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Role of jasmonate signaling in rice resistance to the leaf folder *Cnaphalocrocis medinalis*

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Abstract

Key message **Jasmonate-induced accumulation of anti-herbivore compounds mediates rice resistance to the leaf folder *Cnaphalocrocis medinalis*.**

Abstract The rice leaf folder (LF), *Cnaphalocrocis medinalis*, is one of the most destructive insect pests in the paddy field. LF larvae induces leaf folding and scrapes the upper epidermis and mesophyll tissues reducing photosynthesis and yield in rice. Identifying plant defense pathways and genes involved in LF resistance is essential to understand better this plant–insect interaction and develop new control strategies for this pest. Jasmonate (JA) signaling controls a plethora of plant defenses against herbivores. Using RNA-seq time series analysis, we characterized changes in the transcriptome of wild-type (WT) leaves in response to LF damage and measured the dynamics of accumulation of JA phytohormone pools in time-course experiments. Genes related to JA signaling and responses, known to mediate resistance responses to herbivores, were induced by LF and were accompanied by an increment in the levels of JA pools in damaged leaves. The accumulation of defense compounds such as phenolamides and trypsin proteinase inhibitor (TPI) also increased after LF infestation in WT but not in JA mutant plants impaired in JA biosynthesis (*aoc-2*) and signaling (*myc2-5*). Consistent with all these responses, we found that LF larvae performed better in the JA mutant backgrounds than in the WT plants. Our results show that JA signaling regulates LF-induced accumulation of TPI and phenolamides and that these compounds are likely an essential part of the defense arsenal of rice plants against this insect pest.

Keywords Jasmonate (JA) · Rice leaf folder · Defense response · Phenolamide · Trypsin proteinase inhibitor (TPI)

Introduction

Plants encounter numerous insect herbivores during their lifespan. Considering their feeding types, the damage caused by herbivores varies among species. In order to cope with different herbivore attacks, plants have developed sophisticated defense strategies, including the recognition of different herbivores, signal transduction mechanisms, and the activation of exquisitely fine-tuned defense responses (Erb and Reymond 2019). The jasmonate (JA) phytohormone signaling pathway orchestrates multiple plant responses to herbivory (Howe et al. 2018). Different compounds from oral and oviposition secretions from insects such as metabolites, peptides, and proteins act as ligands for membrane receptors in plant cells, activating signaling cascades that lead to the JA burst at the wounding sites (Arimura 2021).

JA synthesis occurs via the octadecanoid pathway, which has been extensively characterized in the model plant *Arabidopsis*. JA biosynthesis initiates in the chloroplast with the release of α-linolenic acid (18:3) from membranes and the sequential actions of lipoxygenase (LOX), allene oxide synthase (AOS), and allene oxide cyclase (AOC) proteins releasing 12-oxo-phytodienoic acid (OPDA). OPDA is then converted into jasmonic acid in the peroxisome by OPDA reductase 3 (OPR3) via three cycles of beta oxidations (Wasternack and Hause 2013). In addition, JA biosynthesis can also occur through an OPR3-independent pathway (Chini et al. 2018). There are multiple Jasmonic acid

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conjugates in the cytoplasm, of which jasmonoyl-L-isoleucine (JA-Ile) is the bioactive form. In the cell nucleus, the JA co-receptors CORONATINE-INSENSITIVE1 (COI1) and JASMONATE ZIM domain (JAZ) bind to JA-Ile, activating JA-responsive transcription factors (TFs) involved in the regulation of the biosynthesis of plant defense compounds (Fonseca et al. 2009; Sheard et al. 2010).

Plants produce hundreds of thousands of compounds involved in growth, reproduction, and responses to biotic and abiotic stress. Secondary metabolites play a central role in plant–insect interactions (Erb and Kliebenstein 2020). The nitrogen-containing compounds nicotine and glucosinolates (GSs) are potent anti-herbivore compounds in tobacco and plants of the *Brassicaceae* family, respectively. The JA-responsive MYC2 TFs directly regulate the synthesis of nicotine, GSs (Schweizer et al. 2013; Li et al. 2018), and terpenes, another group of secondary metabolites with a function in both direct and indirect defense against herbivores (Xiao et al. 2012; Li et al. 2014). In the wild tobacco plant *Nicotiana attenuata*, the specialist herbivore, *Manduca sexta*, is tolerant to nicotine but susceptible to JA-induced metabolites that result from the association of phenolic acids with aliphatic or aromatic amines, namely phenolamides (Kaur et al. 2010; Kumar et al. 2014; Figon et al. 2021). Thus, different plant species produce specialized metabolites to cope with particular herbivores.

