Physiological and transcriptomic analyses revealed gene networks involved in heightened resistance against tomato yellow leaf curl virus infection in salicylic acid and jasmonic acid treated tomato plants

Peng Wang1, Sheng Sun1*, Kerang Liu2, Rong Peng1, Na Li1, Bo Hu3, Lumei Wang2, Hehe Wang4, Ahmed Jawaad Afzal5 and Xueqing Geng2*

1College of Horticulture, Shanxi Agricultural University, Jinzhong, Shanxi, China, 2School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, China, 3Institute of Quality and Safety Testing Center for Agro-Products, Xining, Qinghai, China, 4Edisto Research and Education Center, Clemson University, Blackville, SC, United States, 5Division of Science, New York University, Saadiyat Island Campus, Abu Dhabi, United Arab Emirates

Tomato yellow leaf curl virus (TYLCV), a member of the genus Begomovirus of the Geminiviridae family, causes leaf curl disease of tomato that significantly affects tomato production worldwide. SA (salicylic acid), JA (jasmonic acid) or the JA mimetic, COR (coronatine) applied exogenously resulted in improved tomato resistance against TYLCV infection. When compared to mock treated tomato leaves, pretreatment with the three compounds followed by TYCLV stem infiltration also caused a greater accumulation of H$_2$O$_2$. We employed RNA-Seq (RNA sequencing) to identify DEGs (differentially expressed genes) induced by SA, JA, COR pre-treatments after Agro-inoculation of TYLCV in tomato. To obtain functional information on these DEGs, we annotated genes using gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) databases. Based on our comparative analysis, differentially expressed genes related to cell wall metabolism, hormone signaling and secondary metabolism pathways were analyzed in compound treated samples. We also found that TYLCV levels were affected in SINPR1 and SICOII silenced plants. Interestingly, compared to the mock treated samples, SA signaling was hyper-activated in SICOII silenced plants which resulted in a significant reduction in viral titer, whereas in SINPR1 silencing tomato plants, there was a 19-fold increase in viral load. Our results indicated that SA, JA, and COR had multiple
Introduction

Tomato yellow leaf curl virus (TYLCV), belonging to the genus *Begomovirus* of the *Geminiviridae* family, contains circular single-stranded DNA (ssDNA) which is 2.7–2.8 kb in length. As a destructive viral pathogen, TYLCV causes a myriad of host disease symptoms ranging from upward curling of leaves and chlorosis to a reduction in leaf size, resulting in significant yield losses (Prasad et al., 2020). In nature, the virus is spread by whiteflies (Huang et al., 2017), and the infection in tomato, as well as cucumber and pepper has been associated with the global spread of the pathogen (Li T. et al., 2019). The spread is further exacerbated due to the high migration rate and reproductive capacity of whiteflies.

Plant hormones play key regulatory roles in defense gene regulation (Du et al., 2017). JA (jasmonic acid) and SA (salicylic acid) are two important defense hormones implicated in the elicitation of plant defenses (Yu et al., 2015). SA plays a key role in resistance against biotrophic pathogens, and induces systemic acquired resistance (SAR) (Glazebrook, 2005; Pieterse et al., 2012, 2014). NPR1 (non-expressor of pathogenesis related genes 1) is one SA receptor that makes essential contributions to SA signaling in *Arabidopsis* (Wu et al., 2012; Ding et al., 2018). In *Arabidopsis*, three proteins (NPR1, NPR3, and NPR4) were proposed to function as SA receptors, as they were shown to bind to SA. NPR1 was proposed to function as a transcriptional co-activator, whereas NPR3/NPR4 were suggested to function as transcriptional co-repressors that induce NPR1 degradation. It was further shown that SA inhibits NPR3/NPR4 function to promote the expression of downstream immune regulators (Ding et al., 2018). On the other hand, JA signaling is activated when plants are attacked by necrotrophic pathogens or herbivores and leads to the activation of induced systemic resistance (ISR) (Pieterse et al., 2014). COI1 (CORONATINE INSENSITIVE1) is a key component of the JA signaling pathway. JA-Ile interacts with a complex of COI1 and jasmonate ZIM-domain transcriptional repressors protein to induce proteasome-mediated degradation of the JAZ protein by the SCF$^{\text{COI1}}$ ubiquitin E3 ligase complex (Thines et al., 2007; Sheard et al., 2010). A previous study showed that the exogenous application of SA could inhibit viral accumulation and enhance the resistance response in susceptible tomato cultivars (Sade et al., 2014). The C4 protein from TYLCV can shift its localization from the plasma membrane to the chloroplast to inhibit SA dependent responses in tomato (Corrales-Gutierrez et al., 2020). Further, the $\delta$C1 protein from the betasatellite of tomato yellow leaf curl China virus suppressed JA dependent plant terpene biosynthesis (Li et al., 2014) and the JA regulated biosynthesis of phytoalexin prevented the virus transmitting from the vector to the plant (Chen et al., 2019). These results show that both SA and JA signaling pathways are involved in plant defense regulation against TYLCV infection. However, the overall mechanism by which SA and JA signaling impact plant resistance to TYLCV are still elusive.

Coronatine (COR) produced by specific strains of *Pseudomonas syringae*, is a structural and functional mimic of the bioactive jasmonic acid conjugate JA-Ile and targets the JA receptor COR-insensitive 1 (COI1) in *Arabidopsis* and tomato (Feys et al., 1994; Katsir et al., 2008; Geng et al., 2014). COR is more active than JA-Ile at stabilizing interactions between JAZ and COI1 protein in tomato (Thilmony et al., 2006; Katsir et al., 2008). COR also acts as a plant growth regulator to elicit the production of secondary metabolites including saponins and phytoalexins (Tamogami and Kodama, 2000; Lee et al., 2018). It has been proposed that the exogenous application COR may promote abiotic resistance in plants (Xie et al., 2015).

In the current study, we discovered that the pre-treatment of tomato plant with SA, JA, and COR followed by Agro-inoculation of TYLCV could decrease TYLCV accumulation at the early stage of infection. DAB staining showed that TYLCV infection that followed the three treatments resulted in a higher level of $\text{H}_2\text{O}_2$ accumulation when compared to mock treatment. We investigated the transcriptome of tomato plants infected by TYLCV under these three treatments. Gene ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases are routinely used to annotate functions of differentially expressed genes (DEGs) in tomato plants. We focused on analysis of genes involved in cell wall biosynthesis as previous reports had shown that tomato leaves infected with TYLCV induced thickening of cell walls (Montasser et al., 2012). We also sought to identify differentially expressed genes in hormone signaling pathways and secondary metabolite regulation. In addition, we silenced $\text{SNP}R_1$, which is a central component in SA signal transduction, and $\text{SI}CO_1$, which functions as a JA-Ile receptor in order to further determine the roles of SA and JA signaling in TYLCV infection. Our
results indicated that SA, JA and COR significantly altered defense signaling during the early stage of TYLCV infection and broaden our understanding of SA and JA signaling pathways involved in mediating resistance to TYLCV in tomato.

Materials and methods

Plant materials

The seeds of Tomato (*Solanum lycopersicum* var. Moneymaker) (susceptible to TYLCV infection) were germinated at 25°C, and then transferred to flats containing soil. Seedlings at the four-leaf stage were moved into plastic pots with soil and grown in a plant growth chamber under a 12 h light (320 µmol·m⁻²·s⁻¹, 26°C)/12 h dark (18°C) cycle. The relative humidity was maintained between 60 and 70%.

