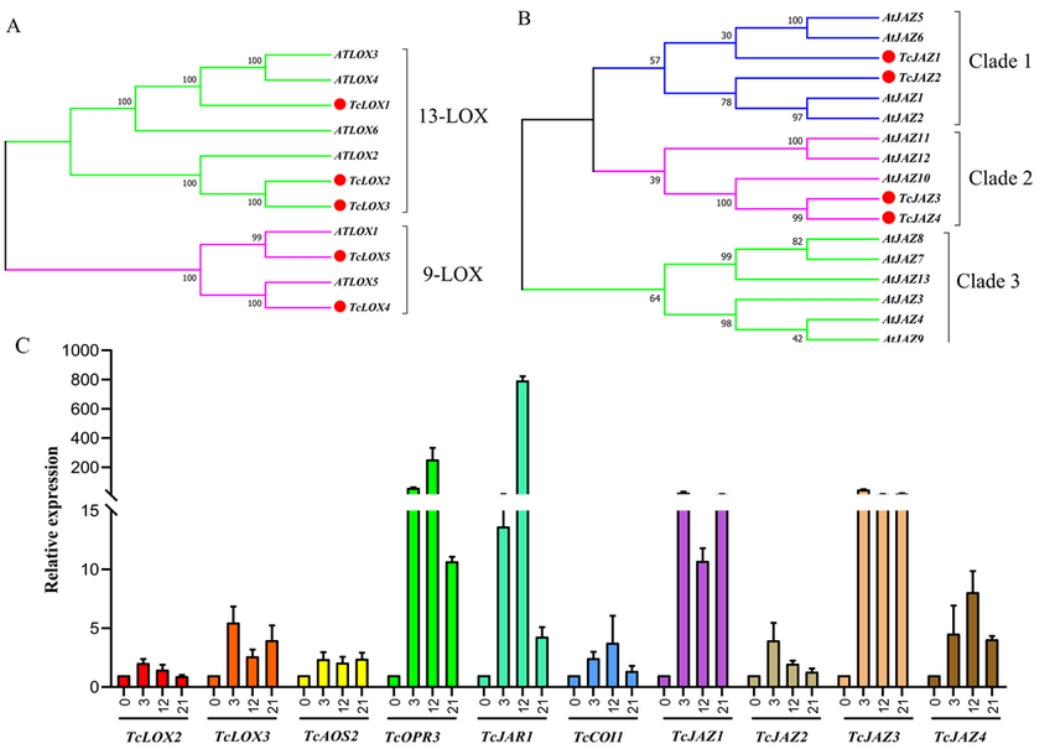

Figure 4

Phylogenetic trees of *T. ciliata LOXs* and *JAZs* and RT-qPCR analysis. The trees was constructed with the Maximum Likelihood method using MEGA program 7.0 with 500 bootstrap. A: Phylogenetic tree of *LOXs* of *T. ciliata* and *Arabidopsis*. Five *LOXs* of *T. ciliata* named *TcLOX1-5* and are divided into 2 subfamilies, including 13-LOX and 9-LOX. B: Phylogenetic tree of *JAZs* of *T. ciliata* and *Arabidopsis.* C: Gene relative expression level of young stems on JA pathway.