Rice is a staple crop worldwide and a model monocot species. In the paddy field, rice plants are frequently attacked by multiple herbivores. The striped stem borer (SSB, *Chilo suppressalis*) is a chewing herbivore that damages rice leaf sheath and stem. Silencing the JA biosynthetic genes herbivore-induced LOX (*HI-LOX*) and phospholipase D (*PLD*) α4/α5 attenuates SSB-mediated induction of trypsin proteinase inhibitor (TPI) accumulation and the release of volatile compounds, compromising plant resistance to this insect pest (Zhou et al. 2009; Qi et al. 2011). JA also mediates root responses to herbivory. JA-deficient rice plants attacked by the cucumber beetle *Diabrotica balteata* or the rice water weevil larva *Lissorhoptrus oryzophilus* lose more root biomass than their control counterparts (Lu et al. 2015). Similarly, JA regulates rice resistance against the brown planthopper (BPH) *Nilaparvata lugens*, a piercing-sucking herbivore. Loss of function mutations in the JA biosynthetic gene *AOC* and the TF *MYC2* reduces the levels of BPH-elicited defensive compounds and rice resistance to BPH both in the laboratory and field conditions (Xu et al. 2021). The rice leaf folder (LF) *Cnaphalocrocis medinalis* is another major insect pest in the paddy field. LF larvae produces the folding of leaves longitudinally by spinning silk and scrapes cells of the upper epidermis and mesophyll tissues, leaving only the lower epidermis, and forming a linear pale white stripe in attacked leaves. Consequently, plants damaged by LF are compromised in their photosynthetic capacity, especially in flag leaves during the booting stage, resulting in yield loss (Padmavathi et al. 2013). Few studies have explored the role of JA in rice defense against LF. Exogenous application of methyl jasmonate (MeJA) was found to increase the mortality of LF larvae and reduced larval weight and leaf damage (Senthil-Nathan 2019). Silencing the JA co-receptor *COI1* by RNA interference decreased the accumulation of peroxidase, polyphenol oxidase and TPI and reduced plant resistance against LF (Ye et al. 2012). The over-expression of the potato proteinase inhibitor II gene in rice enhanced plant resistance to LF, suggesting that endogenous TPI is an anti-LF compound (Kumar et al. 2009). However, other defensive compounds against LF in rice remain to be identified, and a comprehensive study is required to elucidate the role of JA signaling in the rice-LF interaction.

In this study, we address how JA signaling contributes to LF resistance in rice. Using a time-series RNA-seq experiment, we show that JA signaling and responses are up-regulated in response to LF damage, a response that correlates with the increased accumulation of JA pools. Moreover, we found increased LF performance in plants impaired in JA biosynthesis and signaling, and we identified herbivore-induced compounds as potential mediators of this plant–insect interaction. In summary, we show that JA signaling and responses contribute to rice resistance to the leaf miner *C. medinalis*. A result that opens potential avenues to develop new control strategies for this insect pest.

Materials and methods

Plant growth and insect rearing

The rice variety japonica XiuShui 11 (XS11) was used as wild-type (WT) plant. The rice mutants *aoc-2* and *myc2-5* were obtained and screened as previously described in Xu et al. 2021. Seeds were embedded in water for 8 days in an illuminated incubator at 28 ± 2 °C and sown in a hydroponic cultivation system as described in Li et al. 2015. 28-day-old plants were used for all experiments. *C. medinalis* were initially obtained from paddy fields at the Zhejiang University in Hangzhou, China, and kept in a climate chamber at 25.5 ± 1 °C and 65 ± 10% RH under 14 h light.

Plant treatments and sample collections

For LF treatments, a fourth-instar *C. medinalis* larva starved for 2 h was placed into a fully expanded leaf. Treated leaves were collected at 0.5, 1, 3, 8, and 24 h, and leaves from non-treated plants at the same time-points for comparisons were used as controls. The samples were frozen in liquid nitrogen and stored at − 80 °C.

Phytohormone analyses

A total of 100 mg of leaf material was ground and extracted in 1 mL of ethyl acetate containing 20 ng of D6-JA and 5 ng of D6-JA-Ile as internal standards. Extracts were analyzed by liquid chromatography-mass spectrometry using a LCMS-8040 (Shimadzu) as described previously (Xu et al. 2021). JA, OH-JA and OPDA were quantified using the internal standard D6-JA, and JA-Ile and OH-JA-Ile with D6-JA-Ile.

RNA sequencing

Total RNAs were extracted using MiniBEST Plant RNA Extraction Kit (TaKaRa). RNA sequencing was performed by the Novogene company (https://www.novogene.com/). In brief, the total RNA without rRNA was used to construct the double-strand cDNA libraries. Sequencing was performed on an Illumina Hiseq platform. Three replicates were used in each treatment.

Transcriptome analysis

Adaptor sequences and low-quality reads were removed using TRIMMOMATIC (Bolger et al. 2014). Clean reads were mapped to the rice reference genome (http://rice.plantbiology.msu.edu/pub/data/Eukaryotic_Projects/o_sativa/annotation_dbs/pseudomolecules/) using HISAT2 (Kim et al. 2015). STRINGTIE was used to obtain read counts and data normalization using transcripts per million (TPM; Pertea et al. 2015). TPM values of all genes were used for principal component analysis (PCA). PCA was performed using R package GGORD. DEGs were analyzed using the R package Limma (Ritchie et al. 2015) and gene ontology (GO) analysis of up-regulated DEGs was performed using CLUEGO (Bindea et al. 2009). Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of up-regulated DEGs was performed using clusterProfiler (Yu et al. 2012).

Quantitative RT-PCR

800 ng of total RNA for each sample was reverse transcribed using the HiScriptIII QRT SuperMix for qPCR (Vazyme). Five biological replicates were used in each treatment. The primers used are listed in Supplemental Table S1. RT-qPCR was performed on the CFX96 Touch (BioRad) using the Taq Pro Universal SYBR qPCR Master Mix (Vazyme). Relative quantification was performed using *UBI* as a reference gene.

Secondary metabolite measurements

Approximately 50 mg of leaf material (precise mass was recorded) was ground to a fine powder and extracted twice with 800 and 500 µL of 70% methanol. The supernatants were combined, and methanol was evaporated using a nitrogen blower. The remaining aqueous phase was freeze-dried using a vacuum freeze dryer. The dried sample was dissolved with 150 µL of 70% methanol and analyzed via liquid chromatography–mass spectrometry with an electro-spray ionization source with a 6460 Triple Quad Mass Spectrometer (Agilent). All standard phenolamides used in the experiments are chromatographic grade and obtained from Hangzhou Chemipanda Bio-Tech CO., Ltd (China). The standard curve method was used to calculate the compound content. Six biological replicates were used in each treatment.

