Determination of Anthracnose (Colletotrichum fructicola) Resistance Mechanism Using Transcriptome Analysis of Resistant and Susceptible Pear (Pyrus pyrifolia)

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Research Article

Keywords: pear, Colletotrichum fructicola, disease resistance, RNA sequencing, MAPK signaling, calcium signaling

Posted Date: February 26th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-3974403/v1

Additional Declarations: No competing interests reported.

Abstract

Background

Anthracnose, mainly caused by *Colletotrichum fructicola*, leads to severe losses in pear production. However, there is limited information available regarding the molecular response to anthracnose in pears.

Results

In this study, the anthracnose-resistant variety ‘Seli’ and susceptible pear cultivar ‘Cuiguan’ were subjected to transcriptome analysis following *C. fructicola* inoculation at 6 and 24 h using RNA sequencing. A total of 3186 differentially expressed genes were detected in ‘Seli’ and ‘Cuiguan’ using Illumina sequencing technology. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway analyses indicated that the transcriptional response of pears to *C. fructicola* infection included responses to reactive oxygen species, phytohormone signaling, phenylpropanoid biosynthesis, and secondary metabolite biosynthetic processes. Moreover, the mitogen-activated protein kinase (MAPK) signaling pathway and flavonoid biosynthesis were involved in the defense of ‘Seli’. Furthermore, the gene coexpression network data showed that genes related to plant–pathogen interactions were associated with *C. fructicola* resistance in ‘Seli’ at an early stage.

Conclusion

Our results showed that the activation of specific genes in MAPK and calcium signaling pathways was highly related to *C. fructicola* resistance in ‘Seli’ and providing several potential candidate genes for breeding anthracnose-resistant pear varieties.

Introduction

Pear (*Pyrus pyrifolia*) belongs to the *Pyrus* genus in the *Rosaceae* family and is known for its nutritional and health benefits. In the development of the pear industry, the prevalence of pathogens and insects is becoming a major concern. Pear anthracnose, caused by *Colletotrichum* spp., is one of the most devastating fungal diseases in the pear industry. It causes severe necrosis symptoms on leaves and fruits, accelerating the dropping of pear fruits and leaves, resulting in considerable yield losses [1–3]. *Colletotrichum* spp. consists of different strains, with *Colletotrichum fructicola* being the predominant pathogenic species that causes pear anthracnose in China [3]. The control of anthracnose mainly relies on the use of medicaments, which results in the formation of pesticide residues [2]. One of the environmentally and economically friendly approaches is to improve the resistance of host plants.

Host plants have evolved complex resistance mechanisms during their interactions with pathogens [4]. The first line of plant defense is to activate pathogen/microbe-associated molecular patterns

(PAMPs/MAMPs) triggered immunity (PTI) through cell surface transmembrane recognition receptors (PRRs), including the mitogen-activated protein kinase (MAPK) pathway [5–8]. After pathogen recognition, a range of resistance responses are elicited, such as calcium influx pathways, MAPK pathway signaling, phytohormone signaling pathways, generation of reactive oxygen species (ROS), production of antimicrobial compounds [9, 10]. However, for successful invasion, pathogens secrete numerous virulence proteins (effectors) to suppress PTI. In turn, plants have evolved resistance genes that can specifically recognize effectors; this recognition activates the second line of plant immune response known as effector-triggered immunity (ETI) to further restrict pathogen growth [6, 11]. During this process, plants usually produce ROS and elicit a hypersensitive response (HR), which eventually results in host programmed cell death (PCD) [7, 12]. Plant hormones, including salicylic acid (SA), jasmonic acid (JA), ethylene (ET), cytokinins (CKs), abscisic acid (ABA), brassinosteroids (BR), and gibberellin (GA), play synergistic or antagonistic roles in the plant defense system [13, 14].