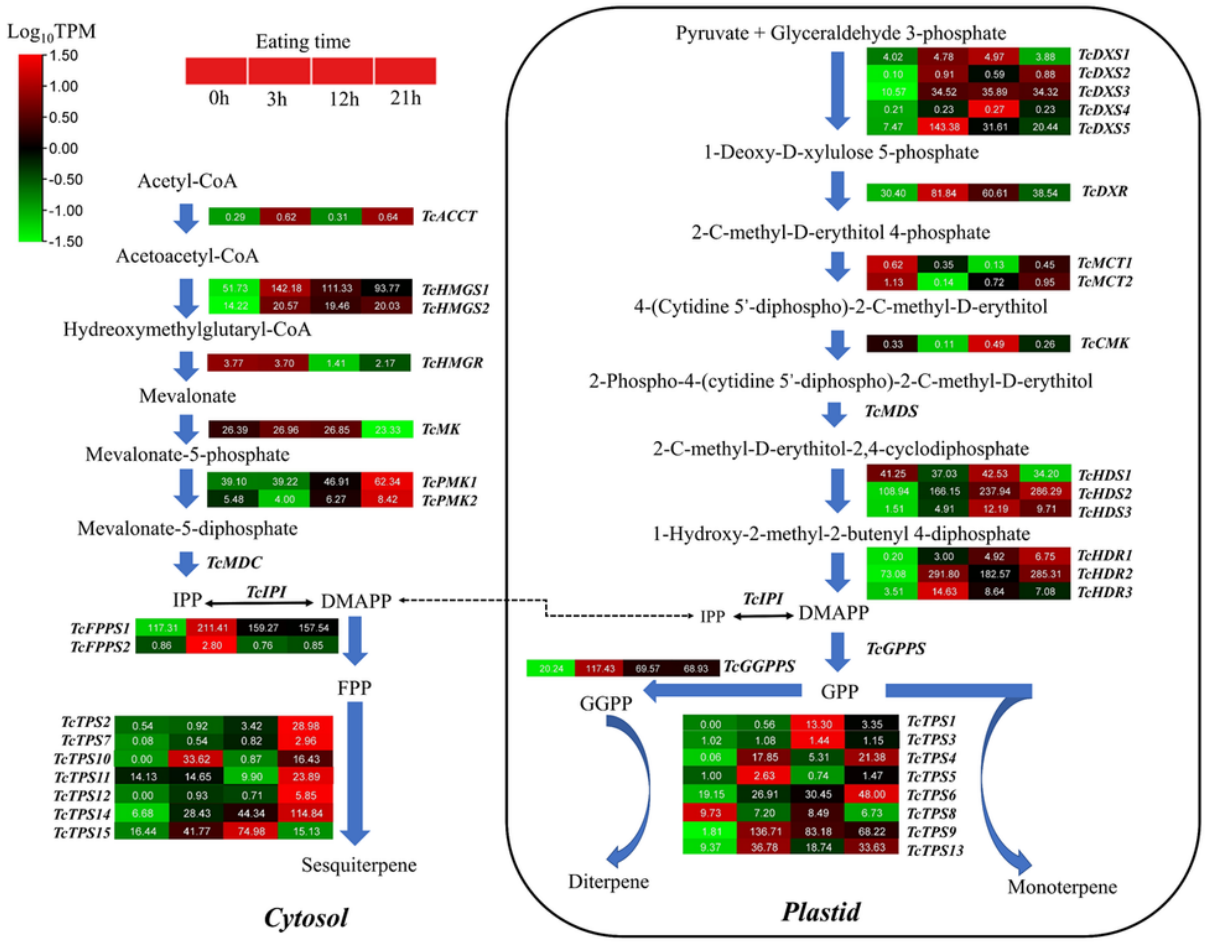

Figure 5

Gene expression patterns related to the biosynthesis of monoterpenes, sesquiterpenes and triterpenes. The names of enzymes in each catalytic stage are represented by abbreviations and italics in bold. Color scales indicate gene expression levels (Log10TPM) at different time stages of HRM stress. Each horizontal band represents a gene, and those with more than one homologous gene are ranked in Arabic order. Gene ID of this pathway all gene are listed Table S2.