TPI analysis

Approximately 50 mg of leaf material was homogenized with 300 µL of cold extraction buffer (0.1 M Tris-C1, pH 7.6, 5% polyvinylpolypyrrolidone, 2 mg/mL phenylthiourea, 5 mg/mL diethyldithiocarbamate, 0.05 M Na_2EDTA). Trypsin proteinase inhibitor (TPI) activity was measured using a radial diffusion assay as previously described (Van Dam et al. 2001). Six biological replicates were used in each treatment.

Bioassays

All the LF bioassays were conducted in a climate chamber at 25.5 ± 1 °C and 65 ± 10% RH under 14 h light. For LF performance assay, one fourth-instar *C. medinalis* larvae starved for 2 h, was weighed and allowed to feed on each treated plant. After 48 h, the mass of each larvae was recorded again. The increased percentage of larval mass on each plant was calculated. Approximately 25 replicates were used for each genotype. For LF feeding assays, a third-instar larva was allowed to feed on the first extended leaf for 24 h. Leaves were then excised, photographed and the consumed leaf area was measured using ImageJ.

Data access

The raw sequence data is available at the Genome Sequence Archive at the Beijing Institute of Genomics (BIG) Data Center (http://bigd.big.ac.cn/gsa) of the Chinese Academy of Sciences, under accession no. CRA004405.

Results

LF induces the up-regulation of JA signaling and defense response genes

To investigate the transcriptional responses associated with LF infestation in rice, we performed a time-course transcriptome experiment. We treated plants with LF larvae, and leaf samples were collected at the early (0.5 h and 1 h) and late (3 h, 8 h and 24 h) time-points after the initiation of the experiment. Non-treated plants at each time point were used as controls. Exploratory analysis of the RNA-seq data using a principal component analysis (PCA) with the gene expression values of all genes showed that biological replicates in each treatment clustered together (Fig. 1a). This unbiased analysis also clearly discriminated LF-treated and control samples at each time-point, indicating that LF infestation causes a large transcriptome rearrangement in attacked leaves. We next analyzed the RNA-seq data using the statistical package Limma to identify genes whose expression varied between LF-treated and control groups. We found a total of 6309 differentially expressed genes (DEGs) (FC > 2; adjusted p value < 0.05; Supplemental Table S2). Among these genes, those with up-regulated trend at all time points were defined as up-regulated DEGs. A total of 2,388 up-regulated DEGs were screened, and the number of genes in each inducible pattern was analyzed. The largest three sets comprised DEGs induced by LF after 8 and 24 h of treatment (Fig. 1b). Only 78 DEGs were up-regulated across all time points. To get functional insights into these up-regulated genes, we performed a gene ontology (GO) analysis. We found that JA and defense-related terms were significantly enriched in the set of up-regulated genes (Fig. 1c), suggesting that the JA signaling pathway is involved in the LF-rice interaction.

LF-infestation increases the accumulation of JAs in leaves

To further delineate the role of JA during LF infestation, we explored the transcriptional changes of genes involved in JA biosynthesis and signaling that were previously published or identified based on sequence homology to known JA genes (Xu et al. 2021). We found multiple biosynthetic and signaling genes largely up-regulated in LF-treated plants (Fig. 2a). To further confirm if JA biosynthesis was affected by LF, we measured different forms of JA in LF-treated and control leaves. Levels of jasmonic acid and JA-Ile were significantly increased at 0.5 h after the initiation of LF treatment, while the concentrations of OH-JA-Ile and OPDA increased from 1 h (Fig. S1a, S1b, S1d and S1e; Fu et al. 2021). The inactive OH-JA levels did not differ between LF-treated and control leaves (Fig. S1c). We found that the pool of JAs significantly increased from 3 h after LF infestation (Fig. 2b). These results indicate that LF feeding leads to rapid and sustained induction of JA biosynthesis.

JA regulates LF-induced TPI levels

To evaluate the function of JA in rice and LF interaction, we measured levels of known and putative defense compounds in JA mutants and WT plants in response to LF attack. Rice genome encodes one copy of *AOC* and *MYC2* genes, and loss-of-function mutations disrupt JA biosynthesis and signaling, respectively (Xu et al. 2021). Therefore, we measured the known anti-LF compound proteinase inhibitor (TPI) levels in WT the JA mutant plants generated by genome editing, namely, *aoc-2* and *myc2-5*. We found that TPI accumulation was dramatically elevated in WT plants at 48 h after LF treatment. LF-induced TPI levels were abolished in *aoc-2* lines and significantly reduced in *myc2-5* lines compared to the WT controls (Fig. 3).

JA mediates the induction of phenylpropanoid biosynthetic genes in LF treated plants

To investigate other secondary metabolites involved in JA-dependent responses in rice to LF infestation, we performed a KEGG enrichment analysis of up-regulated DEGs in response to LF treatment. Interestingly, we found that the phenylpropanoid biosynthetic pathway was enriched in this group of genes (Fig. S2). Cluster analysis of genes in this biosynthetic pathway indicated a set of up-regulated genes in response to LF treatment such as *phenylalanine ammonia-lyases* (*PAL*s), and *spermidine hydroxycinnamoyl transferases* (*SHT*s) (Fig. 4a). Six of these genes showed higher expression levels at 24 h after LF treatment as determined by RT-qPCR. The genes *PAL6*, *PAL7*, and *4-coumarate:coenzyme A ligase-like 6* (*4CL6*) are initial reaction enzymes in the phenylpropanoid pathway, while *tryptamine hydroxycinnamoyl transferase* (*THT1*), *SHT1* and *SHT2* regulate downstream reactions for phenolamide biosynthesis (Dong et al. 2015; Peng et al. 2016). The transcript levels of all six genes were significantly decreased in JA mutants compared with WT plants (Fig. 4b–g). Notably, the expression of these genes was abolished entirely in the *aoc-2* mutant after LF infestation.