Treatments and tomato yellow leaf curl virus infection

Tomato plants containing five to six leaves after 3 weeks of growth were randomly divided into four groups. Solutions of JA (0.5 mM), SA (0.5 mM), COR (0.1 µM), and deionized water (mock) mixed with 0.04% silwet L-77 separately, were exogenously sprayed onto tomato leaves. The TYLCV infectious clone was provided by Professor Zhou Xueping of Zhejiang University (Zhang et al., 2009). After 24 h, all the seedlings were inoculated with *Agrobacterium* (*OD₆₀₀ = 1.0*) carrying TYLCV (the clone was introduced into *Agrobacterium GV3101*) as described previously (Zhang et al., 2009). We injected 1 ml of solution into the plant stem, 5 cm above ground at three points by using a 1.0 ml syringe. Virus infection symptoms were determined visually by observing changes in leaf color and viral infection was confirmed through qPCR. Seven days post inoculation, samples from upper leaves were collected from the same nodes from both three compounds and mock treated plants. One hundred milligrams from each sample was taken for RNA-seq (RNA sequencing) and RT-qPCR verification. Each experiment included three biological replicates.

Detection of tomato yellow leaf curl virus content in tomato plants

Quantitative PCR (qPCR) was used to detect relative TYLCV content from leaves of infected tomato plants (Sade et al., 2014, 2020; Li et al., 2017). All leaf samples from the infected plants were collected and subsequently frozen in liquid N₂. Total DNA was extracted from the leaves using a DNA extraction kit (DNA secure Plant Kit, Shenggong, China). qPCR was carried out in the presence of SYBR Green (TaKaRa, Shanghai, China) by using a RealTime PCR system (Bio-Rad, CA, United States). The tomato *SlACTIN* gene (LOC101260631) (Zhang et al., 2021) was used as the normalizing control. The qPCR reaction was carried out as follows: 30 s at 94°C, followed by 40 cycles consisting of 10 s at 94°C, 30 s at 58°C, and 20 s at 72°C. The specificity of the amplified products was based on melting curves that were generated from 65 to 95°C with increments of 0.5°C/cycle. The melting curve data was analyzed by using the CFX Manager™ software (version 3.0, Bio-Rad, CA, United States). The primers used for detecting TYLCV were: F (5′-CACTCAATTCAGGCAGTAGAATCC-3′), and R (5′-GACCCACTCTTCAAGTTCATCTG-3′). The primers used for *SlACTIN* are listed in Table 1. The TYLCV content in the mock treated leaves was set to 1. Shown are the mean and SEM from three independent biological replicates.

Detection of H₂O₂ accumulation in the leaves of tomatoes

24 hours post salicylic acid, jasmonate acid, and coronatine treatments 24, plants were inoculated with *Agrobacterium* (*OD₆₀₀ = 1.0*) carrying TYLCV. After 7 days, leaves were collected to detect H₂O₂ accumulation by staining with 3,3'-diaminobenzidine (DAB). In brief, leaves were immersed in 0.1% DAB solution and incubated at room temperature for 8 h. Leaf tissues were subsequently immersed in 95% ethanol and boiled for 15 min until the leaves were transparent. For every treatment, 3–4 leaves were collected. Each experiment included three biological replicates. A total of nine slides were made for each treatment and one slide was selected to represent the results.

Transcriptome sequencing and library construction

Leaf samples from the four groups (mock treatment, JA treatment, SA treatment, and COR treatment) were collected at 7 days post TYLCV clone infection. The harvested samples were immediately frozen in liquid nitrogen and stored at −80°C. Total RNA was extracted with Trizol reagent (Invitrogen, Carlsbad, CA, United States). The concentration of RNA was determined using a NanoDrop spectrophotometer and the quality of RNA was assessed by resolving RNA on a 1.5% (w/v) agarose gel (Gao et al., 2015). The mRNA was enriched from a pool of total RNA through Oligo-dT-enriched magnetic beads (Vazyme Biotech, Jiangsu, China). The enriched mRNA was sheared into short pieces by using an RNA fragmentation kit according to manufacturer’s instruction (Illumina, San Diego, CA, United States). The fragments were reverse-transcribed into single stranded cDNA with random N6 primers. The cDNA fragments were subjected to end repair and ligated with adapters, followed by PCR amplification. The amplified PCR products were used to
TABLE 1 Primers used in this manuscript.

| Gene/Gene number | Forward | Reverse | Product length |
|------------------|---------|---------|----------------|
| SlACTIN          | GATGGTGGGTATGGGTCAAA | AGGGGCTTCAGTTAGGAGGA | 199             |
| SlNPR1           | GGTGAGTGTTGCTGGCCTAT | TGAAGGTAAGAGGATGGTG | 150             |
| SlCOI1           | AAGACCTCTTTACTCCGTA | TCAGTGAACAAAACTGACGC | 118             |
| LOC01264227      | GACAGACTTTCTACATGCAAC | ATCCCAATTTCTACATCCA | 111             |
| LOC01267111      | AGCAGCTCTTCTACATGCAAC | TGGTTATAGGAAACGATCAA | 116             |
| LOC01256271      | AAACTCAACAGGATGGTGAGC | GGGGTGAGGAAATAGGACT | 94              |
| LOC01242656      | ACCTGGTGTTGGAACACTTACGAC | GCCCTAGCATCTCACAAGG | 99              |
| LOC01245298      | GTCAAAGCTTTTGAGTTATCG | AGGGACCTCTTGGTTATCAT | 134             |
| LOC012648161     | CAAATTGAATTAAGCCTCGG | CAGATGAAAGCTTACTCCGAG | 142             |
| LOC01258353      | GTTACCTTGCTCTGACCAA | TCCGAGAGTGAACTTGAGT | 138             |
| LOC01265701      | AAACTGATTGAAAGACTTTCTC | CGGGATCATATAATCGTAA | 133             |
| LOC01265854      | GCTCGTGTTGCTAAGCTGGGTT | GACCAAGATGAATCAAGTTCG | 112             |
| LOC01919038      | GATGGCTTCACCTCTCAAAG | TACTCTCCATTTCCTCTCC | 141             |
| LOC01256422      | AGGCTCCCATCTCATATAC | TTGGGCAATAGCTACACAG | 62              |
| LOC019120532     | ATGGACCCCCGTAGAGCTAAAT | TGTGTATTCCCCAAGCTTTGCA | 109             |

generate the cDNA library. cDNA sequencing was carried out on the Huada Gene Technology BGISEQ-500 platform (Wuhan, Hubei, China).

**Read mapping, quality control, and functional annotation**

We obtained raw data from the BGISEQ-500 platform. If more than half of the component bases in the reads had a quality score of <15, they were annotated as low quality reads and filtered by using Soapnuke (v1.5.2) software. Other low-quality regions such as adapter sequences and regions with an excessive number of unknown bases (annotated as "Ns") were also filtered by Soapnuke (Li et al., 2008). The remaining clean reads were mapped onto the reference genome sequence (GCF_000188115.4_SL3.0) using the Hierarchical Indexing for Spliced Alignment of Transcripts HISAT2 (v2.0.4) system (Gao et al., 2014; Kim et al., 2015). Bowtie2 was used to align clean reads to the reference coding genomic sequence and the FPKM (fragments per kilobase of transcript per million mapped reads) was calculated by RSEM (v1.2.12) (Li and Dewey, 2011). Differentially expressed genes (DEGs) were identified by DEseq2 (v1.4.5) (Love et al., 2014), with the following set of conditions ([| log2 Fold Change| ≥ 1, and a False discovery rate (FDR) <0.001. GO (gene ontology) annotation2 and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment3 of DEGs was used to gain insight into the functional classes and pathway information of the DEGs. The RNA-Seq data for SA-treated sample (accession no: SRR19591763), JA-treated sample (accession no: SRR19591764), COR-treated sample (accession no: SRR19591765), and mock sample (accession no: SRR19591766) are available at the NCBI gene expression omnibus server.4

**Validation of candidate genes with virus induced gene silencing**

VIGS (virus induced gene silencing) assays were performed as described (Yu et al., 2019). Briefly, the TRV-mediated VIGS system was used to silence two genes (SINPR1 and SICOI1). Fragments of SINPR1 and SICOI1 amplified using specific primers were inserted into pTRV2 vectors. The constructs were introduced into Agrobacterium GV3101 by electroporation and injected into fully expanded leaves of 2-week-old tomato plants (Li et al., 2013). pTRV1 plus empty pTRV2 served as the negative control, whereas pTRV1 plus pTRV2-phytoene desaturase (SlPDS) served as the positive control. Independent cultures of Agrobacterium GV3101 carrying pTRV1, pTRV2, pTRV2-SINPR1, pTRV2-SICOI1, and pTRV2-SIPDS were liquid-cultured overnight in LB medium separately. Cultures were resuspended in 10 mM MgCl2 to a final OD_{600} = 1.0. Cultures were mixed with a 1:1 ratio. Approximately 1 ml of this suspension was used to inoculate the leaves of tomato plants. Silencing frequency (%) and silencing efficiency were calculated as described previously (Dinesh-Kumar et al., 2003). The primers used for amplification of SINPR1 and SICOI1 are listed in Table 1.

