Protoplasma (2024) 261:499–512
https://doi.org/10.1007/s00709-023-01911-0

ORIGINAL ARTICLE

Transcriptome analysis of *Citrus sinensis* reveals potential responsive events triggered by *Candidatus* Liberibacter asiaticus

Chang Liu[1] · Xiaopeng Chang[1] · Fuxuan Li[1] · Yana Yan[1] · Xiru Zuo[1] · Guiyan Huang[1] · Ruimin Li[1]

Received: 27 July 2023 / Accepted: 1 December 2023 / Published online: 13 December 2023

Abstract

Citrus Huanglongbing (HLB), caused by *Candidatus* Liberibacter asiaticus (CLas), is a devastating immune-mediated disorder that has a detrimental effect on the citrus industry, with the distinguishing feature being an eruption of reactive oxygen species (ROS). This study explored the alterations in antioxidant enzyme activity, transcriptome, and RNA editing events of organelles in *C. sinensis* during CLas infection. Results indicated that there were fluctuations in the performance of antioxidant enzymes, such as ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), peroxidase (POD), and superoxide dismutase (SOD), in plants affected by HLB. Transcriptome analysis revealed 3604 genes with altered expression patterns between CLas-infected and healthy samples, including those associated with photosynthesis, biotic interactions, and phytohormones. Samples infected with CLas showed a decrease in the expression of most genes associated with photosynthesis and gibberellin metabolism. It was discovered that RNA editing frequency and the expression level of various genes in the chloroplast and mitochondrion genomes were affected by CLas infection. Our findings provide insights into the inhibition of photosynthesis, gibberellin metabolism, and antioxidant enzymes during CLas infection in *C. sinensis*.

Keywords *Citrus sinensis* · Huanglongbing · Photosynthesis · RNA editing · Gene expression

Introduction

Cultivation of citrus plants is widespread due to their delicious taste and high nutritional content (Talon et al. 2020). *Citrus sinensis* is especially noteworthy for its high commercial value in subfamily Aurantioideae (Biswas et al. 2020; Liu et al. 2022). However, they are often susceptible to diseases, including Huanglongbing (HLB), which was first identified in Asia and has since spread to South Africa and South America (Ferrarezi et al. 2020). The impact of Huanglongbing (HLB) on the citrus industry is significant, with an estimated 90% of affected areas experiencing damage (Shahzad et al. 2020). The disease is caused by the bacterium *Candidatus* Liberibacter asiaticus (*C*Las), which is transmitted by the *Diaphorina citri* (Hu et al. 2021; Alquézar et al. 2022). At the present time, there is unfortunately no cure available for HLB. Possible treatments include antibiotics, thermotherapy, gibberellin, and the use of citrus cultivars that are resistant to HLB (Munir et al. 2018; Ma et al. 2022). The interactions between citrus and *C*Las have been continuously explored, as several important secreted proteins in *C*Las have been functionally analyzed, including SDE1, SDE3, and SDE15 (Clark et al. 2018; Pang et al. 2020; Shi et al. 2023). Despite extensive research and findings, the pathogenesis of HLB remains unclear.

Plants are faced with environmental stress from abiotic and biotic factors, and the response to this is greatly influenced by the presence of phytohormones, which are responsible for the regulation of multiple functions and intricate biological processes (Ku et al. 2018). Research has shown that phytohormones are necessary for plants to effectively guard themselves against pathogens (Collum and Culver 2016). The major hormones that are functionally active are abscisic acid (ABA), auxin, brassinolide (BR), cytokinin (CK), ethylene (Eth), gibberellin (GA),

Handling Editor: April H Hastwell.

Chang Liu and Xiaopeng Chang contributed equally to this work.

✉ Guiyan Huang
huangguiyan@gnnu.edu.cn

✉ Ruimin Li
liruimin@gnnu.edu.cn

[1] College of Life Sciences, Gannan Normal University, Ganzhou 341000, Jiangxi, China

jasmonic acid (JA), and salicylic acid (SA) (Checker et al. 2018). Utilizing GA can help to improve the consequences of Huanglongbing (HLB) infection, including reducing blotchy mottle and boosting plant growth (Ma et al. 2022). *C. sinensis* displayed a higher tolerance to HLB, likely due to the augmented levels of auxins, CKs, and JA, found in citrus affected by HLB (Peng et al. 2021; Suh et al. 2021). However, the levels of SA varied between different citrus varieties during CLas infection (Suh et al. 2021; Zou et al. 2021).

Photosynthesis in plants takes place in chloroplasts, which are capable of converting the sun's light energy into chemical energy effectively (Kruse et al. 2005). Furthermore, they are instrumental in defending against pathogen infection (Lu and Yao 2018). Consequently, chloroplasts are indispensable for both photosynthesis and independent immune responses when the plant is exposed to pathogen (Jensen and Leister 2014). Research has revealed that CLas infection has an impact on carbon metabolism, resulting in an increase of starch in leaves and the destruction of chloroplasts (Etxeberria et al. 2009). Transcriptome analysis of *Citrus* spp. showed that the photosynthesis processes were impeded when citrus plants were infected with HLB (Zhong et al. 2016; Hu et al. 2017; Tang et al. 2018; Liu et al. 2019; Zhao et al. 2019).

RNA editing events in plant organellar were documented and studied in the early 2000s (Shikanai 2006). In recent years, the importance of RNA editing in plant development and defense has been increasingly recognized (Hao et al. 2021). It has been suggested that RNA editing is a key mechanism for the regulation of gene expression in chloroplasts (Wang et al. 2022). RNA editing has been shown to be involved in the regulation of chloroplast-localized defense responses, including the expression of defense-related genes, the accumulation of defense-related metabolites, and the modulation of JA and ABA signaling pathways (Jiang and Dehesh 2021; Zhang et al. 2022). Moreover, it has been suggested that RNA editing is involved in the abiotic stress response in *C. reticulata* (Pan et al. 2021). Nevertheless, a few investigations have indicated correlations between RNA editing events and the emergence of HLB.

RNA-seq offers a swift approach to distinguishing differentially expressed genes (DEGs) and discovering gene clusters that are co-expressed (Han et al. 2015; Conesa et al. 2016). Moreover, RNA-seq data can be utilized to identify single nucleotide polymorphisms and can be used to detect RNA-editing events (Pinto and Levanon 2019). To comprehend the events that may be elicited by CLas and uncover the molecular foundation during interaction between CLas and *C. sinensis*, we conducted a study to investigate the transcriptomic alterations, including the identification of DEGs, the enrichment of gene functions, and the detection of RNA-editing events in mitochondria and chloroplasts during CLas infection in *C. sinensis* using RNA-seq, which provides valuable insight for further research.

Materials and methods

Measurement the activities of antioxidant enzymes

Leaves from CLas free and -infected two years old *C. sinensis* cv. "Newhall" plants, grown in the citrus germplasm of Gannan Normal University, were collected for this study. To determine whether the citrus plants were CLas free or -infected, qPCR was employed as previously described (Li et al. 2023). The leaves were immediately ground in phosphate buffer saline solutions and centrifuged to obtain the supernatant for determining the activities of antioxidant enzymes. The activities of ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), peroxidase (POD), and superoxide dismutase (SOD) were measured using commercially available APX activity detection assay kits (BC0225, Solarbio), CAT activity detection assay kits (BC4785, Solarbio), GR activity detection assay kits (BC1165, Solarbio), POD activity detection assay kits (BC0095, Solarbio), and SOD activity detection assay kits (BC0175, Solarbio), respectively.

RNA extraction and sequencing

Total RNA was extracted from citrus leaves using the TaKaRa MiniBEST Plant RNA Extraction Kit (9769, TaKaRa) according to the manufacturer's instructions. The concentration, purity, and integrity of the extracted total RNA were assessed using NanoDrop one Spectrophotometer (Thermo Fisher Scientific) and Agilent Bioanalyzer 2100 system (Agilent Technologies), respectively. For library construction, the TruSeq RNA Library Prep Kit v2 was used, and the resulting libraries were subsequently sequenced on an Illumina HiSeq 4000 platform.

Transcriptome analysis

Raw sequencing reads were subjected to quality control using trimmomatic v0.36 to remove adapter sequences and low-quality sequences. Subsequently, the clean reads were aligned to the reference genome of *C. sinensis* (version 3.0) obtained from the CPBD database using Hisat2 with default parameters (Kim et al. 2019; Liu et al. 2022). To estimate the Fragments Per Kilobase of exon model per million mapped fragments (FPKM) value for each gene, a count matrix was generated using StringTie (Pertea et al. 2015). For the analysis of gene expression patterns in the chloroplast and mitochondrion genomes, the reference genomes of chloroplast (NC_008334.1) and mitochondrion (NC_037463.1)

were downloaded from the NCBI genome database since this information was not available in the CPBD database (Bausher et al. 2006; Yu et al. 2018). Differential expressed genes (DEGs) were identified using DESeq2 with a significance threshold of |log2(fold change, FC)|≥ 1 and false discovery rate (FDR) < 0.05 (Love et al. 2014). The gene trend cluster was performed using R package "Mfuzz" (Kumar and Futschik 2007).