LF induces phenolamide accumulation in a JA-dependent manner

To further examine if the altered expression of phenolamide biosynthetic genes could influence the accumulation

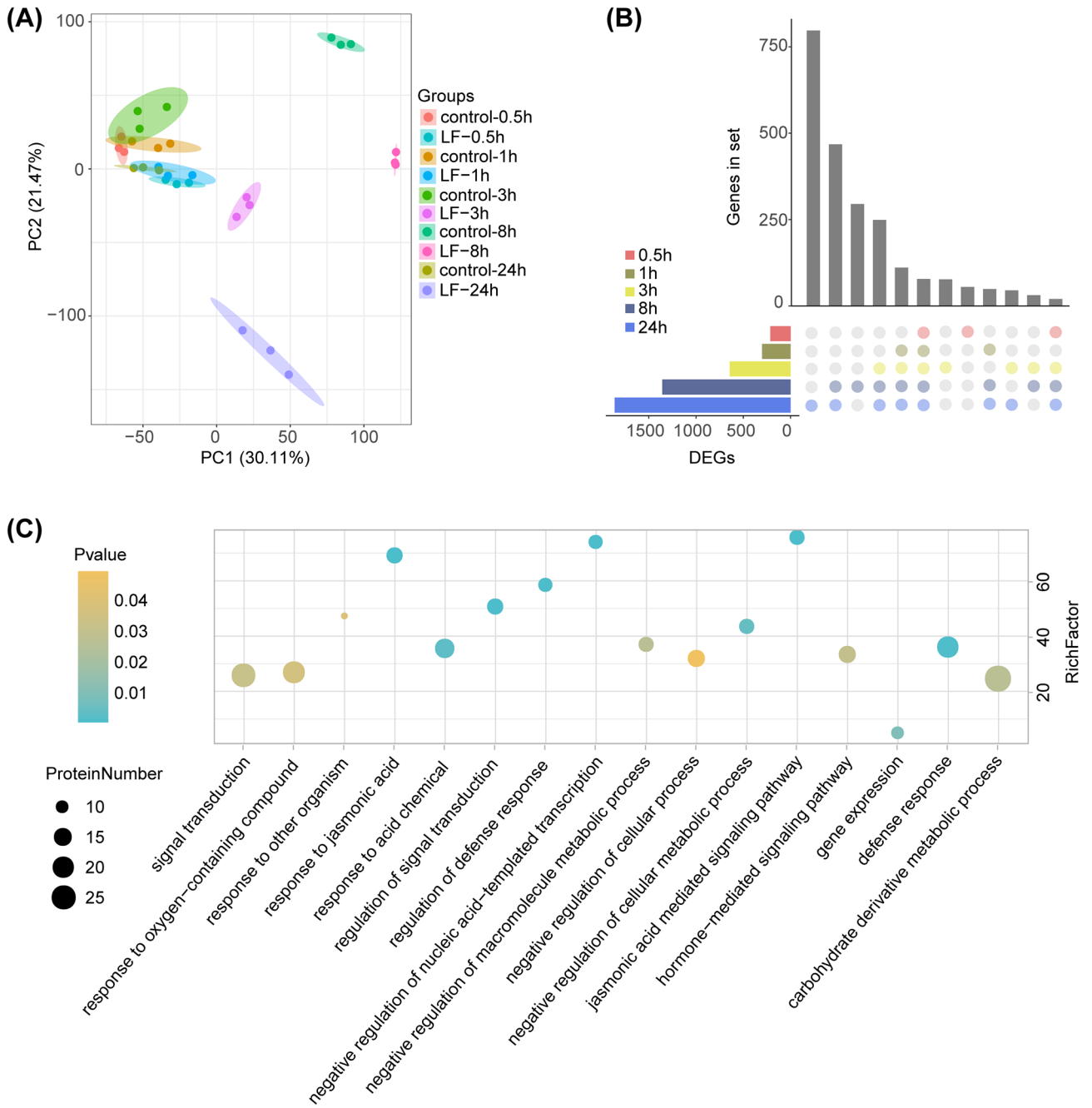

Fig. 1 Transcriptional responses in leaves of LF-treated plants. **a** Principal component analysis (PCA) of RNA-seq data on samples of control and LF-treated plants. Three biological replicates at each time point were used for RNA-seq analysis. **b** UpSet diagram showing the number of differentially expressed genes (DEGs) up-regulated at each time point of treatment (left) and the top 12 interactions (bottom right) by size (top right). The cutoff of DEGs was fold-change > 2 and adjusted p value < 0.05. **c** Gene ontology (GO) analysis of DEGs up-regulated upon LF attack. Only GO terms with a $p < 0.05$ are listed

of these secondary metabolites, we quantified nine different phenolamides present in rice plants. The levels of all examined phenolamides were significantly increased in WT plants 48 h after LF infestation, and the basal levels of cinnamoyl putrescine, feruloyl putrescine, and mustard acyl putrescine were lower in *aoc-2* lines than in WT plants (Fig. 5a–c). Consistent with the gene expression, LF-elicited phenolamide levels were decreased in the two JA mutant plants compared to the controls (Fig. 5a–i) except for p-coumaroyl agmatine, which was reduced in *aoc-2* but not in *myc2-5* (Fig. 5d). Overall, we found a considerable reduction in all measured phenolamides in