---

1 https://www.ncbi.nlm.nih.gov/assembly/GCF_000188115.4
2 http://geneontology.org/
3 https://www.genome.jp/kegg/
4 https://www.ncbi.nlm.nih.gov/geo/
FIGURE 1
The relative tomato yellow leaf curl virus (TYLCV) contents in tomato leaves. Pre-treatment of coronatine (COR) (0.1 µM), salicylic acid (SA) (0.5 mM), jasmonate acid (JA) (0.5 mM), or mock was followed by the inoculation of the TYLCV infectious clones. Leaf samples were collected 7 days post infection. TYLCV levels were determined using qPCR and the TYLCV levels in the mock leaves were set to 1. Shown are the mean and SEM from three independent biological replicates.

FIGURE 2
Detection of H$_2$O$_2$ by 3,3-diaminobenzidine (DAB) staining. Leaf samples were collected 7 days after inoculation with the tomato yellow leaf curl virus (TYLCV) infectious clone that were pre-treated with coronatine (COR), salicylic acid (SA), jasmonate acid (JA), or mock. Brown color in the leaves serves as a proxy for H$_2$O$_2$ production. Shown are representative pictures from three biological repeats.

Validation of differentially expressed genes with quantitative real-time RT-PCR

To access the reliability of our transcriptome data, we determined the expression profile of 12 randomly selected genes through quantitative real-time PCR. RNA was extracted using the Trizol reagent (Invitrogen, United States) whereas first-stand cDNA synthesis was carried out using the PrimeScript$^\text{TM}$ RT and the gDNA Eraser (TaKaRa, Shanghai, China) kits. Quantitative real-time RT-PCR (qRT-PCR) was carried out on a RealTime PCR System (Bio-Rad, CA, United States) using the SYBR Premix Ex Taq II kit (Takara, China). The following reaction conditions were used: pre-denaturation at 95°C for 30 s, denaturation at 95°C for 5 s, extension at 60°C for 30 s for a total of 40 cycles. Relative gene expression was monitored in realtime and measured using the 2$^{-\Delta\Delta CT}$ method as previously described (Gao and Chen, 2018; Zhang et al., 2021). The gene LOC101260631, which encodes SlACTIN was used as the internal reference. qRT-PCR primer sequences for the 12 selected genes are shown in Table 1. Each treatment employed three independent biological replicates.

Results

The relative tomato yellow leaf curl virus content reduced in tomato plants under treatments of jasmonate acid, salicylic acid, and coronatine

We treated tomato plants with solutions of COR, SA, JA, or mock through foliar spray. Twenty-four hours post spray, we inoculated plants with the TYLCV infectious clone detected TYLCV titer 7 days post infection. TYLCV levels in compound treated leaves were significantly lower than those in mock treated leaves. Compared to the mock treatment, JA treatment caused a 50-fold reduction in TYLCV
TABLE 2 Data mapped quality of the transcriptome.

| Sample   | Total raw reads (M) | Total clean reads (M) | Clean reads Q20 (%) | Clean reads Q30 (%) | Clean reads ratio (%) | Total mapping (%) |
|----------|---------------------|-----------------------|---------------------|---------------------|-----------------------|-------------------|
| MOCK1    | 25.91               | 24.20                 | 98.82               | 95.41               | 93.43                 | 97.10             |
| MOCK2    | 23.75               | 23.65                 | 98.63               | 94.65               | 99.59                 | 96.59             |
| MOCK3    | 23.75               | 23.61                 | 98.73               | 95.06               | 99.42                 | 97.13             |
| JA_T1    | 23.75               | 23.60                 | 98.74               | 95.04               | 99.40                 | 96.71             |
| JA_T2    | 23.75               | 23.60                 | 98.71               | 94.95               | 99.40                 | 96.86             |
| JA_T3    | 23.75               | 23.61                 | 98.52               | 94.19               | 99.44                 | 96.69             |
| SA_T1    | 23.75               | 23.65                 | 98.65               | 94.71               | 99.60                 | 97.18             |
| SA_T2    | 23.92               | 23.75                 | 98.44               | 95.25               | 99.29                 | 97.42             |
| SA_T3    | 23.75               | 23.66                 | 98.59               | 94.50               | 99.63                 | 97.15             |
| COR_T1   | 24.09               | 22.51                 | 98.78               | 95.29               | 93.46                 | 97.17             |
| COR_T2   | 23.92               | 23.61                 | 98.39               | 95.35               | 98.69                 | 96.81             |
| COR_T3   | 23.75               | 23.67                 | 98.63               | 94.60               | 99.67                 | 97.00             |

FIGURE 3
Volcano plots for differentially expressed genes (DEGs) including jasmonate acid (JA) treatment vs. mock treatment, salicylic acid (SA) treatment vs. mock treatment and coronatine (COR) treatment vs. mock treatment. The log₂ fold change (X-axis) is plotted against the log₁₀ FDR (Y-axis). Red dots and green dots represent DEGs that were either up-regulated or down-regulated. Gray dots denote genes that were not significantly different between treatments and mock.

accumulation. COR treatment resulted in a 10-fold reduction, whereas SA treatment resulted in a 4-fold reduction in virus content (Figure 1). A typical symptom of TYLCV infection is leaf curling, which we observed 7 days post virus inoculation. The extent of curling of leaves for all three treatments was lower when compared with mock treated leaves (data not showed). These results indicated that COR, SA, and JA induced tomato defense responses to inhibit TYLCV accumulation at the early stage of infection.

Reactive oxygen species production of tomato leaves under the exogenous application of jasmonate acid, salicylic acid, and coronatine

ROS (reactive oxygen species) production is an early plant response to biotic and abiotic stress (O’Brien et al., 2012). ROS, especially H₂O₂, impacts plant tolerance through regulating signal recognition and transduction. In this study, we used 3,3-diaminobenzidine (DAB) staining to detect the accumulation of H₂O₂ 7 days after the Agro-inoculation of the TYLCV infectious clone. JA treatment resulted in robust accumulation of H₂O₂, whereas SA and COR treatments resulted in the accumulation of hydrogen peroxide, albeit to reduced levels (Figure 2). As a signaling molecule that activates defense responses against virus infection, H₂O₂ accumulation in JA treated samples showed the greatest reduction in TYLCV (Figure 1).