Functional and pathway analysis of DEGs

The Gene Ontology (GO) enrichment analysis of DEGs was performed using the "GO enrichment" modules in TBtools, with a significance threshold of a p value < 0.05 (Chen et al. 2020). Similarly, the KEGG enrichment analysis of DEGs was conducted using the "KEGG enrichment analysis" modules in TBtools, also with a significance threshold of a p value < 0.05 (Chen et al. 2020). Additionally, MapMan was utilized to display the DEGs on diagrams representing specific metabolic pathways (Thimm et al. 2004).

RNA editing event identification

REDO was used to detect the RNA editing events in chloroplast and mitochondrion (Wu et al. 2018). BWA was employed for RNA-seq mapping (Li and Durbin 2009), while GATK and SAMtools were employed for genome variant calling (Li et al. 2009; McKenna et al. 2010). The output variant call format files and the sequence and gene annotation file of the reference genome were then used as input files for REDO. The output files generated by REDO were manually settled.

RT-qPCR analysis

Total RNA of the *C. sinensis* leaves was extracted using M5 HiPer Plant RNeasy Complex Mini Kit (Mei5bio, China). Then, first strand cDNA was reverse translated by EasyScript® First-Strand cDNA Synthesis SuperMix (Transgen, China). The qPCR experiments were conducted on ABI StepOne PLUS Real-Time PCR System (ABI, USA) with 2X M5 HiPer UltraSYBR Mixture (Low ROX) (Mei5bio, China). All primers were listed in Table S1. The relative expression profiles of candidate genes were calculated using $2^{-\Delta\Delta CT}$ method with *CsGAPDH* as internal gene (Livak and Schmittgen 2001).

Statistical analysis

The statistical significance of experimental data was import into SPSS 25.0 and analyzed by one-way ANOVA using Tukey's (HSD) test or by Student's unpaired two-sided t-test.

Results

CLas infection caused alterations in physiological indices of *C. sinensis*

To assess the changes in physiological indices of *C. sinensis* during CLas infection, the levels of chlorophyll and the activities of APX, CAT, GR, POD, and SOD were evaluated (Fig. 1). Results showed that the chlorophyll content of leaves of citrus plants affected by HLB was significantly reduced (Fig. 1A). Upon CLas infection, the activities of APX and GR were diminished (Fig. 1A, D), whereas CAT, POD, and SOD activities were enhanced (Fig. 1C, E, F).

Analysis of transcriptome data obtained from RNA-seq

In this study, the amount of filtered clean reads of sequenced samples for transcriptome analysis varied from 35,920,788 to 51,884,560. The correlation and PCA analysis revealed that biological repeatability is of a good quality (Fig. 2A, B). The DEGs were identified based on a significance threshold of |log2(fold change, FC)|≥ 1 and a false discovery rate (FDR) of < 0.05 (Fig. 2C). A total of 3604 DEGs were identified between CLas free and -infected citrus plants, with 1371 downregulated and 2233 upregulated (Fig. 2D and Table S2).

Clustering and GO enrichment analysis of DEGs

The R package "Mfuzz" was used to analyze the expression tendency of DEGs, and the tendency was discernible. Analysis revealed that DEGs were mainly divided into two distinct categories, with either down- or upregulation, although there were genes with sample specificity (Fig. 3A). The GO enrichment analysis revealed that the majority of the top 20 enriched GO terms were associated with either "chloroplast" or "photosynthesis," suggesting a significant effect on photosynthesis (Fig. 3B and Table S3). The enriched GO terms included "GO:0009535, chloroplast thylakoid membrane," "GO:0055035, plastid thylakoid membrane," "GO:0019684, photosynthesis, light reaction," and "GO:0015979, photosynthesis," (Fig. 3B).

KEGG enrichment analysis of DEGs

To investigate the particular pathway of the downregulated and upregulated DEGs, KEGG enrichment analysis was conducted (Fig. 4). The top 25 KEGG enrichment pathways of downregulated DEGs, including "photosynthesis," "Fructose and mannose metabolism," "Carotenoid biosynthesis," and

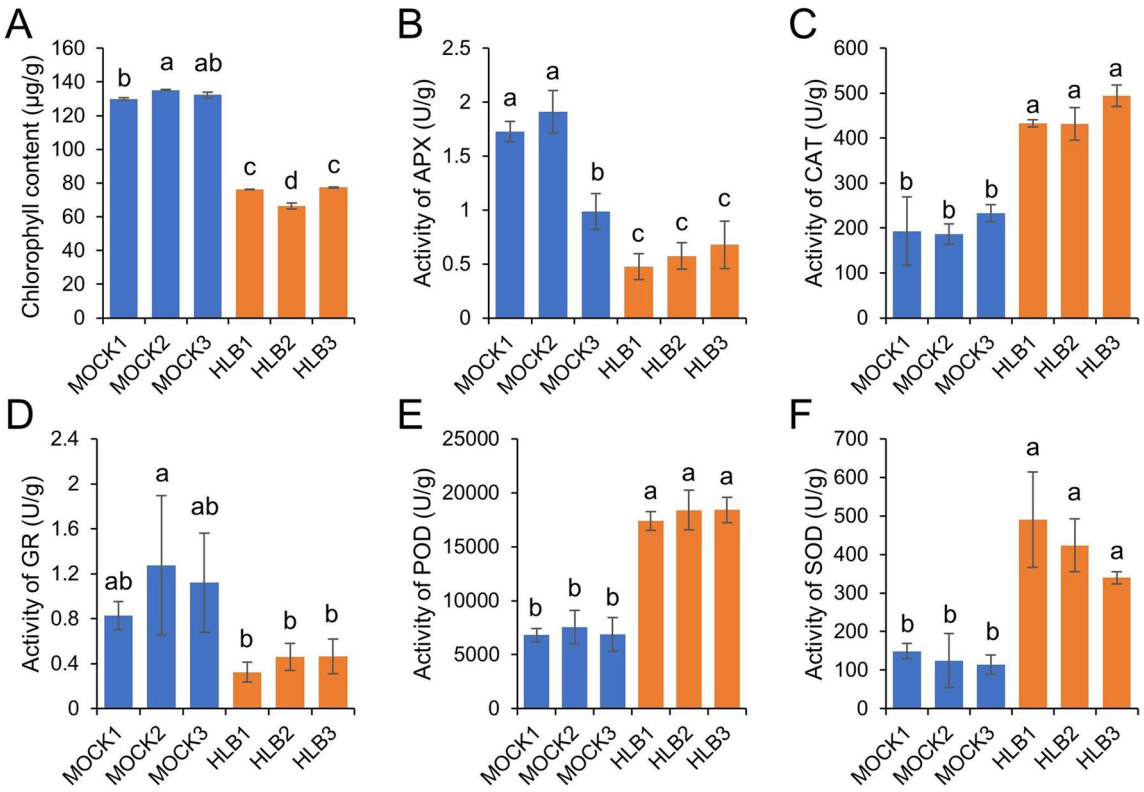


Fig. 1 Determination analysis of chlorophyll content and oxidation–reduction enzyme activity of CLas free and -infected citrus plants. **A** Content of chlorophyll, **B** activity of ascorbate peroxidase (APX), **C** activity of catalase (CAT), **D** activity of glutathione reductase (GR), **E** activity of peroxidase (POD), **E** activity of superoxide dismutase (SOD). Statistical analysis revealed significant differences (p value < 0.05) between the groups, as indicated by different letters

"Nitrogen metabolism," suggest that these pathways were inhibited during CLas infection (Fig. 4A and Table S4). Conversely, the top 25 KEGG enrichment pathways of upregulated DEGs encompass "Amino sugar and nucleotide sugar metabolism," "Phenylpropanoid biosynthesis," "Oxidative phosphorylation," and "Starch and sucrose metabolism," signifying that these pathways were augmented during CLas infection (Fig. 4B and Table S5).

DEGs associated with biotic stress in *C. sinensis* during CLas infection

Analysis of *C. sinensis* during CLas infection uncovered a range of DEGs associated with biotic stress, including those involved in hormone signaling, cell wall, proteolysis, redox state, transcription factors, and secondary metabolites (Fig. 5A and Table S6). Specifically, analysis of differentially expressed genes (DEGs) involved in gibberellin signaling (Fig. 5A and Table S6) revealed that two ent-kaurene oxidase genes were upregulated, while the third showed downregulation. Additionally, two gibberellin 20-oxidase genes were downregulated, while two gibberellin receptor GID1 genes were upregulated and one gibberellin 2-oxidase gene demonstrated downregulation. Furthermore, the isochorismate synthase gene was downregulated, while two salicylic acid 3-hydroxylase genes were upregulated in relation to salicylic acid metabolism. Lastly, the majority of DEGs connected to cell wall metabolism and redox state, such as expansin, peroxidases, and glutathione S-transferase genes, showed an upregulation (Fig. 5A and Table S6).

DEGs associated with photosynthesis in *C. sinensis* during CLas infection

In order to evaluate the influence of photosynthesis on CLas infection, MapMan software was used to analyze the expression levels of DEGs in photosynthesis (Fig. 5B and Table S7). Results indicated a decrease in expression of DEGs associated with Photosystem I and II, such as *LHCa1/2/3/4*, *PsaK*, *PsaL*, *PsaN*, *LHCb1/2/3*, *LHCb4/5/6*, *PsbTn*, *PsbW*, and *PsbQ* (Fig. 5B and Table S7). Most of the DEGs that form part of the Calvin cycle also showed a decrease in expression, apart from a gene encoding the RuBisCo heterodimer small subunit and a gene encoding fructose 1,6-bisphosphate aldolase (Fig. 5B and Table S7).