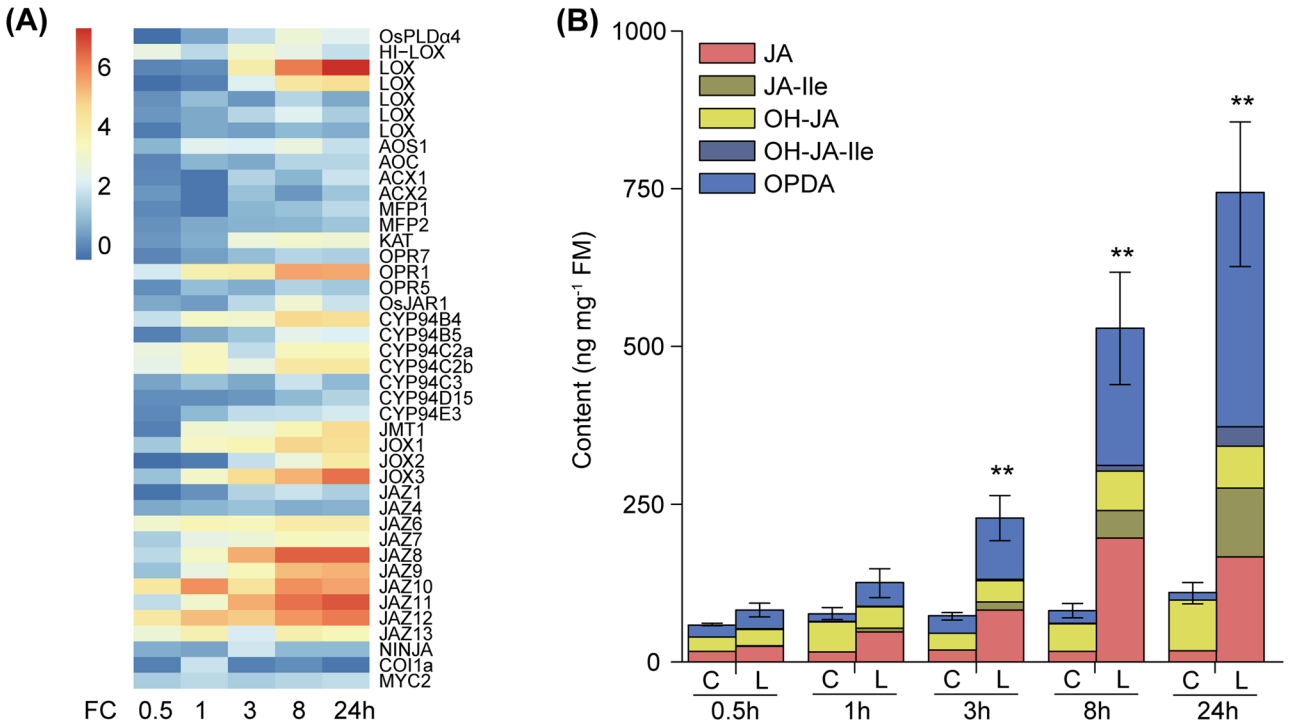

Fig. 2 Changes in JA signaling and biosynthesis in LF-treated rice leaves. **a** Heat map representing the transcript levels of genes in the jasmonate (JA)-signaling pathway in LF-treated samples compared to their respective controls. The color gradient represents $\log_2$ fold changes between control and treated plants. FC, fold change. **b** Mean concentration (SE, n = 5) of JAs in LF-treated plants (L) and control plants (C). Asterisks indicate significant differences in treated plants compared to control plants (**$p < 0.01$; Student's t-test). FM, fresh mass; JA, jasmonic acid; JA-Ile, jasmonyl-L-isoleucine; OH-JA-Ile, 12-hydroxy-JA-Ile; OPDA, 12-oxo-phytodienoic acid; OH-JA, hydroxy-JA

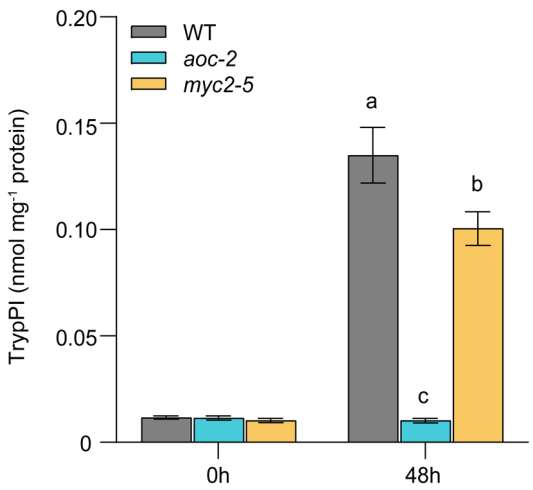

Fig. 3 LF-induced trypsin proteinase inhibitor levels in WT and JA mutants. Mean trypsin proteinase inhibitor (TPI) contents (±SE, n = 6) in *aoc-2*, *myc2-5* and wild type (WT) plants upon LF attack. Letters indicate significant differences among different plants ($p < 0.05$, Duncan's multiple range test)

aoc-2 and lower, but significant differences, in the *myc2-5* mutant relative to their control counterparts. As phenolamides are also elicited by herbivores and act as anti-herbivore defense compounds in other plant species (Kaur et al. 2010), it is plausible that the LF and JA-dependent induction of phenolamide accumulation in rice may also play a role in resistance to this insect pest.