Understanding the mechanism by which *Colletotrichum* species interact with plants is crucial for anthracnose control. Comparative transcriptome sequencing has been widely used to determine the mechanisms underlying plant–pathogen interactions, and many studies have investigated the mechanism of anthracnose resistance. For example, the MAPK-mediated activation of resistance genes involved in HR and H_2O_2 accumulation is a critical mechanism in tea plants in response to *C. fructicola* infection, which was identified using comparative transcriptome sequencing of the susceptible tea cultivar ‘Longjing 43’ and resistant cultivar ‘Zhongcha 108’ [15]. In soybean, the resistance of ‘Zhechun No. 2’ to *C. truncatum* was related to JA, auxin, MAPK, and calcium signaling; resistance gene expression; and terpenoid metabolism [16].

Despite these advances, the complex molecular mechanisms underlying the interactions between pear and *C. fructicola* remain poorly understood. Research has revealed that pear anthracnose caused by the PAFQ31 strain is mainly related to JA, ET, and ABA signaling pathways, especially the increased endogenous JA levels, as estimated by analyzing the transcriptomic data of pear leaves after infection with PAFQ31 and mutant PAFQ32 strains [17]. Another recent study revealed that resistance to early pear defoliate disease was associated with expression levels of WRKY and ERF transcription factors [18].

To better understand the mechanism of pear anthracnose resistance, we performed transcriptome analysis of the anthracnose-resistant variety ‘Seli’ and highly susceptible variety ‘Cuiguan’ after infection with *C. fructicola* and sterile water for 6 and 24 h using RNA sequencing (RNA-seq). We identified several potential candidate resistant genes and preliminarily revealed that the activation of the PTI signaling pathway plays a crucial role in pear *C. fructicola* resistance.

Results

P. pyrifolia ‘Seli’ exhibits higher resistance to C. fructicola than ‘Cuiguan’

Leaves of ‘Seli’ and ‘Cuiguan’ were inoculated with *C. fructicola* to evaluate their disease resistance. Disease symptoms appeared in both varieties after 3 days of inoculation. However, the lesion area of ‘Seli’ was significantly smaller than that of ‘Cuiguan’ at 3, 5, and 7 days after inoculation (Fig. 1a and b). The paraffin section of inoculated leaves revealed that the palisade and spongy tissues of ‘Cuiguan’ leaves were severely damaged, whereas those of ‘Seli’ leaves remained largely unaffected (Fig. 1c).

Enzyme activity analysis

To elucidate the early physiological response, the ‘Seli’ and ‘Cuiguan’ samples were collected after inoculation with *C. fructicola* at 0, 6, and 24 h and analyzed. The enzyme activity illustrated that the polyphenol oxidase (PPO) activity, and H_2O_2 content in ‘Seli’ leaves were notably higher than those in ‘Cuiguan’ leaves at 0, 6, and 24 h after inoculation (Fig. 2a and b). Furthermore, peroxidase (POD) activity was remarkably lower in ‘Seli’ leaves than in ‘Cuiguan’ leaves at 0, 6, and 24 h after inoculation (Fig. 2c).

Differentially expressed genes in ‘Seli’ and ‘Cuiguan’ in response to C. fructicola infection

To determine the molecular basis for ‘Seli’ resistance to anthracnose, samples were collected from leaves inoculated with *C. fructicola* (treatment) and sterile water (control) at 6 and 24 h. A total of 24 sample libraries were constructed, each with 40–55 million reads. The GC content and the number of repeat sequence reads were calculated using FastQC software (Table S1 in Additional File 1). More than 90% of the clean reads were mapped to the reference genome of *P. pyrifolia* (Gao et al., 2021), thus indicating that the quality of the transcriptome sequencing data was reliable. Principal component analysis, and Pearson’s correlation coefficient analysis results showed good reproducibility for each treatment (Fig. 3a and b). The total mapped reads of all genes were used for differential expression analysis using DESeq $|\log2(\text{fold change})| \geq 2$ and false discovery rate FDR ≤ 0.05. A total of 3186 differentially expressed genes (DEGs) were identified in ‘Seli’ and ‘Cuiguan’ at 6 and 24 h after inoculation (Fig. 3c). Comparative analysis of DEGs between the two *C. fructicola* infection time points in ‘Seli’ revealed that they shared 145 DEGs (i.e. SL-6 and SL-24) (Fig. 3d). A similar comparison for ‘Cuiguan’ revealed 131 common genes at the two time points (Fig. 3d). In addition, the DEGs were analyzed between ‘Seli’ and ‘Cuiguan’ at 6 and 24 h post-infection, and we found that only 24 DEGs overlapped across all treatment samples (Fig. 3d).