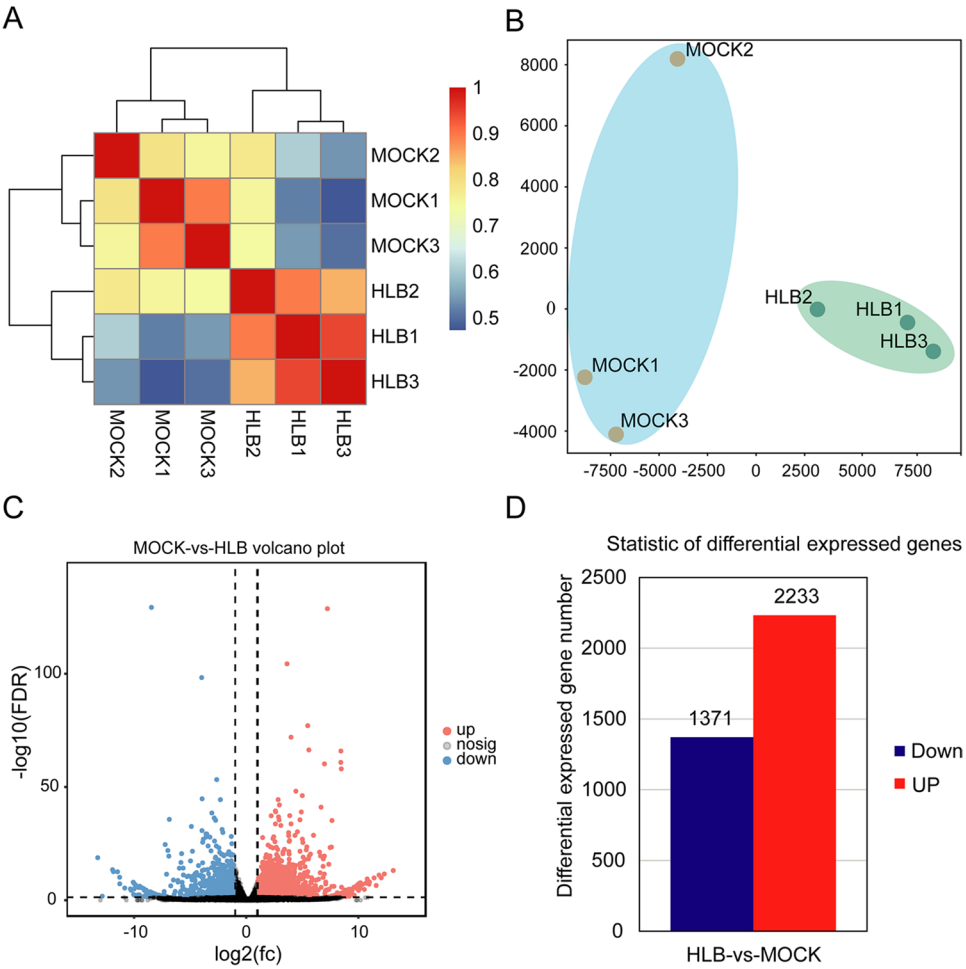


Fig. 2 Comparison transcriptome analysis of CLas free and -infected citrus plants. **A** Cluster analysis, **B** principal component analysis, **C** volcano map analysis, **D** number of differentially expressed genes. "MOCK" referred to CLas free *Citrus sinensis* samples and "HLB" referred to CLas infected samples. the number after MOCK or HLB indicated the number of biological replicates

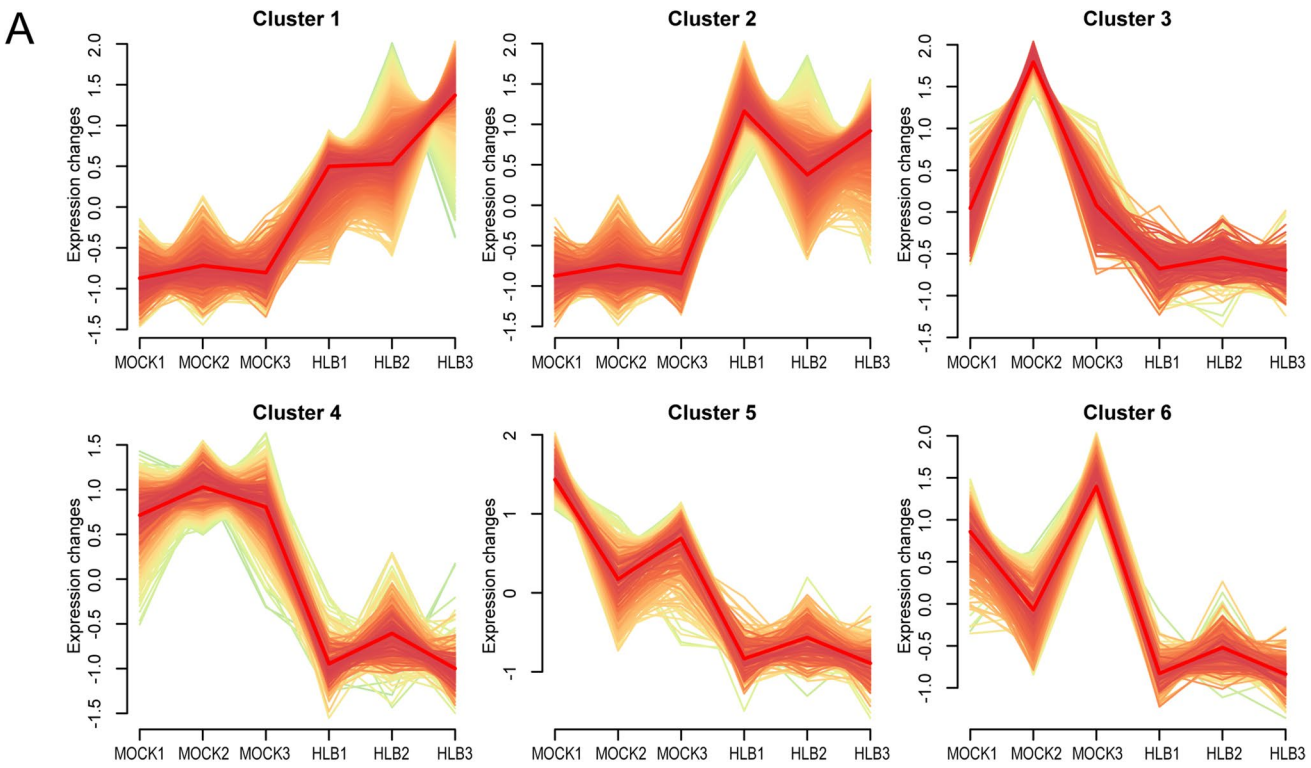
Moreover, four out of five DEGs associated with ATP synthase had decreased expression.

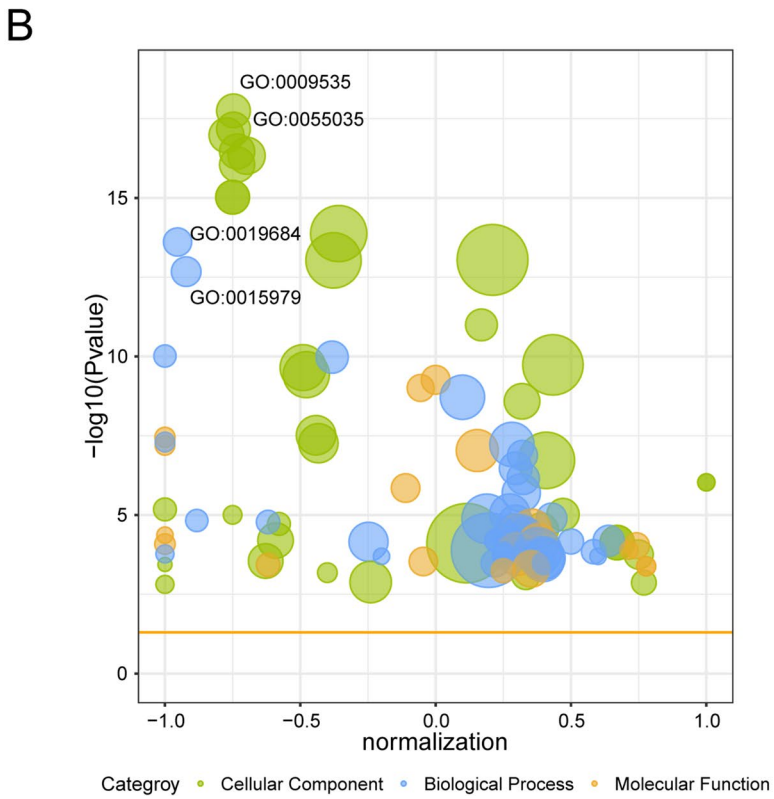
Examination of RNA editing events and their frequency alteration in chloroplast and mitochondrion of *C. sinensis* during CLas infection

A systematic analysis of DEGs encoded by the genome of chloroplast and mitochondrion were conducted, and 13 and 9 DEGs were identified respectively (Fig. 6 and Figure S1). The DEGs in the chloroplast were associated with photosystem I and II, including *psbK*, *ndhB*, *petA*, and *psaJ* genes, all of which were downregulated (Fig. 6C). In contrast, the only DEG in the mitochondrion, nad6, which is involved in oxidative phosphorylation, was upregulated (Figure S1C). To generate reliable RNA editing events, the common RNA editing events from MOCK and HLB samples were utilized for further study (Fig. 7A and S2A). Most of the common RNA editing events were cytimidine mutant to uracil, which was marked as thymine (Table S8, S9, and S10). Significantly decreased RNA editing frequencies were observed for some RNA editing events, such as the *rps14* gene in chloroplast and the *cob* gene in mitochondrion, during CLas infection (Fig. 7B and S2B). Bioinformatic analysis generated RNA editing events, which were verified by PCR amplification and Sanger sequencing. The peaks of the sequencing results indicated the presence of the editing events (Fig. 8).