Abrogation of JA biosynthesis or signaling enhances LF larval performance

To evaluate the role of JA-induced defenses in rice responses to LF attack, we first measured the damaged leaf area by third-instar LF larvae, but we did not observe significant differences in leaf damage between JA mutants and WT plants (Fig. S3a and S3b). The LF larval performance was then conducted, and we found that the weight of LF larvae was increased by 11.83% and 28.48% on *myc2-5* and *aoc-2* lines, respectively, compared with that on WT plants (Fig. 6). These results show that JA regulates rice defense against LF.

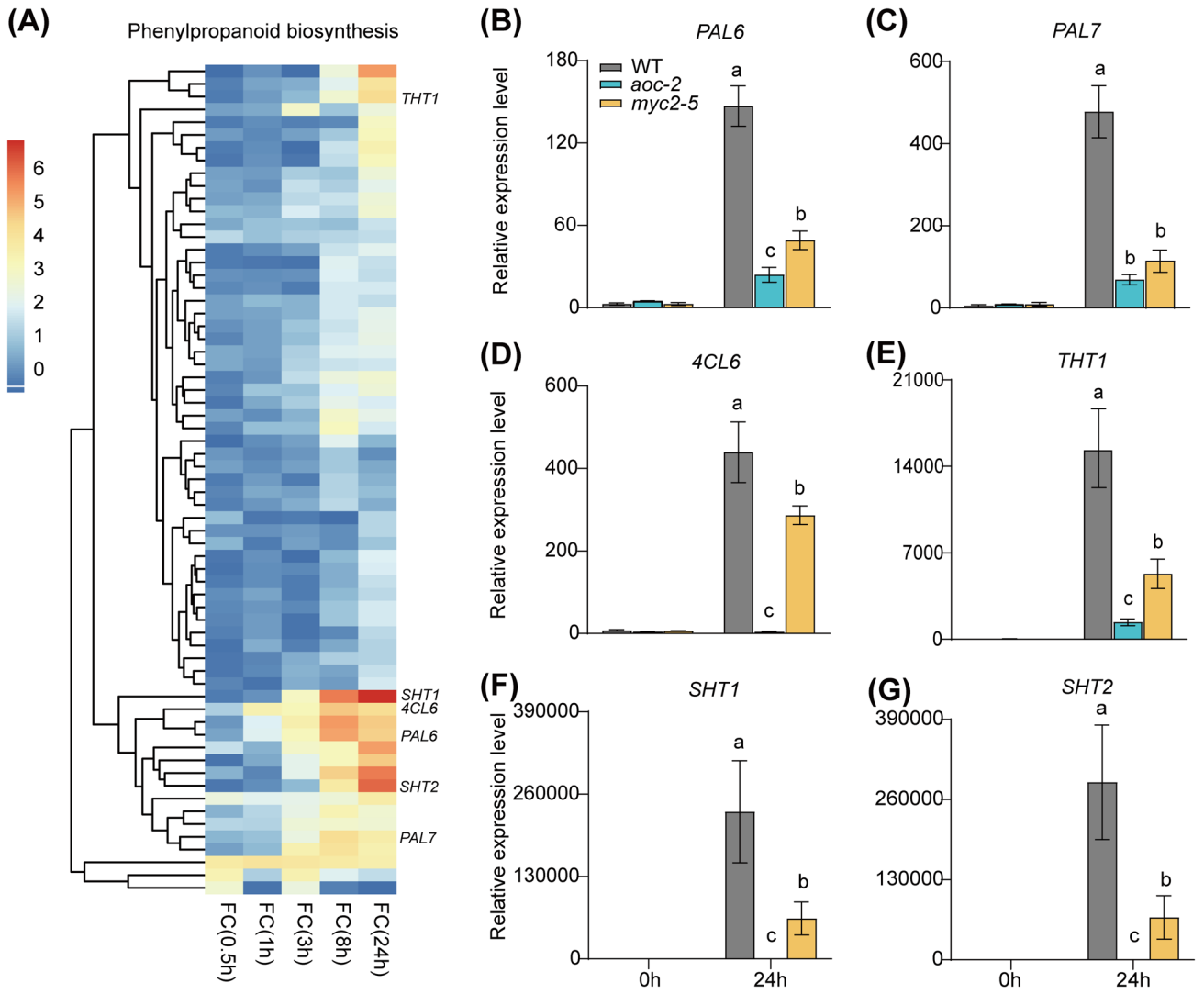

Fig. 4 Induced transcript levels of phenylpropanoid biosynthetic genes in WT and JA mutants in response to LF infestation. **a** Heat map representing the transcript levels of phenylpropanoid biosynthetic genes in LF-treated samples compared with control samples. The color gradient represents $\log_2$ fold changes between control and treated plants. Mean transcript abundance (SE, n = 6) of *PAL6* (**b**), *PAL7* (**c**), *4CL6* (**d**), *THT1* (**e**), *SHT1* (**f**), and *SHT2* (**g**) in *aoc-2*, *myc2-5*, and WT plants upon LF attack. Letters indicate significant differences among treatments ($p < 0.05$, Duncan's multiple range test)