Functional annotation of DEGs and pathway enrichment analysis

To fully understand the biological process of the identified DEGs in ‘Seli’ and ‘Cuiguan’ at 6 and 24 h after *C. fructicola* infection, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed. GO enrichment analysis of the DEGs revealed that there were six common pathways in the comparisons of CG-D versus CG-CF and SL-D versus SL-CF, which included response to hypoxia, response to ROS, reduction in ROS levels, oxylipin biosynthetic process, phenylpropanoid biosynthesis, and secondary metabolite biosynthetic process. In addition, the DEGs of SL-D versus SL-CF were enriched in response to chitin, response to wound, and immune effector

processes (Fig. 4a and b). The KEGG enrichment results revealed that most DEGs of CG-D versus CG-CF and SL-D versus SL-CF were enriched in the biosynthesis of secondary metabolites and metabolic pathways. However, according to the enrichment pathway results, MAPK signaling, and flavonoid biosynthesis pathways were uniquely enriched in SL-D versus SL-CF (Fig. 4c and d). We speculated that the DEGs involved in the pathways of secondary metabolites, biosynthetic processes, and MAPK signaling pathway are closely related to 'Seli' resistance to *C. fructicola* infection.

Genes potentially involved in 'Seli' resistance to C. fructicola infection

We further analyzed the gene expression levels in four KEGG pathways: MAPK signaling pathway, phenylpropanoid biosynthesis, alpha-linolenic acid metabolism, and plant hormone signal transduction. The plant MAPK signaling pathway plays a pivotal role in plant disease resistance [15, 19]. Herein, we revealed that genes related to the PTI pathway were only induced in 'Seli,' which included leucine-rich repeat (LRR) receptor-like kinase (*FLS2*), BRI1-associated receptor kinase 1 (*BAK1*), *MPK3*, WRKY-type transcription factor 29 (*WRKY29*), oxidative signal inducible 1 (*OXI1*), MAP kinase substrate 1 (*MKS1*), and NADPH oxidase (*RBOHD*) (Fig. 5a). We inferred that the expression of these early defense genes in 'Seli' could induce ROS accumulation and cell death [11], thus restricting *C. fructicola* expansion in the early stage.

Phenylpropanoid biosynthesis is an important secondary metabolism pathway in plant disease resistance [20]. We found that the expression of genes related to flavonoid biosynthesis, including 4-coumarate:coenzyme A ligase (*4CL*), phenylalanine ammonia lyase, and p-coumarate 3-hydroxylase (*C3'H*), was remarkably upregulated in 'Seli' and 'Cuiguan' leaves after *C. fructicola* infection. However, the expression of genes related to fatty acid and flavonoid biosynthesis, including fatty acid desaturase, cinnamon alcohol dehydrogenase 5 (*CAD5*), anthocyanidin reductase (*ANR*), leucoanthocyanidin reductase (*LAR*), and two shikimate hydroxycinnamoyl transferases (*HCT*), was substantially upregulated in 'Seli' when inoculated with *C. fructicola* at 6 h (Fig. 5b).

Furthermore, transcription factors play an important role in the plant's response to abiotic and biotic stresses by regulating the plant's immune defense system [21]. In this study, we found that *WRKY6*, *WRKY75*, and *WRKY76* were remarkably upregulated in 'Seli' and 'Cuiguan' at 6 and 24 h after *C. fructicola* infection, whereas *WRKY9*, *WRKY50*, and *WRKY72* were particularly upregulated in 'Seli'. Other transcription factors such as *ERF95*, *ERF14*, *bHLH030*, and *MYB108* were also upregulated after *C. fructicola* infection (Fig. 5c).