RT-qPCR verification of transcriptome data

To validate the accuracy of the transcriptome results, we conducted RT-qPCR analysis and compared the expression tendency of nine candidate genes using their RT-qPCR and RNA-seq data. Results showed that eight of the nine genes were upregulated and one gene, *Cs_ont_6g016140*, was downregulated (Fig. 9). The expression patterns of these genes were consistent with the FPKM value of RNA-seq analysis. For instance, *Cs_ont_5g006680*, a WRKY transcription factor, was induced about 3.50-fold during CLas infection as determined by RT-qPCR analysis (Fig. 9). Similarly, the log2FC of *Cs_ont_5g006680* was 3.79-fold according to the RNA-seq analysis. This suggests that the RNA-seq data is highly reliable.





GO term Top 20

ID	Descrption
GO:0009535	chloroplast thylakoid membrane
GO:0055035	plastid thylakoid membrane
GO:0044436	thylakoid part
GO:0009534	chloroplast thylakoid
GO:0009579	thylakoid
GO:0031976	plastid thylakoid
GO:0034357	photosynthetic membrane
GO:0042651	thylakoid membrane
GO:0009536	plastid
GO:0016020	membrane
GO:0009507	chloroplast
GO:0019684	photosynthesis, light reaction
GO:0015979	photosynthesis
GO:0048046	apoplast
GO:0071944	cell periphery
GO:0044434	chloroplast part
GO:0044435	plastid part
GO:0005576	extracellular region
GO:0009765	photosynthesis, light harvesting
GO:0006091	generation of precursor metabolites and energy

Fig. 3 Gene expression tendency and GO enrichment analysis of differentially expressed genes between CLas free and -infected citrus plants. **A** Gene expression tendency analysis and **B** GO enrichment analysis. The size of each circle was proportional to the gene numbers of the related GO term

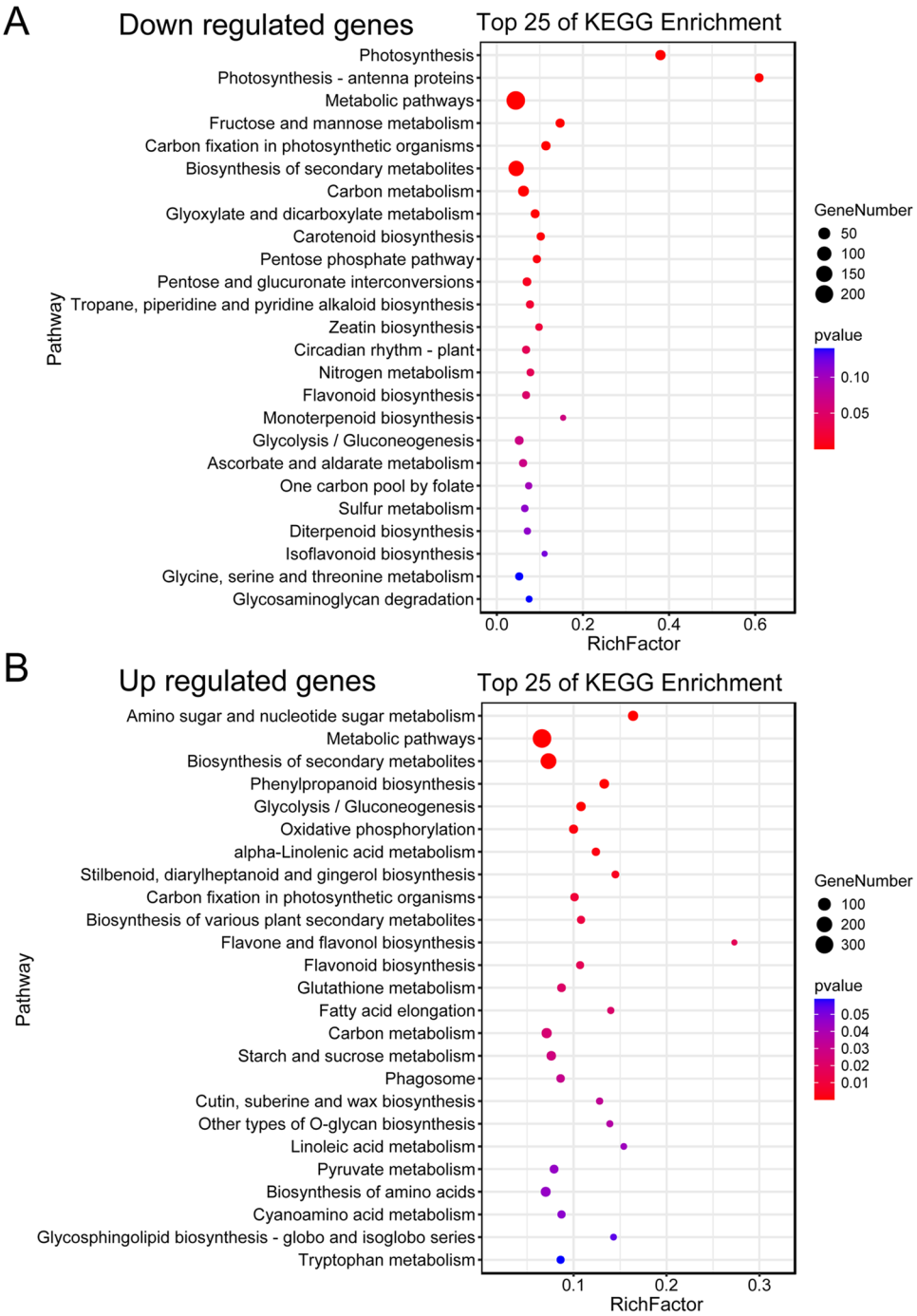


Fig. 4 KEGG enrichment analysis of differentially expressed genes between CLas free and -infected citrus plants. **A** Downregulated genes and **B** upregulated genes

Discussion

The utilization of high-throughput sequencing technology has enabled researchers to analyze the interactions between pathogens and plants, providing an effective means of analyzing HLB (Wang et al. 2016; Naidoo et al. 2018). Studies on this subject have revealed that photosynthesis, phytohormone metabolism, and signaling pathways are significantly altered due to HLB, with marked responses observed in biotic stress related differentially expressed genes (DEGs) (Balan et al. 2018; Arce-Leal et al. 2020; Weber et al. 2022; Liu et al. 2023).

CLas infection caused a significant decrease in photosynthesis process of *C. sinensis*

The starch accumulation in citrus plants infected with HLB is abnormally high, resulting in the destruction of chloroplasts and degradation of chlorophyll (Etxeberria et al. 2009). Accordingly, CLas infection in citrus plants caused a blockage