Discussion

LF attack occurs through all rice developmental stages, affecting plant growth and fertility, but is more significant during the reproductive stage (Padmavathi et al. 2013). In the paddy field, pesticides are commonly used to control LF population even in the early season, when the foliar damage caused by LF is conspicuous, as symptoms prompt farmers to apply insecticides rapidly. However, the early application of pesticides has a negative side effect: it also kills natural enemies of LF and other insect pests (Gurr et al. 2012). In addition, LF can develop resistance to insecticides, and secondary breakouts can occur long-term after pesticide application. Thus, identifying LF resistance genes and LF-elicited defense pathways could help find new control strategies for this insect pest. The JA signaling pathway is involved in rice resistance to BPH and SSB (Zhou et al. 2009; Qi et al. 2011; Li et al. 2015; Xu et al. 2021). In this study, we investigated the role of JA in rice and LF interaction. Based on transcriptome data, genetic manipulation experiments, and chemical analysis, we found that (1) JA and defense pathway genes are up-regulated in LF attacked leaves; (2) JAs, TPI, and phenolamide levels accumulate to high levels after LF infestation; (3) Mutation in *AOC* and *MYC2* genes reduce LF-elicited TPI and phenolamide accumulation, and (4) LF larvae grow better on *aoc* and *myc2* mutants than on WT plants.

Here we found that LF-induced defense compounds were lower in JA mutant plants than in WT plants, albeit this

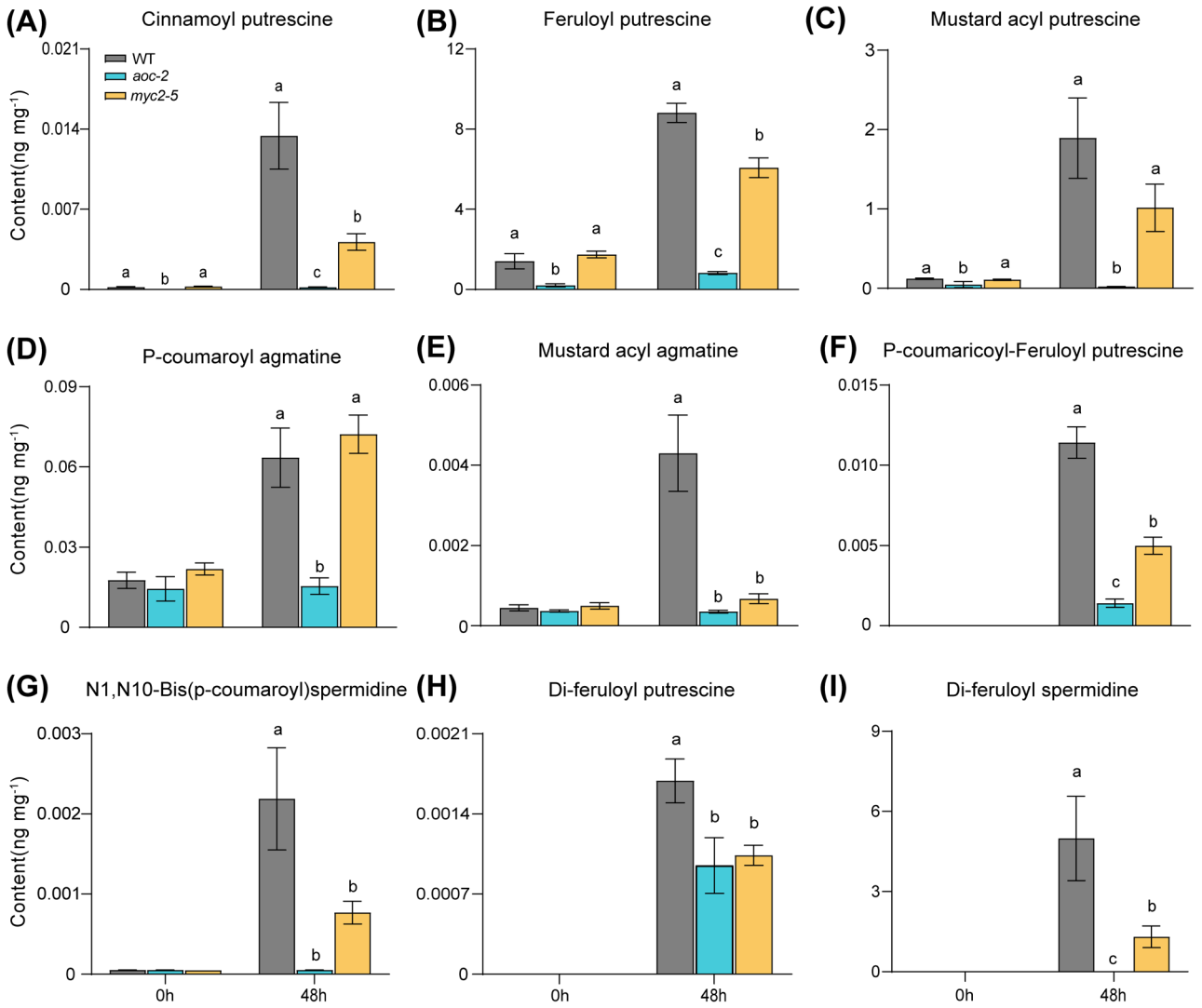

Fig. 5 LF-induced phenolamide accumulation in WT and JA mutants. Mean levels (SE, n = 6) of cinnamoyl putrescine (**a**), feruloyl putrescine (**b**), mustard acyl putrescine (**c**), p-coumaroyl agmatine (**d**), mustard acyl agmatine (**e**), p-coumaricoyl-feruloyl putrescine (**f**), N1,N10-bis(p- coumaroyl)spermidine (**g**), di-feruloyl putrescine (**h**), and di-feruloyl spermidine (**i**) in *aoc-2*, *myc2-5*, and wild type (WT) plants upon LF attack. Letters indicate significant differences among different plants ($p < 0.05$, Duncan's multiple range test)