Overview of the Solanum lycopersicum transcriptome

Next-Generation RNA sequencing (RNA-Seq) is a powerful tool used to study global transcriptional changes under biotic stress. In order to ascertain gene expression changes in tomato
plants against TYLCV under the exogenous application of JA, SA, and COR, we performed transcriptome analysis on 12 samples. The transcriptome data was generated using DNBSEQ. After filtering out low-quality reads with SOAP nuke (v1.5.2) (Li et al., 2008), we obtained an average of 23.59 M high-quality reads per sample, accounting for >93%
of the raw reads per sample (Table 2). The clean reads with a quality value of Q30 accounted for >94% of the total reads and a quality value of Q20 accounted for >98% of total clean reads (Table 2). The clean data were aligned to the genome sequence of S. lycopersicum (GCF_000188115.3_SL2.50) using the Hierarchical Indexing for Spliced Alignment of Transcripts (HISAT) system. More than 96% of reads were successfully mapped to the reference genome and the unique mapping rate for each sample was over 90%, indicating that the RNA-Seq data was of high quality. Based on transcriptome analysis, DEGs (differentially expressed genes) ([log2 Fold Change] ≥ 1, FDR < 0.001) from different comparative groups were identified. Briefly, a total number of 2,574 (958 up-regulated and 1,616 down-regulated), 3,864 (2,170 up-regulated and 1,694 down-regulated) and 1,406 DEGs (993 up-regulated and 413 down-regulated) genes were identified in JA vs. mock, SA vs. mock, and COR vs. mock comparisons. To make the visualization more intuitive, we have drawn a volcanic map to show the distribution of DEGs under three different treatments (Figure 3).

**Gene ontology analysis of differentially expressed genes**

Gene ontology is a system of standardized scientific classification used to systematically annotate gene function. To determine the potential function of tomato DEGs involved in TYLCV infection under the exogenous application of JA, SA, and COR, we performed GO analysis. Our aim was to decipher potential biological processes and genes regulated under the three treatments. A total of 25, 32, and 30 GO terms were significantly enriched with DEGs regulated by JA, SA and COR respectively (P < 0.01) (Figure 4). Intriguingly, there were commonly enriched GO terms for both up-regulated and down-regulated DEGs among the three treatments. Up-regulated DEGs were commonly enriched in GO terms such as oxylipin biosynthetic process (GO:0031408), defense response (GO:0006952), and oxidation-reduction process (GO:0055114), while down-regulated DEGs were commonly enriched in terms such as auxin-activated signaling pathway (GO:0009734), plant-type cell wall organization (GO:00098664), and DNA replication initiation (GO:0006270). Moreover, DEGs from the three treatments also enriched for the “cell wall” category. This is not surprising as the first physical and defensive barrier against pathogens, the plant cell wall undergoes dynamic structural changes to prevent pathogen ingress (Nafisi et al., 2015).

**Important Kyoto encyclopedia of genes and genomes pathways influenced by the three treatments**

The KEGG database is a very useful resource for understanding high-level function of a biological system. To better understand the systemic roles of the three treatments, we also performed KEGG pathway enrichment analysis on the identified DEGs. There was a significant enrichment in 15, 11, and 19 KEGG pathways (P < 0.05) for DEGs induced by COR, SA and JA, respectively (Tables 3, 4). We identified 213 shared DEGs which were up-regulated in the three treatment groups (Figure 5A and Supplementary Table 1). Three pathways, including “Plant-pathogen interaction” (ko04075), “alpha-Linolenic acid metabolism” (ko00592), and “Phenylpropanoid biosynthesis” (ko00940) were the mostly enriched pathways on the basis of up-regulation. Meanwhile, there were 156 shared DEGs which were all down-regulated by the three treatments (Figure 5B and Supplementary Table 2) and significantly enriched for the “Plant hormone signal transduction” (ko04075) pathway. In particular, up-regulated DEGs induced by JA were enriched in many metabolic pathways such as “Tyrosine metabolism” (ko00350), and “Phenylalanine metabolism” (ko00360), which will be further characterized later. These results indicated that while the three treatments mostly induce different DEGs, they do share some common genes/pathways in response to TYLCV invasion.

**Differentially expressed genes related to cell wall synthesis regulated by salicylic acid, jasmonate acid, and coronatine**

Pathogen attack can result in plant cell wall fortification which prevents further pathogen ingress (O’Brien et al., 2012). Polysaccharides are the main component of plant cell walls. In our study, 25, 28, and 36 DEGs were related to carbohydrate metabolism under JA, SA, and COR treatments. Cellulose, a kind of polysaccharide is the central component in the plant secondary wall. Cellulose synthesis is a highly regulated process involving multi protein complexes including the cellulose synthase (CESA) complex from the glycosyltransferase GT-2 family (Decker et al., 2012). In our analysis, genes related with cellulose biosynthesis were up-regulated in all three compound treated samples (Supplementary Table 3). The gene GALE (LOC101266745) encoding UDP-glucose 4-epimerase (UGE) which catalyzes the conversion of UDP-Galactose to UDP-Glucose was 2-fold up-regulated by JA, whereas genes encoding cellulose degradation enzymes such as LOC101247302, CEI7 (LOC543583), and CELI8...
TABLE 3  The Kyoto encyclopedia of genes and genomes (KEGG) pathways (top 10) enriched by up-regulated differentially expressed genes (DEGs) under the three treatments.

| Treatment | Pathway ID | KEGG pathway                                      | Gene number | P-value   |
|-----------|-----------|---------------------------------------------------|-------------|-----------|
| JA        | 00940     | Phenylpropanoid biosynthesis                      | 28          | 4.52E-08  |
|           | 04626     | Plant-pathogen interaction                        | 24          | 5.42E-06  |
|           | 00945     | Stilbenoid, diaryleptanoid, and gingerol biosynthesis | 10          | 1.18E-05  |
|           | 00941     | Flavonoid biosynthesis                            | 10          | 1.05E-04  |
|           | 00592     | alpha-Linolenic acid metabolism                   | 9           | 1.76E-04  |
|           | 00350     | Tyrosine metabolism                               | 7           | 0.003754  |
|           | 00280     | Valine, leucine, and isoleucine degradation       | 8           | 0.003862  |
|           | 00591     | Linoleic acid metabolism                          | 4           | 0.005518  |
|           | 00480     | Glutathione metabolism                            | 11          | 0.00604   |
|           | 00071     | Fatty acid degradation                             | 7           | 0.00833   |
| SA        | 04626     | Plant-pathogen interaction                        | 45          | 1.84E-08  |
|           | 04016     | MAPK signaling pathway-plant                      | 35          | 4.98E-06  |
|           | 00592     | alpha-Linolenic acid metabolism                   | 12          | 0.001178  |
|           | 00562     | Inositol phosphate metabolism                     | 15          | 0.002055  |
|           | 00565     | Ether lipid metabolism                             | 8           | 0.002609  |
|           | 04075     | Plant hormone signal transduction                 | 39          | 0.003307  |
|           | 04070     | Phosphatidylinositol signaling system              | 14          | 0.005498  |
|           | 00280     | Valine, leucine, and isoleucine degradation       | 12          | 0.008001  |
|           | 00564     | Glycerophospholipid metabolism                    | 16          | 0.013576  |
|           | 00591     | Linoleic acid metabolism                          | 5           | 0.017725  |
| COR       | 00500     | Starch and sucrose metabolism                     | 16          | 2.00E-04  |
|           | 04016     | MAPK signaling pathway-plant                      | 17          | 0.001028  |
|           | 04626     | Plant-pathogen interaction                        | 19          | 0.001129  |
|           | 00908     | Zeatin biosynthesis                               | 8           | 0.005088  |
|           | 00591     | Linoleic acid metabolism                          | 4           | 0.005518  |
|           | 00950     | Isoquinoline alkaloid biosynthesis                | 5           | 0.005555  |
|           | 00053     | Ascorbate and aldarate metabolism                 | 7           | 0.006927  |
|           | 00360     | Phenylalanine metabolism                          | 7           | 0.007606  |
|           | 00562     | Inositol phosphate metabolism                     | 8           | 0.011395  |
|           | 00350     | Tyrosine metabolism                               | 6           | 0.015016  |

(LOC543583) were all down-regulated by JA and SA (Supplementary Tables 3, 4). This result indicated that SA and JA treatments induced cellulose synthesis to prevent TYLCV invasion. In addition, many genes involved in sugar metabolism that were differentially regulated by the three compounds. For example, Wiv-1 (LOC543502) encoding beta-fructofuranosidase, TPS1 (LOC100135703) encoding trehalose 6-phosphate synthase were up-regulated in all three compound treated samples (Supplementary Table 3). LOC101257623 that encodes raffinose synthase, was up-regulated under JA and SA treatments. Specifically, for COR treatment, XET2 (xylolucan endotransglycosylase 2), XET4 (xylolucan endotransglycosylase 4), XTH7 (xylolucan endotransglucosylase-hydrolase 7), and XTH1 (endo-xylolucan transferase 1), all of which are involved in the plant cell wall development (Decker et al., 2012) were up-regulated after TYLCV invasion (Supplementary Table 3). Taken together, all three treatments activated plant defense against TYLCV through regulating cell wall related physiological processes.