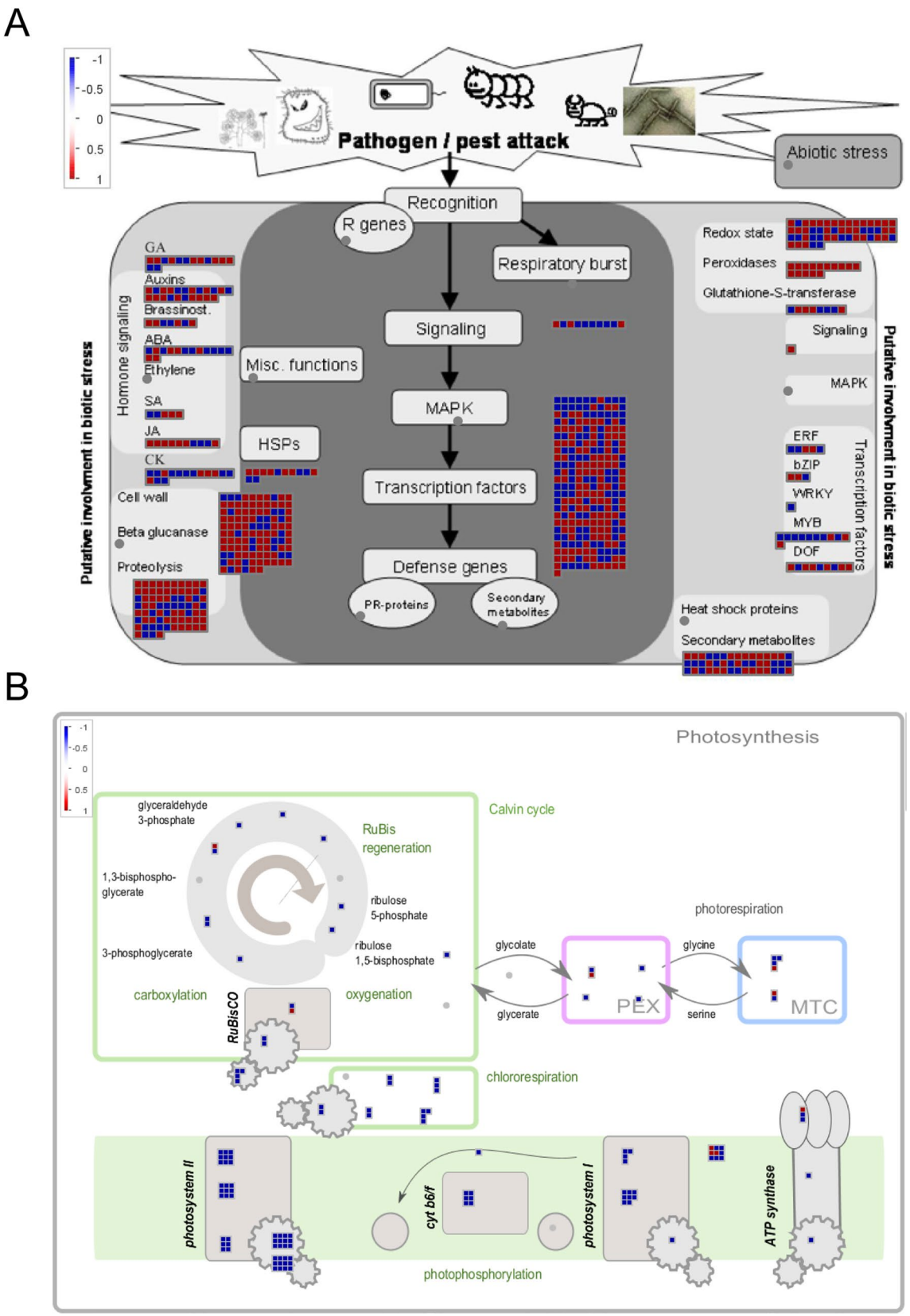


Fig. 5 MapMan analysis of differentially expressed genes between HLB-uninfected and -infected citrus plants. **A** Biotic stress-related genes and **B** photosynthesis-related genes. PEX referred to peroxisome and MTC referred to mitochondria

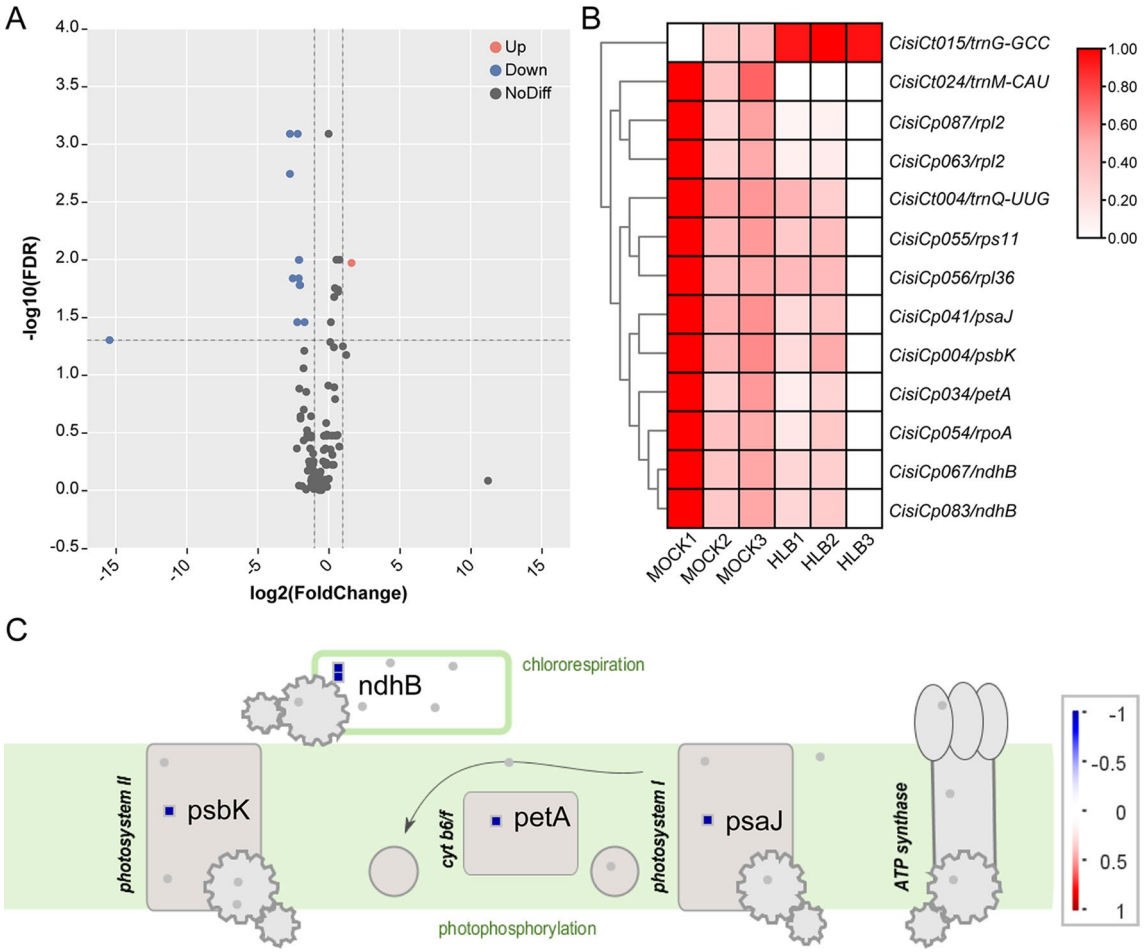
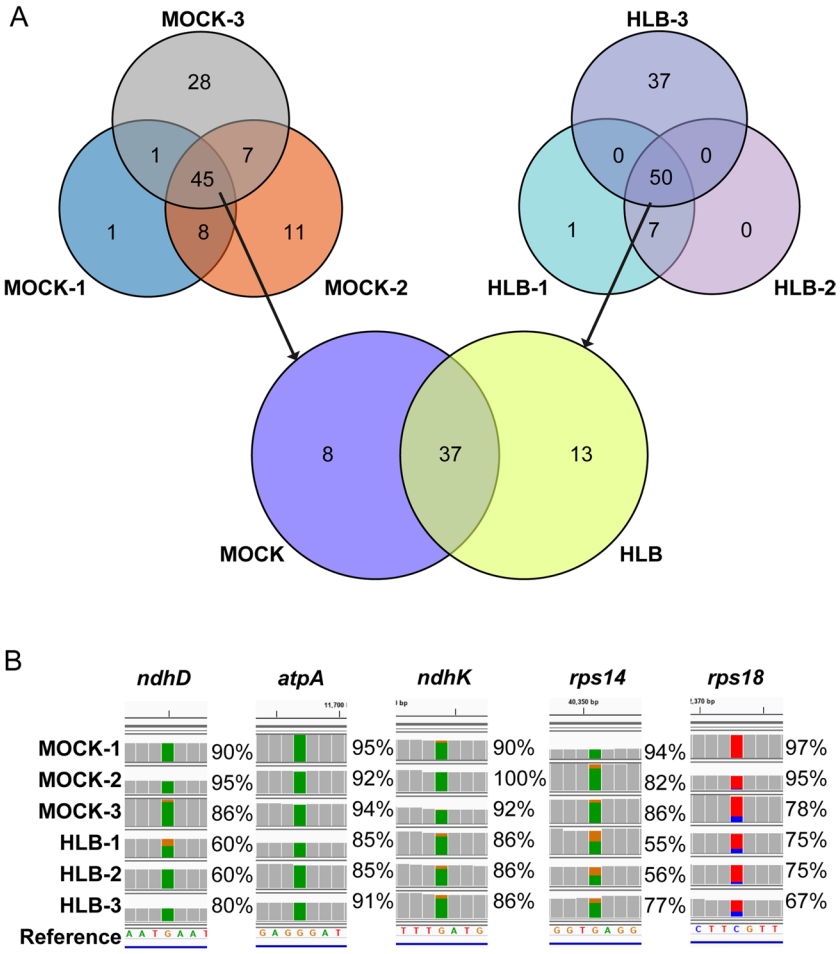


Fig. 6 Analysis of differentially expressed genes in the chloroplast genome between CLas free and -infected citrus plants. **A** Volcano map analysis, **B** heatmap analysis, and **C** MapMan analysis

in photosynthesis processes. The expression profiles of photosynthesis related genes were dramatically influenced. The photosynthesis processes enriched by DEGs in MapMan were downregulated in HLB-diseased fruits (Zhao et al. 2019). Remarkably, the DEGs associated with the light reactions of photosynthesis displayed a decrease in susceptible *C. sinensis*, while not showing any alteration in tolerant *C. hystrix* under CLas infection (Hu et al. 2017). Most DEGs related to photosynthesis were downregulated in CLas infected periwinkle (Liu et al. 2019). Through our study, we identified 125 DEGs that are related to photosynthesis (Table S6). Out of these, only nine were upregulated, while the rest associated with photosystem I and II were downregulated, suggesting that photosynthesis in the leaves of *C. sinensis* "Newhall" has been significantly suppressed.