response was stronger in *aoc-2* than in *myc2-5*. We hypothesize that this difference could be due to *myc2-5* being a weak allele of the *myc2* mutant (Xu et al. 2021). Alternatively, other JA-responsive transcription factors could be involved in LF resistance. Along these lines, several TFs are known to regulate different JA-induced defense responses (Chini et al. 2016). We found that levels of LF-induced defense compounds were dramatically decreased in the *aoc-2* mutant compared to WT plants. However, we did not observe a proportional reduction in the increment of LF larval mass feeding on *aoc-2*. A similar phenotype has been reported in another study with an *aoc* mutant (Lu et al. 2015). It is conceivable that LF may have evolved some mechanism to counter rice JA-induced defenses similar to that found in another plant–herbivore system, in which the HARP1 protein from cotton bollworm oral secretion could directly interact with JAZ proteins to suppress JA-mediated defense responses (Chen et al. 2019). Alternatively but not exclusively, this may include LF-mediated detoxification of rice defense compounds.

The levels of JA-Ile and PAL increase in both LF-resistant and -susceptible varieties after LF infestation, but the induced levels were higher in the resistant variety (Guo et al. 2019). Similarly, a proteomics study showed that LOX and PAL have higher expression in a resistant variety than in the susceptible one (Cheah et al. 2020). Silicon element has been documented to mediate rice resistance to LF. The application of Si enhances rice resistance to LF, while the

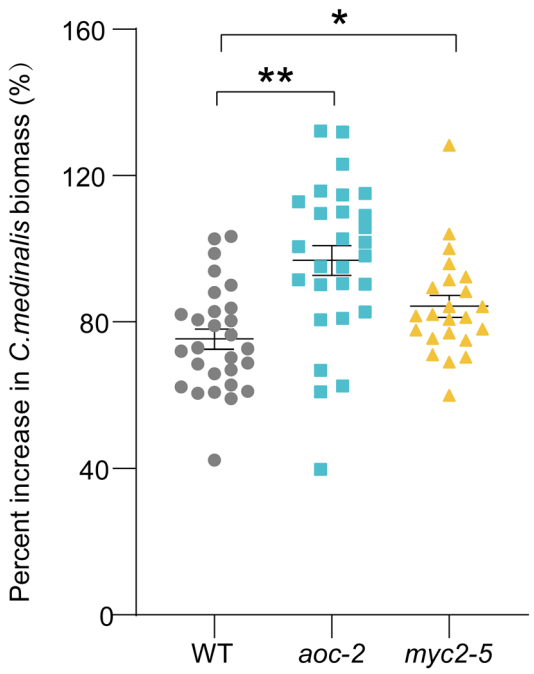

Fig. 6 LF enhanced performance in JA mutants. Mean larval growth rates (±SE, n = 23–28) in *aoc-2*, *myc2-5*, and WT plants. Asterisks indicate significant differences in WT plants compared with JA mutants (*p < 0.05; **p < 0.01; Student's *t* test)

disruption of Si transporter compromises LF resistance (Ye et al. 2013; Lin et al. 2019). Mechanistic studies indicated that Si could prime rice JA-mediated defense responses, and the accumulation of Si in rice leaves is under the control of JA (Ye et al. 2013). Therefore, JA is underlaying some resistance traits of rice against LF.

The anti-herbivore function of phenolamides has been investigated in the wild tobacco plant *N. attenuata*. Silencing the TF *NaMYB8* dramatically reduced phenolamide levels and plant resistance to the specialist herbivore *M. sexta* (Kaur et al. 2010). Phenolamides have been frequently identified in rice tissues in the context of plant–herbivore interactions (Alamgir et al. 2016; Xu et al. 2021). Here we found, nine phenolamides that were responsive to LF infestation and regulated by JA signaling. The effect of phenolamides in LF resistance needs to be further investigated, and functional characterization of NaMYB8 orthologs in rice will be instrumental in future studies. In addition to phenolamides, monolignol, phenolic acid, and flavonoids are also derived from the phenylpropanoid pathway. Monolignols are the basic units of lignin, a major component of plant secondary cell wall, and bound phenolic compounds modify wall strength and digestibility (Deng and Lu 2017). Since LF larva scrapes the upper epidermis and mesophyll tissues, strengthened cell walls could provide a physical barrier against LF feeding. A recent study found that a mutation in a flavonoid O-glucosyltransferase altered rice defense against BPH and rice grasshopper, suggesting that flavonoids are also involved in herbivore resistance (Yang et al. 2021). Thus, JA-mediated regulation of phenylpropanoid biosynthesis may play a multi-faceted role in rice resistance to LF by regulating the synthesis of phenolamides, flavonoids and modifying cell wall properties.

In summary, we found that LF attack leads to significant rearrangement of the leaf transcriptome and JA biosynthesis activation. Downstream components of the JA-signaling pathways (MYC2) promote the accumulation of defensive compounds, such as TPI and phenolamides, reducing LF performance. In this study we unveil the mechanisms and function of the defense arsenal of rice against LF, which opens new avenues to develop targeted and biological control strategies for this insect pest.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11103-021-01208-x.

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Author contributions RL designed the research. YZ and XW performed experiments. RL, LCL, JL and YL analyzed data. RL and LCLwrote the manuscript.

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Data availability The raw sequence data reported in this paper has been deposited in the Genome Sequence Archive at the BIG Data Center (http://bigd.big.ac.cn/gsa), Beijing Institute of Genomics (BIG), Chinese Academy of Sciences, under Accession No. CRA004405.

Declarations

Conflict of interest The authors declare no potential conflict of interest.

Code availability Not applicable.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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