Impact on plant hormone signal transduction pathways

Plant hormones play key regulatory roles during the activation of specific defense signaling pathways in response to pathogen attack. Pathogens can also manipulate plant hormone signaling pathways to evade host defenses. The exogenous application of SA enhanced the resistance of plants infected with TYLCV by inducing the expression of PR (pathogenesis related) genes (Li T. et al., 2019). Our data also showed that SIPR1B1, which serves as a readout for SA signaling (Li et al., 2017) was strongly up-regulated by SA and down-regulated by
TABLE 4 The Kyoto encyclopedia of genes and genomes (KEGG) pathways (top 10) enriched by down-regulated differentially expressed genes (DEGs) under the three treatments.

| Treatment | Pathway ID | KEGG pathway                             | Gene number | P-value   |
|-----------|------------|------------------------------------------|-------------|-----------|
| JA        | 04075      | Plant hormone signal transduction         | 36           | 3.18E-06  |
|           | 00860      | Porphyrin and chlorophyll metabolism      | 10          | 2.25E-04  |
|           | 03030      | DNA replication                           | 10          | 3.19E-04  |
|           | 00500      | Starch and sucrose metabolism             | 19          | 3.65E-04  |
|           | 04141      | Protein processing in endoplasmic reticulum | 24         | 0.001211  |
|           | 00906      | Carotenoid biosynthesis                    | 6           | 0.015049  |
|           | 00944      | Flavone and flavonol biosynthesis         | 2           | 0.016211  |
|           | 00591      | Linoleic acid metabolism                   | 4           | 0.016937  |
|           | 00904      | Diterpenoid biosynthesis                   | 5           | 0.034046  |
|           | 00920      | Sulfur metabolism                          | 5           | 0.042292  |
| SA        | 03010      | Ribosome                                  | 103         | 2.08E-40  |
|           | 03030      | DNA replication                            | 12          | 3.67E-04  |
|           | 00100      | Steroid biosynthesis                       | 10          | 4.34E-04  |
|           | 00941      | Flavonoid biosynthesis                     | 11          | 0.006102  |
|           | 00860      | Porphyrin and chlorophyll metabolism      | 8           | 0.031731  |
|           | 00944      | Flavone and flavonol biosynthesis         | 2           | 0.031943  |
|           | 00780      | Biotin metabolism                          | 4           | 0.06257   |
|           | 00904      | Diterpenoid biosynthesis                   | 5           | 0.116215  |
|           | 00920      | Sulfur metabolism                          | 5           | 0.139336  |
|           | 00942      | Anthocyanin biosynthesis                   | 1           | 0.148024  |
| COR       | 04075      | Plant hormone signal transduction         | 23          | 8.96E-10  |
|           | 00280      | Valine, leucine and isoleucine degradation | 6           | 9.85E-04  |
|           | 00073      | Cutin, suberine, and wax biosynthesis     | 4           | 0.002791  |
|           | 00640      | Propanoate metabolism                      | 4           | 0.010436  |
|           | 00904      | Diterpenoid biosynthesis                   | 3           | 0.021341  |
|           | 04146      | Peroxisome                                 | 5           | 0.024155  |
|           | 00100      | Steroid biosynthesis                       | 3           | 0.028613  |
|           | 00591      | Linoleic acid metabolism                   | 2           | 0.043216  |
|           | 00900      | Terpenoid backbone biosynthesis            | 3           | 0.081637  |
|           | 00565      | Ether lipid metabolism                     | 2           | 0.09718   |

JA after TVLCV invasion (Supplementary Tables 5, 6), thereby suggesting that SA and JA might induce tomato resistance against TYLCV through different mechanisms. brassinosteroids (BRs) can enhance plants resistance by inducing ROS generation in *Nicotiana benthamiana* (Deng et al., 2016). As the negative regulator in BR signaling pathway, BKI1 (BRI1 kinase inhibitor 1) prevents the activation of BR signaling transduction by interaction with BRI1 (brassinosteroid insensitive 1), the plasma membrane-localized receptor of BRs (Wang and Chory, 2006). In our study, CYCD3 (cyclin D3), the gene encoding a D-type plant cyclin protein, involved in the BR pathway, was down-regulated in all three treatments (Supplementary Table 6), whereas BKI1 (LOC101262109) was strongly up-regulated only by SA treatment (Supplementary Table 5).

In our study, many genes encoding auxin-responsive proteins (IAA), such as IAA2, IAA3, IAA19, IAA21, and LOC101055550, were all down-regulated in the presence of JA, SA and COR (Supplementary Table 6). Meanwhile, SAUR (small auxin-up RNA) genes (LOC101252930, LOC101247499, and LOC101249503), which are involved in tryptophan metabolism, were also down-regulated by JA, SA and COR (Supplementary Table 6). These results indicate that the three compound treatments perturbed auxin and tryptophan signaling, as these negatively regulate defenses against TYLCV invasion.

**Secondary metabolism regulated by salicylic acid, jasmonate acid, and coronatine**

In tomato plants infected with TYLCV, many secondary metabolites play a key role in response to the virus. 46 DEGs
regulated by JA were involved in the Phenylalanine pathway. Among the 46 DEGs involved in phenolic metabolism, genes encoding phenylalanine ammonia-lyase (PAL), 4-coumarate:CoA ligase (4CL), shikimate O-hydroxy cinnamoyl transferase (HCT) and caffeoyl-CoA O-methyltransferase were induced by JA (Supplementary Table 7). Interestingly, the expression of 4CL was up-regulated under all three treatments with the highest induction observed in JA treated samples (Supplementary Table 7). The genes involved in flavonoid biosynthesis such as F3H (flavanone-3-dioxygenase), F3′5′H (flavonoid-3′,5′-hydroxylase), CHI1 (chalcone–flavonone isomerase 1), CHS1 (chalcone synthase 1) and CHS2 (chalcone synthase 2) were all down-regulated in the presence of the compounds (Supplementary Table 8) with the most significant reduction in transcript abundance observed under SA and COR treatments.

Some amino acids, such as serine, proline, and leucine, have been showed as precursors for the synthesis of secondary metabolites, which play pivotal roles in response to pathogens (Hildebrandt et al., 2015). In this study, the expression of three genes (LOC101259064, LOC101261704, and LOC101258774) involved in tyrosine metabolism were up-regulated by JA (Supplementary Table 7). Three genes involved in the linoleic acid metabolism pathway, two genes encoding lipoxigenase (LoxC and LOC101262081) were also up-regulated by JA and down-regulated in the presence of SA and COR (Supplementary Tables 7, 8). The expression of Lox genes under the exogenous application of JA was induced but repressed by the exogenous application of SA and COR. Taken together, the expression of genes involved in phenylpropanoid metabolism and amino acids metabolism was affected by the exogenous application of JA.