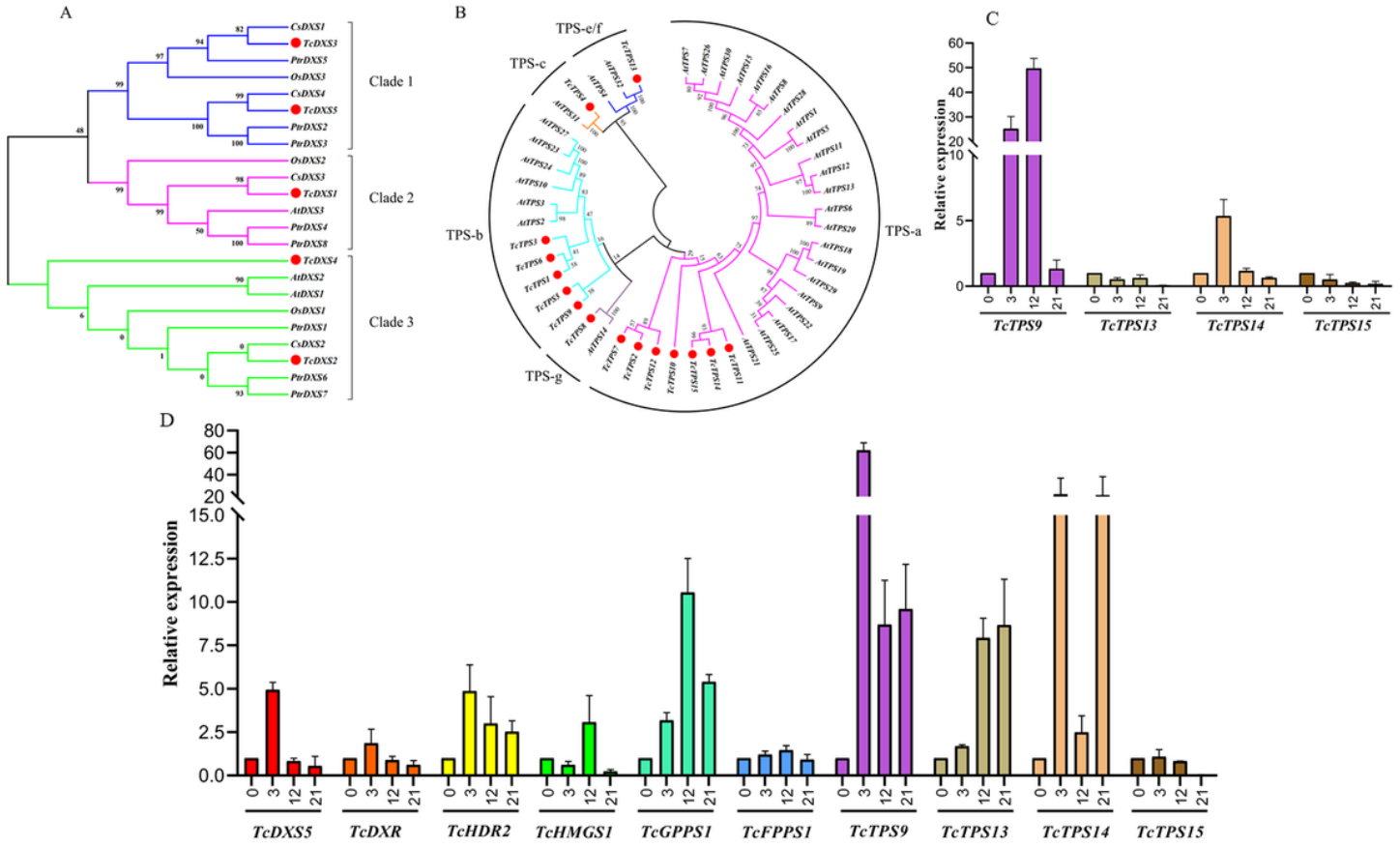

Figure 6

Phylogenetic trees of *T. ciliata DXSs* and *TPSs* and RT-qPCR analysis. The trees was constructed with the Maximum Likelihood method using MEGA program 7.0 with 500 bootstrap. A: Phylogenetic tree of plant *DXSs*. Five *DXSs* of *T. ciliata* (named *TcDXS1-5*); *At, Arabidopsis; Cs, Citrus sinensis; Os, Oryza sativa; Ptr, Populus trichocarpa*. B: Phylogenetic tree of *TPSs* of *T. ciliata* and *Arabidopsis*; All *TPSs* are divided into 5 subfamilies, including TPS-a, b, c, g and e/f. : Gene relative expression level of leaves (C) and young stems (D) on MEP or MVA pathway.