CLas infection caused a significant dynamic change in hormone signaling of *C. sinensis*

Plant hormones are essential for plants, as they are involved in the responses to a variety of pathogenic infections, insect infestations, and abiotic stresses (Bari and Jones 2009). Our study revealed a great number of DEGs related to hormone signaling were observed during CLas infection (Table S7). Auxin functions as a balancing agent between JA and SA, connecting the defense response and development of plants when they interact with pathogens (Kazan and Manners 2009). The *Aux/IAA* genes act as transcriptional repressors, controlling the auxin signaling transduction process (Reed 2001). The PIN proteins facilitate the movement of auxin (Adamowski and Friml 2015). We identified 22



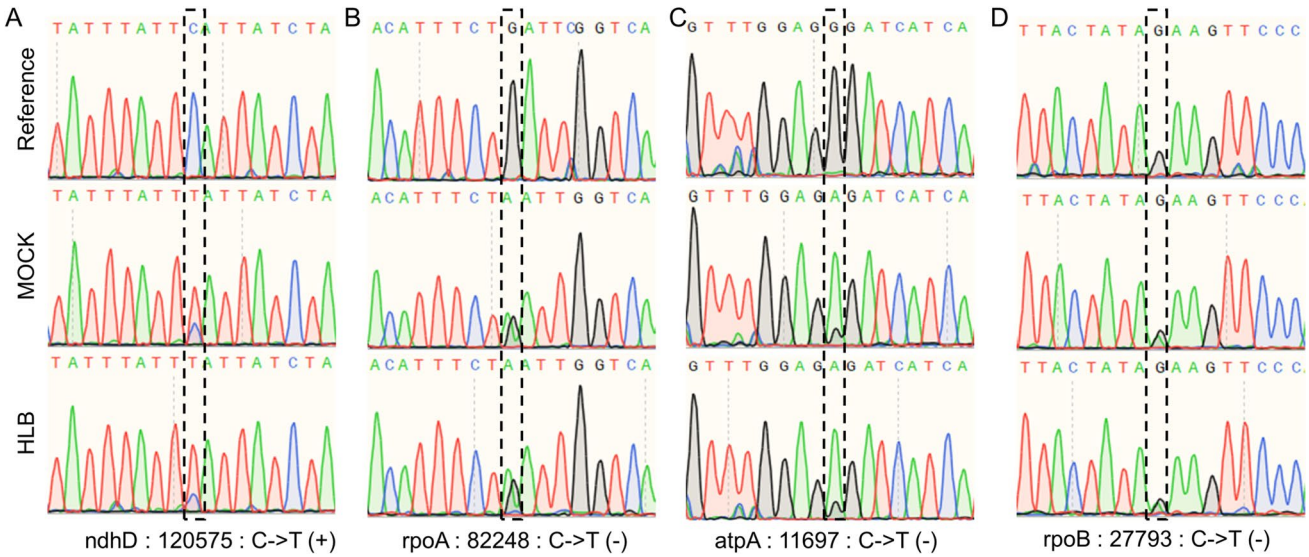


Fig. 7 RNA editing events observed in genes coded in the chloroplast genome. **A** Venn diagram analysis and **B** example genes with RNA editing events. "MOCK" referred to CLas free *Citrus sinensis* samples and "HLB" referred to CLas infected samples. The percentage number that followed each bar graph was representative of the frequency of RNA editing events

Fig. 8 PCR verification of RNA editing events in the chloroplast genome. **A** ndhD, **B** rpoA, **C** atpA, and **D** ropB. Labels indicating the genome location of RNA editing events were placed beneath the sequencing peak diagrams. "MOCK" referred to PCR products amplified from the cDNA of CLas free *Citrus sinensis* samples while "HLB" referred to CLas infected samples. "Reference" referred to PCR products amplified from the genomic DNA of CLas free *C. sinensis* samples

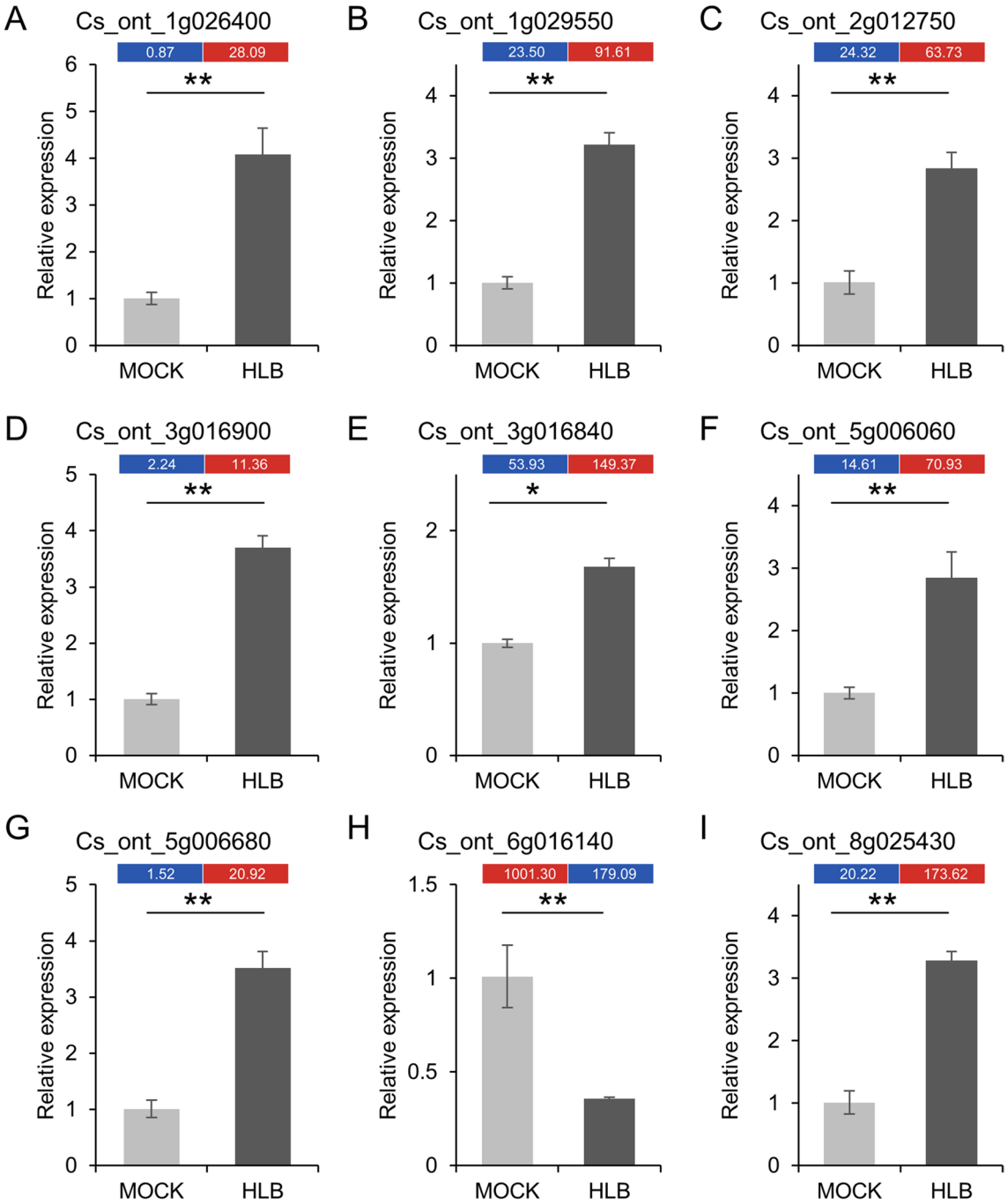


Fig. 9 RT-qPCR verification analysis of differentially expressed genes between CLas free and -infected citrus plants. **A** *Cs_ont_1g026400*, **B** *Cs_ont_1g029550*, **C** *Cs_ont_2g012750*, **D** *Cs_ont_3g016900*, **E** *Cs_ont_3g016840*, **F** *Cs_ont_5g006060*, **G** *Cs_ont_5g006680*, **H** *Cs_ont_6g016140*, and **I** *Cs_ont_8g025430*. The heatmaps above the histograms displayed the average FPKM value of both "MOCK" and "HLB" samples. "MOCK" referred to PCR products amplified from the cDNA of CLas free *Citrus sinensis* samples while "HLB" referred to CLas infected samples. Statistical analysis revealed significant differences (p value < 0.05) between the groups, as indicated by asterisks

auxin-related DEGs (Table S7), 8 of which were downregulated, including *YUCCA*, *Aux/IAA*, *MES17*, and *PIN*. The other 14 upregulated DEGs also contained these genes, demonstrating a divergence in gene function during CLas infection in auxin signaling metabolism. Five SA-related DEGs were identified, including one *ICS*, two *NPR3/4*, and two *DLO*, as well as ten JA-related DEGs with seven upregulated, such as *LOX*, *AOC*, *JAT*, and *ACS* (Table S7). There

were 14 DEGs related to GA, 6 of which were downregulated, and 14 DEGs related to ABA, 8 of which were downregulated. Additionally, 15 DEGs related to CK were identified, 10 of which were downregulated, including *IPT*, *LOG*, *AHP*, and *IPT*. Plant hormones play an important role in balancing the development and defense responses of plants when they encounter pathogens (Kazan and Manners 2009). Generally, SA and JA related DEGs were both affected due to the close relationship between these two plant hormones and plant immunity (Hou and Tsuda 2022); however, in our study, we found that the majority of DEGs were related to auxin, followed by CK, GA, and ABA. It is possible that HLB is caused by an immune response that has been provoked by a pathogen (Ma et al. 2022).