The tomato response to tomato yellow leaf curl virus infection in SINFRI and SICOII silenced plants

To further investigate the role of JA, COR, and SA induced defense against TYLCV invasion, we silenced SINFRI, an essential contributor to SA signaling, and SICOII, a key component of the JA singling pathway. We proceeded to detect TYLCV levels in the silenced plants, even though our data analysis showed that SICOII expression levels remained largely unaffected and SINFRI expression was only weakly up-regulated following compound treatments. The positive control (plant with pTRV1 + pTRV2-SIPDS) showed bleached areas in leaves after 2 weeks of agroinfiltration (Figure 6A). We measured the relative expression level of SINFRI and SICOII after silencing the two genes. Compared to the control plants, the expression level of SINFRI and SICOII in the silenced plants decreased by approximately 40% and 50%, respectively (Figures 6B,C). Further, we detected TYLCV accumulation 1 week after inoculation of the viral clone in the silenced plants. Our results showed that the amount of TYLCV accumulation in SINFRI silencing plants was approximately 19 times greater than in the control plants (Figure 6D). This indicated to us that SINFRI is required for SA mediated defense activation.
FIGURE 6
Tomato yellow leaf curl virus (TYLCV) levels were affected by silencing essential genes in the salicylic acid (SA) and jasmonate acid (JA) signaling pathways. (A) The first picture (from left to right) shows the negative control plant carrying pTRV1 + empty pTRV2. The second picture shows the positive control plant carrying pTRV1 + pTRV2-SlPDS. The bleached areas in leaves were visible 2 weeks after Agro-inoculation. The third picture shows plants Agro-infiltrated with pTRV1 + pTRV2-SlNPR1 while the forth picture depicts plants Agro-infiltrated with pTRV1 + pTRV2-SlCOI1. (B) The relatively expression levels of SlNPR1 in pTRV1 + pTRV2-SlNPR1 Agro-inoculated plants. (C) The relatively expression levels of SlCOI1 in pTRV1 + pTRV2-SlCOI1 Agro-inoculated plants. The SlNPR1 or SlCOI1 expression profile in the pTRV1 + empty pTRV2 inoculation plants (CK) was set to 1 and served as the baseline. Shown is representative data from three independent biological replicates. (D,E) The relative levels of TYLCV in pTRV1+pTRV2-SlNPR1 plants and pTRV1 + pTRV2-SlCOI1 plants 7 days post inoculation of TYLCV. CK represents the baseline TYLCV levels in plants with pTRV1 + empty pTRV2 agroinfiltration. The mean and SEM are derived from pooled data corresponding to three independent biological replicates.

in response to TYLCV infection. Surprisingly, a dramatic reduction in the level of TYLCV accumulation was not observed in the SlCOI1 silenced plants (Figure 6E). Based on previous reports, coi1 mutant plants exhibited enhanced resistance and higher SA accumulation at early stages of bacterial infection (Kloek et al., 2001). We therefore speculate that partial SlCOI1 silencing may have led to hyper-activated SA signaling thereby resulting in a reduction in TYLCV accumulation.

Validation with quantitative real-time RT-PCR of RNA sequencing results
To validate the reliability of the RNA-seq data, 12 differentially expressed genes were randomly selected for qRT-PCR validation (Figure 7). These genes included LOC101264227, LOC101267111, LOC101256271, LOC101243656, LOC101245298, LOC104648161, LOC101258353,
FIGURE 7

RT-PCR verification of RNA-seq results. (A) RT-qPCR and RNA-seq comparison between 12 randomly selected differentially expressed genes (DEGs). The RT-qPCR values represent mean ± SEM from three biological replicates. (B) Regression analysis of gene expression ratios compared by RT-qPCR and RNA-seq analysis.

LOC101265701, LOC101265854, LOC109119038, LOC10126422, and LOC109120532. Our results showed a linear correlation ($R^2 > 0.887$) between the fold change value from the RNA-seq and qRT-PCR experiments, which suggested that the RNA-seq data was reliable.

Discussion

Tomato yellow leaf curl virus is a devastating pathogen that has caused significant reduction in yield worldwide. The initial response to pathogen ingress includes the production of ROS (Lamb and Dixon, 1997). Previous reports suggested that one of the main sources of ROS during the oxidative burst is peroxidase-derived H$_2$O$_2$ (O’Brien et al., 2012). We found that JA, SA, and COR treatments could reduce TYLCV accumulation (Figure 1), and that all three treatments resulted in H$_2$O$_2$ production at the early stage of infection (Figure 2). It is known that H$_2$O$_2$ production is caused by the partial influx of Ca$^{2+}$ (O’Brien et al., 2012). As expected, the genes (LOC101055527 and LOC101260391) encoding CDPK (calcium-dependent protein kinase) and (LOC109120532) encoding calmodulin (CAM) were highly up-regulated by JA in response to TYLCV infection and weakly upregulated by SA and COR. This suggests that the production of Ca$^{2+}$ which is dependent on CDPK activation contributes to ROS production. In addition, several genes involved in peroxidase metabolism were upregulated only in the presence of JA. These included FAR (fatty acyl-CoA reductase) and XDH (xanthine dehydrogenase/oxidase). These results might help explain why JA treatment induced higher level of ROS production than either SA or COR.

The cell wall provides rigidity to the cell and serves as a passive barrier that limits the access of pathogens to the cells. It is therefore not surprising that our data analysis showed that many genes in cell wall category were enriched in JA, SA and COR treated samples. Some common genes were shared between the three treatments. These included genes related to the cell wall biosynthesis such as Wiv-1 (LOC543502), TPS1, GALE, and UGE (Rosti et al., 2007). We identified 369 DEGs in common under the three treatments (Figure 5A and Supplementary Tables 1, 2). Interestingly many genes involved in auxin signaling
was down-regulated in the presence of the compounds (Supplementary Table 6). Considering the trade-off between plant growth and plant defense, it is not surprising that genes related to auxin signal pathways were suppressed. Viral accumulation shifts the nutrient allocation from plant growth to defense. Thus, the activation of plant defenses affects plant growth and development, resulting in a reduction in overall yield.

In plants, many secondary metabolites with roles in plant defense are derived from the different branches of the phenylpropanoid pathway (Naoumkina et al., 2010; Piasecka et al., 2015). In this study, we identified many genes related to the phenylpropanoid pathway that were induced in the presence of all three compounds. For instance PAL and 4CL, two genes in the PAL pathway were up-regulated, while genes involved in flavonoid biosynthesis such as F3H, F3′5′H, were all down-regulated. These results indicated that while the phenylpropanoid pathway might be induced upon the three treatments, there are multiple downstream branches which might be involved in many intermediated products that function as phytoalexins to defend against the virus. The C2 protein, which is encoded by TYLCV, has been showed to suppress JA responses by interacting with plant ubiquitin, which results in the degradation of JAZ1 protein, thereby leading to a reduction in JA mediated defenses (Li P. et al., 2019). βC1 protein encoded by the betasatellite of tomato yellow leaf curl China virus (TYLCCNB-βC1), suppresses JA dependent terpene biosynthesis in plants (Li et al., 2014). Our analysis showed that COR, as a mimic of JA, induced many genes that overlapped with JA treatment during TYLCV infection. Our results are consistent with many previous reports that showed that COR and JA share similar functions such as the activation of JA responses and the elicitation of secondary metabolism (Thilmony et al., 2006). The identification of secondary compounds is an emergent area of research and will contribute to advancements in agricultural research through the development of effective plant defense strategies.