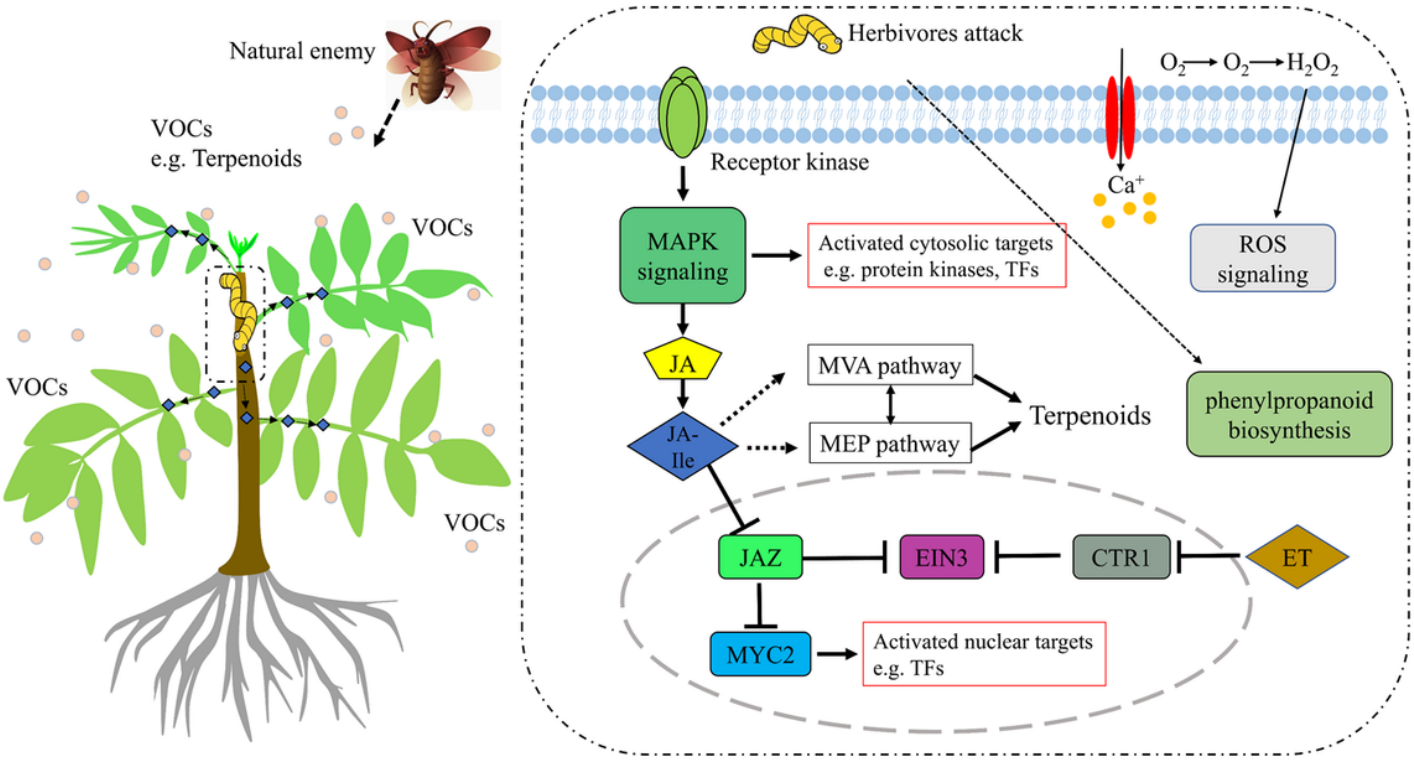

Figure 7

A hypothetical model of *T. ciliata* in repones to HRM. The response pathways include MAPK signaling, Plant hormone signaling (JA, ET), ROS signaling, and phenylpropanoid biosynthesis. MAPK: mitogen-activated protein kinases, ET: ethylene, EIN3: ethylene insensitive 3, CTR1: constitutive triple response 1, ROS: Reactive Oxygen Species. The dark blue diamond represents JA-Ile

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