CLas infection caused activation in ROS-related genes of *C. sinensis*

ROS are highly toxic and play a key role in various processes related to plant growth, development, and defense (Shetty et al. 2008). ROS are also responsible for systemic reactions during plant-pathogen interactions (Bolwell and Daudi 2009). In *C. sinensis*, ROS were elicited in response to CLas infection (Ma et al. 2022). A previous study documented that the expression of *RbohB*, *RbohC*, *RbohD*, and *RbohF* was increased, while *SOD*, *APX*, *CAT*, and *GR* were decreased in both Valencia and SB mandarin (Ribeiro et al. 2023). Results of our study demonstrated a significant increase in the activity of CAT, POD, and SOD and a decrease in the activity of APX and GR in response to CLas infection (Fig. 1). According to a recent study, CLas infection stimulated the activity of CAT, POD, and SOD in *C. maxima* and *C. reticulata* (Wu et al. 2023). However, another study revealed that CAT and SOD activities decreased, with no change in POD activity in *C. junos* (Chen et al. 2022). It appears that various *Citrus* species reacted differently to CLas infection. Correspondingly, the expression of *Rboh*, *APX*, *MDAR*, *Trxh*, and multiple *GST* genes was upregulated in the presence of HLB (Fig. 5A and Table S6).

RNA-editing events were influenced during CLas infection in the plant organellar of *C. sinensis*

RNA-editing events were ubiquitous in cells and participated in a lot of biological processes. Studies conducted by Yang et al. (2020) found that *NbMORF8* reduces plant immunity to *Phytophthora* via RNA editing, whereas *AtSLO2* improves plant resistance to *Botrytis cinerea* through the same mechanism (Zhu et al. 2014). In our research, we identified a substantial amount of RNA editing events in the chloroplast and mitochondrion and observed that the editing frequencies of some of these events were impacted by CLas infection. Pentatricopeptide repeat (PPR) proteins were responsible for plant organellar RNA editing (Small et al. 2020). However, few PPR proteins were identified and functional analyzed in *C. sinensis*. We hypothesized that CLas infection could disrupt the functioning of PPR proteins during RNA editing. Nevertheless, the correlation between these RNA-editing events and the pathogenesis of CLas is yet to be determined, and further experiments must be conducted to gain a better understanding of this relationship.

Conclusions

Our research has revealed that citrus plants affected by HLB experience considerable physiological changes, including chlorophyll content and the activity of APX, CAT, GR, POD, and SOD. In addition, HLB affects the expression of genes related with the metabolism of phytohormone such as Auxin, CK, GA, and JA and inhibited most of the genes participated in the photosynthesis pathway. Furthermore, the RNA editing events were disturbed as the editing frequency of specific nucleotide sites was changed by CLas infection. Our discovery provides a new perspective on revealing the pathogenic mechanism of HLB.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00709-023-01911-0.

Acknowledgements We thank academic editors from TopEdit for their help in grammar improvement.

Author contribution CL and XC participated in the design of the experiments, prepared RNA for sequencing, analyzed the data, and drafted the manuscript; FL, YY, and XZ participated in the real-time-quantitative PCR (RT-qPCR) validation and statistical analyses. GH and RL designed the experiments, analyzed the data, and drafted the manuscript.

Funding This work was supported by the National Natural Science Foundation of China (32160621 and 32260659) and Innovation Fund Designated for Graduate Students of Jiangxi Province (YC2022-S928).

Data availability The raw reads for this project have been submitted to the Sequence Read Archive with the accession numbers SRR24101860, SRR24101859, SRR24101858, SRR24101857, SRR24101856, and SRR24101855.

Declarations

Competing interests The authors declare no competing interests.

References

Adamowski M, Friml J (2015) PIN-dependent auxin transport: action, regulation, and evolution. Plant Cell 27(1):20–32

Alquézar B, Carmona L, Bennici S, Miranda MP, Bassanezi RB, Peña L (2022) Cultural management of huanglongbing: current status and ongoing research. Phytopathology 112(1):11–25

Arce-Leal ÁP, Bautista R, Rodríguez-Negrete EA, Manzanilla-Ramírez MÁ, Velázquez-Monreal JJ, Santos-Cervantes ME, Méndez-Lozano J, Beuzón CR, Bejarano ER, Castillo AG (2020) Gene expression profile of Mexican lime (Citrus aurantifolia) trees in response to Huanglongbing disease caused by Candidatus Liberibacter asiaticus. Microorganisms 8(4):528

Balan B, Ibáñez AM, Dandekar AM, Caruso T, Martinelli F (2018) Identifying host molecular features strongly linked with responses to huanglongbing disease in citrus leaves. Front Plant Sci 9:277

Bari R, Jones JDG (2009) Role of plant hormones in plant defence responses. Plant Mol Biol 69:473–488

Bausher MG, Singh ND, Lee S-B, Jansen RK, Daniell H (2006) The complete chloroplast genome sequence of Citrus sinensis (L.) Osbeck var 'Ridge Pineapple': organization and phylogenetic relationships to other angiosperms. BMC Plant Biol 6:1–11

Biswas MK, Bagchi M, Deng X, Chai L (2020) Genetic Resources of Citrus and Related Genera. In: Gentile A, La Malfa S, Deng Z (eds) The Citrus Genome. Springer International Publishing, Cham, pp 23–31

Bolwell GP, Daudi A (2009) Reactive oxygen species in plant–pathogen interactions. In: Rio LA, Puppo A (eds) Reactive Oxygen Species in Plant Signaling. Springer, Berlin Heidelberg, Berlin, Heidelberg, pp 113–133

Checker VG, Kushwaha HR, Kumari P, Yadav S (2018) Role of Phytohormones in Plant Defense: Signaling and Cross Talk. In: Singh A, Singh IK (eds) Molecular Aspects of Plant-Pathogen Interaction. Springer Singapore, Singapore, pp 159–184

Chen C, Chen H, Zhang Y, Thomas HR, Frank MH, He Y, Xia R (2020) TBtools: an integrative toolkit developed for interactive analyses of big biological data. Mol Plant 13(8):1194–1202

Chen Q, Min A, Luo S, He J, Wu R, Lin X, Wang Y, He W, Zhang Y, Lin Y, Li M, Zhang Y, Luo Y, Tang H, Wang X (2022) Metabolomic analysis revealed distinct physiological responses of leaves and roots to huanglongbing in a citrus rootstock. Int J Mol Sci 23(16):9242

Clark K, Franco JY, Schwizer S, Pang Z, Hawara E, Liebrand TWH, Pagliaccia D, Zeng L, Gurung FB, Wang P (2018) An effector from the Huanglongbing-associated pathogen targets citrus proteases. Nat Commun 9(1):1718

Collum TD, Culver JN (2016) The impact of phytohormones on virus infection and disease. Curr Opin Virol 17:25–31

Conesa A, Madrigal P, Tarazona S, Gomez-Cabrero D, Cervera A, McPherson A, Szcześniak MW, Gaffney DJ, Elo LL, Zhang X, Mortazavi A (2016) A survey of best practices for RNA-seq data analysis. Genome Biol 17:13

Etxeberria E, Gonzalez P, Achor D, Albrigo G (2009) Anatomical distribution of abnormally high levels of starch in HLB-affected Valencia orange trees. Physiol Mol Plant Pathol 74(1):76–83

Ferrarezi RS, Vincent CI, Urbaneja A, Machado MA (2020) Editorial: Unravelling Citrus Huanglongbing Disease. Frontiers in Plant Science 11, Editorial

Han Y, Guo S, Muegge K, Zhang W, Zhou B (2015) Advanced applications of RNA sequencing and challenges. Bioinform Biol Insights 9:29

Hao W, Liu G, Wang W, Shen W, Zhao Y, Sun J, Yang Q, Zhang Y, Fan W, Pei S, Chen Z, Xu D, Qin T (2021) RNA editing and its roles in plant organelles. Front Genet 12:757109

Hou S, Tsuda K (2022) Salicylic acid and jasmonic acid crosstalk in plant immunity. Essays Biochem 66(5):647–656

Hu Y, Zhong X, Liu X, Lou B, Zhou C, Wang X (2017) Comparative transcriptome analysis unveils the tolerance mechanisms of Citrus hystrix in response to 'Candidatus Liberibacter asiaticus' infection. PLoS ONE 12(12):e0189229

Hu B, Rao MJ, Deng X, Pandey SS, Hendrich C, Ding F, Wang N, Xu Q (2021) Molecular signatures between citrus and Candidatus Liberibacter asiaticus. PLoS Pathog 17(12):e1010071

Jensen PE, Leister D (2014) Chloroplast evolution, structure and functions. F1000prime reports 6:40

Jiang J, Dehesh K (2021) Plastidial retrograde modulation of light and hormonal signaling: an odyssey. New Phytol 230(3):931–937

Kazan K, Manners JM (2009) Linking development to defense: auxin in plant–pathogen interactions. Trends Plant Sci 14(7):373–382

Kim D, Paggi JM, Park C, Bennett C, Salzberg SL (2019) Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. Nat Biotechnol 37(8):907–915

Kruse O, Rupprecht J, Mussgnug JH, Dismukes GC, Hankamer B (2005) Photosynthesis: a blueprint for solar energy capture and biohydrogen production technologies. Photochem Photobiol Sci 4(12):957–970

Ku Y-S, Sintaha M, Cheung M-Y, Lam H-M (2018) Plant hormone signaling crosstalks between biotic and abiotic stress responses. Int J Mol Sci 19(10):3206