We report that SINPRI silenced plant accumulated more TYLCV than wild type plants after Agro-inoculation of TYLCV. However, in comparison with wild type plants, in SICOI silenced plants, there was a diminution in TYLCV levels. We speculate that SA signaling pathway might by hyper-activated when SICOI is partly silenced, thereby resulting in a reduction in TYLCV levels. To date, several key regulatory proteins involved in SA-JA crosstalk have been identified in Arabidopsis. NPR1, the major positive regulator of SA signaling, is a possible JA-SA crosstalk mediator (Dong, 2004) and requires nuclear localization for efficient function (Spoel et al., 2003). Interestingly coi1 mutant plants display enhanced resistance against biotrophic pathogens, and accumulated a greater amount of SA at early stages of bacterial infection (Kloek et al., 2001). Based on our transcriptomics data, SIPRIb expression was inhibited by JA treatment, which indicated that SA signaling pathway was suppressed by JA treatment. Further, considering there was only 50% reduction in SICOI expression through the VIGS system, JA signaling would still be partially activated upon TYLCV invasion. To conclude, in SICOI silenced plants, low levels of JA accumulation combined with a hyper-activated SA response may lead to a robust immune response against TYLCV accumulation. In future, the generation of null SICOI lines would further aid in determining the function of SICOI during TYLCV tomato interactions.

**Conclusion**

Tomato yellow leaf curl virus infection causes substantial losses in tomato production worldwide. Based on our findings, SA, JA, and COR inhibit TYLCV accumulation at the early stage of TYLCV infection. We carried out RNA-Seq to identify DEGs induced under three different treatments. In addition, we silenced SINPRI and SICOI in tomato to investigate the role of SA and JA induced systemic resistance in defense against TYLCV infection. Our findings will serve as a valuable resource for understanding the mechanistic details underlying TYLCV tomato interactions.

**Data availability statement**

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

**Author contributions**

PW and SS: conceptualization. PW, SS, and KL: formal analysis. SS and XG: funding acquisition and supervision. PW, KL, RP, NL, and BH: methodology. SS, AJA, and XG: writing—original draft. AJA and XG: writing—review and editing. All authors contributed to the article and approved the submitted version.

**Funding**

This research was funded by the Shanghai Agricultural and Rural Committee Foundation (Grant No. 2021-02-08-00-12-F00799), the Key Research and Development Project of Shanxi Province (Grant No. 202102140601015), the Shanghai International Scientific and Technological Cooperation...
Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Chen, X. Y., Wang, D. D., Fang, X., Chen, X. Y., and Mao, Y. B. (2019). Plant specialized metabolism regulated by jasmonate signaling. Plant Cell Physiol. 60, 2638–2647. doi: 10.1093/pcp/pcz126

Corrales-Gutierrez, M., Medina-Puche, L., Yu, Y. L., Wang, L. P., Ding, X., Luna, A. P., et al. (2020). The C4 protein from the geminivirus Tomato yellow leaf curl virus confers drought tolerance in Arabidopsis through an ABA-independent mechanism. Plant Biotechnol. J. 18, 1121–1123. doi: 10.1111/pbi.13280

Decker, D., Meng, M., Gornicka, A., Hofer, A., Wilczynska, M., and Kleczkowski, A. W. (2012). Substrate kinetics and substrate effects on the quaternary structure of barley UDP-glucose pyrophosphorylase. Phytochemistry 79, 39–45. doi: 10.1016/j.phytochem.2012.04.002

Deng, X.-G., Zha, T., Peng, X.-J., Xi, D.-H., Guo, H., Yin, Y., et al. (2016). Role of brassinosteroid signaling in modulating Tobacco mosaic virus resistance in Nicotiana benthamiana. Sci. Rep. 6:20579. doi: 10.1038/srep20579

Dharmasiri, N., and Estelle, M. (2004). Auxin signaling and regulated protein degradation. Trends Plant Sci. 9, 302–308. doi: 10.1016/j.tplants.2004.08.001

Dinesh-Kumar, S. P., Anandalakshmi, R., Marathe, R., Schiff, M., and Liu, Y. (2003). Virus-induced gene silencing. Methods Mol. Biol. 126, 287–294.

Ding, Y. L., Sun, T. J., Ao, K., Peng, Y. J., Zhang, Y. X., Li, X., et al. (2018). Opposite roles of salicylic acid receptors NPR1 and NPR3/NPR4 in transcriptional regulation of plant immunity. Cell 173, 1454–1467.e15. doi: 10.1016/j.cell.2018.03.044

Dong, X. N. (2004). NPR1, all things considered. Curr. Opin. Plant Biol. 7, 547–552. doi: 10.1016/j.pop.2004.07.005

Du, M. M., Zhao, J. H., Tseng, D. W. T., Liu, Y. Y., Deng, L., Yang, T. X., et al. (2017). MYC2 orchestrates a hierarchical transcriptional cascade that regulates jasmonate-mediated plant immunity in tomato. Plant Cell 29, 1883–1906. doi: 10.1105/tpc.16.00953

Fey, B. J. F., Benedetti, C. E., Penfold, C. N., and Turner, J. G. (1994). Arabidopsis mutants selected for resistance to the phytotoxin coronatine are male sterile, insensitive to mifetyl jasmonate, and resistant to a bacterial pathogen. Plant Cell 6, 751–759. doi: 10.1105/tpc.6.5.751

Gao, J. X., and Chen, J. (2018). Transcriptome analysis identifies candidate genes associated with melanin and toxin biosynthesis and pathogenicity of the maize pathogen, Curvularia lunata. Phytobiology. 166, 233–241. doi: 10.1111/jph.12680

Gao, J. X., Jing, J., Liu, T., and Chen, J. (2015). Identification of Cbf-1-regulated proteins associated with the production of non-host-specific toxin and pathogenicity in Cochliobolus lunatus. J. Phytopathol. 163, 33–41. doi: 10.1111/jph.12278

Gao, S. G., Li, Y. Q., Gao, J. X., Suo, J. J., Fu, K. H., Li, Y. Y., et al. (2014). Genome sequence and virulence variation-related transcriptome profiles of Curvularia lunata, an important maize pathogenic fungus. BMC Genomics 15:627. doi: 10.1186/1471-2164-15-627

Geng, X. Q., Jin, L., Shimada, M., Kim, M. G., and Mackey, D. (2014). The phytotoxin coronatine is a multifunctional component of the virulence armament of Pseudomonas syringae. Planta 240, 1149–1165. doi: 10.1007/s00425-014-2151-x

Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu. Rev. Phytopathol. 43, 203–227. doi: 10.1146/annurev.phyto.43.040204.135923

Hildebrandt, T. M., Nesi, A. N., Araujo, W. L., and Braun, H.-P. (2015). Amino acid catabolism in plants. Mol. Plant 8, 1563–1579. doi: 10.1016/j.molp.2015.09.005

Huang, Y., Li, T., Xu, Z. S., Wang, F., and Xiong, A. S. (2017). Six NAC transcription factors involved in response to TYLCV infection in resistant and susceptible tomato cultivars. Plant Physiol. Biochem. 120, 61–74. doi: 10.1016/j.plaphy.2017.09.020

Katris, L., Schimpler, A. L., Staswick, P. E., He, S. Y., and Howe, G. A. (2008). COI1 is a critical component of a receptor for jasmonate and the bacterial virulence factor coronatine. Proc. Natl. Acad. Sci. U.S.A. 105, 7180–7185. doi: 10.1073/pnas.0802323105

Kim, D., Langmead, B., and Salzberg, S. L. (2015). HISAT: A fast spliced aligner with low memory requirements. Nat. Methods 12, 357–360. doi: 10.1038/nmeth.3317

Kloek, A. P., Verbovy, M. L., Sharma, S. B., Schoela, J. E., Vogel, J., Klessig, D. F., et al. (2001). Resistance to Pseudomonas syringae conferred by an Arabidopsis thaliana coronatine-insensitive (coi1) mutation occurs through two distinct mechanisms. Plant J. 26, 509–522. doi: 10.1046/j.1365-313x.2001.01050.x

Lamb, C., and Dixon, R. A. (1997). The oxidative burst in plant disease resistance. Annu. Rev. Plant Biol. 48, 251–275.