Kumar L, Futschik ME (2007) Mfuzz: a software package for soft clustering of microarray data. Bioinformation 2(1):5

Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25(14):1754–1760

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, Genome Project Data Processing S (2009) The sequence alignment/map format and SAMtools. Bioinformatics 25(16):2078–2079

Li T, Liu C, Chang X, Li F, Wang T, Huang G, Li R (2023) Genome-wide identification of papain-like cysteine proteases in Citrus sinensis and expression analysis in response to Candidatus Liberibacter asiaticus. J Plant Interact 18(1):2238785

Liu X, Zheng Y, Wang-Pruski G, Gan Y, Zhang B, Hu Q, Du Y, Zhao J, Liu L (2019) Transcriptome profiling of periwinkle infected with Huanglongbing ('Candidatus Liberibacter asiaticus'). Eur J Plant Pathol 153:891–906

Liu H, Wang X, Liu S, Huang Y, Guo Y-X, Xie W-Z, Liu H, ulQamar MT, Xu Q, Chen L-L (2022) Citrus Pan-genome to breeding database (CPBD): a comprehensive genome database for citrus breeding. Mol Plant 15(10):1503–1505

Liu Y, Dong L, Ran D, Wang S, Qu R, Zheng L, Peng A, He Y, Chen S, Zou X (2023) A Comparative analysis of three rutaceae species reveals the multilayered mechanisms of citrus in response to huanglongbing disease. J Plant Growth Regul 42(12):7564–7579

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. Methods (San Diego, Calif) 25(4):402–408

Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15(12):1–21

Lu Y, Yao J (2018) Chloroplasts at the crossroad of photosynthesis, pathogen infection and plant defense. Int J Mol Sci 19(12):3900

Ma W, Pang Z, Huang X, Xu J, Pandey SS, Li J, Achor DS, Vasconcelos FNC, Hendrich C, Huang Y (2022) Citrus Huanglongbing is a pathogen-triggered immune disease that can be mitigated with antioxidants and gibberellin. Nat Commun 13(1):529

McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M (2010) The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 20(9):1297–1303

Munir S, He P, Wu Y, He P, Khan S, Huang M, Cui W, He P, He Y (2018) Huanglongbing control: perhaps the end of the beginning. Microb Ecol 76:192–204

Naidoo S, Visser EA, Zwart L, Du Toit Y, Bhadauria V, Shuey LS (2018) Dual RNA-seq to elucidate the plant–pathogen duel. Curr Issues Mol Biol 27(1):127–142

Pan R, Chen Y, Deng X, Xu M (2021) Digital gene expression analysis of Huanglongbing affected mandarins (Citrus reticulata Blanco) in response to thermotherapy. Horticultural Plant J 7(1):1–12

Pang Z, Zhang L, Coaker G, Ma W, He S-Y, Wang N (2020) Citrus CsACD2 is a target of Candidatus Liberibacter asiaticus in Huanglongbing disease. Plant Physiol 184(2):792–805

Peng A, Zou X, He Y, Chen S, Liu X, Zhang J, Zhang Q, Xie Z, Long J, Zhao X (2021) Overexpressing a NPR1-like gene from Citrus paradisi enhanced Huanglongbing resistance in C. sinensis. Plant Cell Rep 40(3):529–541

Pertea M, Pertea GM, Antonescu CM, Chang T-C, Mendell JT, Salzberg SL (2015) StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. Nat Biotechnol 33(3):290–295

Pinto Y, Levanon EY (2019) Computational approaches for detection and quantification of A-to-I RNA-editing. Methods 156:25–31

Reed JW (2001) Roles and activities of Aux/IAA proteins in Arabidopsis. Trends Plant Sci 6(9):420–425

Ribeiro C, Xu J, Hendrich C, Pandey SS, Yu Q, Gmitter FG Jr, Wang N (2023) Seasonal transcriptome profiling of susceptible and tolerant citrus cultivars to citrus Huanglongbing. Phytopathology® 113(2):286–298

Shahzad F, Chun C, Schumann A, Vashisth T (2020) Nutrient uptake in huanglongbing-affected sweet orange: transcriptomic and physiological analysis. J Am Soc Hortic Sci 145(6):349–362

Shetty NP, Jørgensen HJL, Jensen JD, Collinge DB, Shetty HS (2008) Roles of reactive oxygen species in interactions between plants and pathogens. Eur J Plant Pathol 121(3):267–280

Shi J, Gong Y, Shi H, Ma X, Zhu Y, Yang F, Wang D, Fu Y, Lin Y, Yang N, Yang Z, Zeng C, Li W, Zhou C, Wang X, Qiao Y (2023) "Candidatus Liberibacter asiaticus" secretory protein SDE3 inhibits its host autophagy to promote Huanglongbing disease in citrus. Autophagy 19(9):2558–2574

Shikanai T (2006) RNA editing in plant organelles: machinery, physiological function and evolution. Cell Mol Life Sci CMLS 63:698–708

Small ID, Schallenberg-Rüdinger M, Takenaka M, Mireau H, Ostersetzer-Biran O (2020) Plant organellar RNA editing: what 30 years of research has revealed. Plant J 101(5):1040–1056

Suh JH, Tang X, Zhang Y, Gmitter FG Jr, Wang Y (2021) Metabolomic analysis provides new insight into tolerance of huanglongbing in citrus. Front Plant Sci 12:710598

Talon M, Caruso M, Gmitter jr FG (2020) Chapter 2 - The origin of citrus. In: Talon M, Caruso M, Gmitter FG (eds) The Genus Citrus. Woodhead Publishing, pp 9–31

Tang J, Ding Y, Nan J, Yang X, Sun L, Zhao X, Jiang L (2018) Transcriptome sequencing and ITRAQ reveal the detoxification mechanism of Bacillus GJ1, a potential biocontrol agent for Huanglongbing. PLoS ONE 13(8):e0200427

Thimm O, Bläsing O, Gibon Y, Nagel A, Meyer S, Krüger P, Selbig J, Müller LA, Rhee SY, Stitt M (2004) MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. Plant J 37(6):914–939

Wang Y, Zhou L, Yu X, Stover E, Luo F, Duan Y (2016) Transcriptome profiling of Huanglongbing (HLB) tolerant and susceptible citrus plants reveals the role of basal resistance in HLB tolerance. Front Plant Sci 7:933

Wang Y, Yang Z, Zhang M, Ai P (2022) A chloroplast-localized pentatricopeptide repeat protein involved in RNA editing and splicing and its effects on chloroplast development in rice. BMC Plant Biol 22(1):1–18

Weber KC, Mahmoud LM, Stanton D, Welker S, Qiu W, Grosser JW, Levy A, Dutt M (2022) Insights into the mechanism of Huanglongbing tolerance in the Australian finger lime (Citrus australasica). Front Plant Sci 13:1019295

Wu S, Liu W, Aljohi HA, Alromaih SA, Alanazi IO, Lin Q, Yu J, Hu S (2018) REDO: RNA editing detection in plant organelles based on variant calling results. J Comput Biol 25(5):509–516

Wu F, Liu L, Fox EGP, Deng X, Xu M, Zheng Z, Li X, Fu J, Zhu H, Huang J, Deng T (2023) Physiological variables influenced by "candidatus liberibacter asiaticus" infection in two citrus species. Plant Dis 107(6):1769–1776

Yang Y, Fan G, Zhao Y, Wen Q, Wu P, Meng Y, Shan W (2020) Cytidine-to-Uridine RNA Editing Factor NbMORF8 Negatively Regulates Plant Immunity to Phytophthora Pathogens. Plant Physiol 184(4):2182–2198

Yu F, Bi C, Wang X, Qian X, Ye N (2018) The complete mitochondrial genome of Citrus sinensis. Mitochondrial DNA Part B 3(2):592–593

Zhang A, Xiong Y, Fang J, Liu K, Peng H, Zhang X (2022) Genome-wide identification and expression analysis of peach multiple organellar RNA editing factors reveals the roles of RNA editing in plant immunity. BMC Plant Biol 22(1):583

Zhao W, Baldwin EA, Bai J, Plotto A, Irey M (2019) Comparative analysis of the transcriptomes of the calyx abscission zone of sweet orange insights into the huanglongbing-associated fruit abscission. Hortic Res 6(1):71

Zhong Y, Cheng C, Jiang B, Jiang N, Zhang Y, Hu M, Zhong G (2016) Digital gene expression analysis of Ponkan mandarin (Citrus reticulata Blanco) in response to Asia citrus psyllid-vectored Huanglongbing infection. Int J Mol Sci 17(7):1063

Zhu Q, Dugardeyn J, Zhang C, Mühlenbock P, Eastmond PJ, Valcke R, De Coninck B, Öden S, Karampelias M, Cammue BPA (2014) The Arabidopsis thaliana RNA editing factor SLO2, which affects the mitochondrial electron transport chain, participates in multiple stress and hormone responses. Mol Plant 7(2):290–310

Zou X, Zhao K, Liu Y, Du M, Zheng L, Wang S, Xu L, Peng A, He Y, Long Q (2021) Overexpression of salicylic acid carboxyl methyltransferase (Cssamt1) enhances tolerance to huanglongbing disease in wanjincheng orange (citrus sinensis (l.) osbeck). Int J Mol Sci 22(6):2803