Lee, J. K., Eom, S. H., and Hyun, T. K. (2018). Enhanced biosynthesis of sauponins by coronaetin in cell suspension culture of Kalopanax septemlobus. 3 Biotech 8:59. doi: 10.1007/s13205-018-1090-9

Li, B., and Dewey, C. N. (2011). RSEM: Accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics 12:323. doi: 10.1186/1471-2105-12-323

Li, C., Yan, J. M., Li, Y. Z., Zhang, Z. C., Wang, Q. L., and Liang, Y. (2013). Silencing the SpMPK1, SpMPK2, and SpMPK3 genes in tomato reduces abscisic acid-mediated drought tolerance. Int. J. Mol. Sci. 14, 21983–21996. doi: 10.3390/ijms14121983

Li, P., Liu, C., Deng, W. H., Yao, D. M., Pan, L. L., Li, Y. Q., et al. (2019). Plant begomoviruses subvert ubiquitination to suppress plant defenses against insect vectors. PLoS Pathog. 15:e1007607. doi: 10.1371/journal.ppat.1007607

Li, R., Weldegergis, B. T., Li, J., Jung, C., Qi, J., Sun, Y. W., et al. (2014). Virulence factors of geminivirus interact with MYC2 to subvert plant resistance and promote vector performance. Plant Cell 26, 4991–5008. doi: 10.1105/tpc.114.133181
susceptible tomatoes. Plant Sci. Solanum habrochaites resistance with their wild progenitor curl virus (TYLCV)-resistant tomatoes share molecular mechanisms sustaining Arabidopsis et al. (2007). UDP-glucose 4-epimerase isoforms UGE2 and UGE4 cooperate in Trends Plant Sci. lycopersicum. Mol. Plant Pathol. pathogens. Phytochemistry. Can. J. Plant Pathol. of Tomato yellow leaf curl bigeminivirus (TYLCV) infection on tomato cell Wall and phytohormones in interaction between plants and necrotrophic Can. J. Plant Pathol. metabolic effects of coronatine on cotton seedling growth under salt stress. J. Plant Growth Regul. growth and development of tomato and tomato cell Wall. Planta 230, 1565–1579. doi: 10.1007/s00425-12-0936-3 New Phytol. 206, 948–964. doi: 10.1111/nph.13325 Pieterse, C. M. J., Van der Does, D., Zamioudis, C., Leon-Reyes, A., and Van Wees, S. C. M. (2012). Hormonal modulation of plant immunity. Annu. Rev. Cell Dev. Biol. 28, 489–521. Pieterse, C. M. J., Zamioudis, C., Berendse, R. L., Weller, D. M., Van Wees, S. M. C., and Bakker, P. (2014). Induced systemic resistance by beneficial microbes. Annu. Rev. Phytopathol. 52, 347–375. Prasad, A., Sharma, N., Hari-Gowtham, G., Muthamilarasan, M., and Prasad, M. (2020). Tomato yellow leaf curl virus: Impact, challenges, and management. Trends Plant Sci. 25, 897–911. doi: 10.1016/j.tplants.2020.03.015 Rosti, I., Barton, C. J., Albrecht, S., Dupree, P., Pauly, M., Findlay, K., et al. (2007). UDP-glucose 4-epimerase isoforms UGE2 and UGE4 cooperate in providing UDP-galactose for cell wall biosynthesis and growth of Arabidopsis thaliana. Plant Cell 19, 1565–1579. doi: 10.1105/tpc.106.049619 Sade, D., Sade, N., Brotman, Y., and Cinosnek, H. (2020). Tomato yellow leaf curl virus (TYLCV)-resistant tomatoes share molecular mechanisms sustaining resistance with their wild progenitor Solanum habrochaites but not with TYLCV-susceptible tomatoes. Plant Sci. 295:110439. doi: 10.1016/j.plantsci.2020.110439 Sade, D., Sade, N., Shriki, O., Lerner, S., Gehremedhin, A., Karavani, A., et al. (2014). Water balance, hormone homeostasis, and sugar signaling are all involved in tomato resistance to tomato yellow leaf curl virus. Plant Physiol. 165, 1684–1697. doi: 10.1104/pp.114.243402 Sheard, L. B., Tan, X., Mao, H. B., Withers, J., Ben-Nissan, G., Hinds, T. R., et al. (2010). Jasmonate perception by inositol-phosphate-potentiated COI1-JAZ co-receptor. Nature 468, 400–405. doi: 10.1038/nature09430 Spool, S. H., Kosnerneq, A., Claessens, S. M. C., Korzunis, J. P., Van Pelt, J., Mueller, M. J., et al. (2003). NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. Plant Cell 15, 760–770. doi: 10.1105/tpc.010919 S. P. Tamagomi, S., and Kodama, O. (2000). Coronatine elicits phytoalexin production in rice leaves (Oryza sativa L.) in the same manner as jasmonic acid. Phytochemistry 54, 689–694. doi: 10.1016/s0031-9422(00)00190-4 Thilmony, R., Underwood, W., and He, S. Y. (2006). Genome-wide transcriptional analysis of the Arabidopsis thaliana interaction with the plant pathogen Pseudomonas syringae pv. tomato DC3000 and the human pathogen Escherichia coli O157: H7. Plant J. 46, 34–53. doi: 10.1111/j.1365-313X.2006.02725.x Thines, B., Katsir, L., Melotto, M., Niu, Y., Mandaokar, A., Liu, G. H., et al. (2007). JAZ repressor proteins are targets of the SCFCOI1 complex during jasmonate signaling. Nature 448, 661–665. doi: 10.1038/nature06960 Wang, X. L., and Chory, J. (2006). Brassinosteroids regulate dissociation of BIK1, a negative regulator of BRI1 signaling, from the plasma membrane. Science 313, 1118–1122. doi: 10.1126/science.1127593 Wu, Y., Zhang, D., Chu, J. Y., Boyle, P., Wang, Y., Brindile, I. D., et al. (2012). The Arabidopsis NPR1 protein is a receptor for the plant defense hormone salicylic acid. Cell Rep. 1, 639–647. doi: 10.1016/j.celrep.2012.05.008 Xie, Z. X., Duan, L. S., Li, Z. H., Wang, X. D., and Liu, X. J. (2015). Dose-dependent effects of coronatine on cotton seedling growth under salt stress. J. Plant Growth Regul. 34, 651–664. doi: 10.1007/s10640-015-9501-1 Yu, C. J., Fan, L. L., Gao, J. X., Wang, M., Wu, Q., Tang, J., et al. (2015). The platelet-activating factor acetylhydrolase gene derived from Trichoderma harzianum induces maize resistance to Curvularia lunata through the jasmonic acid signaling pathway. J. Environ. Sci. Health B 50, 708–717. doi: 10.1080/03602234.2015.1048104 Yu, G., Xian, L., Sang, Y. Y., and Macho, A. P. (2019). Cautionary notes on the use of Agrobacterium-mediated transient gene expression upon SGT1 silencing in Nicotiana benthamiana. New Phytol. 222, 14–17. doi: 10.1111/nph.15601 Zhang, H., Gong, H. R., and Zhou, X. P. (2009). Molecular characterization and pathogenicity of tomato yellow leaf curl virus in China. Virus Genes 39, 249–255. doi: 10.1007/s11262-009-0384-8 Zhang, Z. C., He, B., Sun, S., Zhang, X., Li, T., Wang, H. H., et al. (2021). The phytoalexin COI1 induces transcriptional reprogramming of photosynthetic, hormonal and defence networks in tomato. Plant Biol. 23, 69–79. doi: 10.1111/plb.13239