Genes involved in different plant hormone signal transduction pathways were also characterized. We found that transcriptional repressors such as jasmonate zim-domain (*JAZ*) in JA signaling as well as SCF-type E3 ligase complex (*GID2*), and DELLA in the GA signaling pathway were upregulated after inoculation with *C. fructicola*, and their expression in 'Cuiguan' was remarkably greater than that in 'Seli'. AOS is the second enzyme in the biosynthesis of the plant defensive hormone JA [22]. We found that the expression of *AOS1* (EVM0031709) was upregulated in 'Seli' and 'Cuiguan' at 6 h, whereas the expression of *AOS1* was specifically induced in the resistant variety of 'Seli' at 24 h after inoculation (Fig. 5d).

Weighted correlation network analysis (WGCNA) for the DEGs of 'Seli' and 'Cuiguan' in response to C. fructicola infection

To determine the gene regulatory network of 'Seli' and 'Cuiguan' in response to *C. fructicola* infection, a weighted gene coexpression network was constructed based on 3186 DEGs identified in the RNA-seq data. A network heatmap plot was constructed incorporating different gene dendrograms and modules to visualize the topological overlap matrix of the DEGs post-infection (Fig. 6a). In total, the network was divided into 19 modules according to the correlations between the modules and samples (Fig. 6b). The expression of genes in the brown module was upregulated in 'Seli' and downregulated in 'Cuiguan' (Fig. 6c). We then analyzed the biological processes of the DEGs in the brown module and found that these DEGs were enriched in the plant–pathogen interaction and MAPK signaling pathway (Fig. 6d). We further revealed that these DEGs were highly related to *C. fructicola* infection. Combining the KEGG enrichment results and gene function prediction, *FLS2*, *MPK3*, *MKS1*, and *WRKY29* in the brown module appeared to be the hub genes that play important roles in plant–pathogen interaction (Fig. 6e).

Validation of RNA-seq data via quantitative reverse transcription polymerase chain reaction (qRT–PCR)

To confirm the quality of the transcriptome data, qRT–PCR was conducted for the nine candidate genes involved in phenylpropanoid metabolism, and the MAPK signaling pathway was also validated via qRT–PCR. The expression of these genes such as *BAK1*, *FLS2*, *WRKY29*, *MPK3*, and *MKS1* were upregulated in 'Seli' at 6 and 24 h after *C. fructicola* inoculation, whereas their expression in 'Cuiguan' was only upregulated at 6 h after inoculation. Meanwhile, the expression of male discoverer 1-interacting receptor-like kinase 2 (*MIK2*) was upregulated in 'Seli' and 'Cuiguan' at 24 h after inoculation. Furthermore, the expression of calmodulin-like protein 19 (*CML19*), calcium-dependent protein kinase (*CDPK*), and *CAD5* was induced and upregulated in 'Seli' leaves after *C. fructicola* inoculation. Thus, all selected genes showed similar expression patterns as those of the RNA-seq data (Fig. 2a, Fig. 6, Table S2 in Additional File 1).

Discussion

C. fructicola is a severe disease-causing pathogen in many economic fruits, such as mango, banana, apple, citrus, and pear [2, 23–26]. It has been reported that most *Pyrus* varieties are susceptible to anthracnose, and most of the resistant varieties are wild germplasm [27]. The molecular mechanisms of *C. fructicola* resistance in pear remain unclear. Shan et al. (2023) investigated early leaf defoliation in 155 pear varieties and reported 19 resistant varieties (*P. pyrifolia*). They employed comparative transcriptomics and revealed the response of the resistant variety 'Whasan.' However, studies on the early defense responses to anthracnose are limited. 'Seli,' an excellent wild pear variety with local characteristics, is grown in Shexian, Anhui Province, China. Pears have high medicinal value ('Shexian chronicle' in 1995), and the leaves were found to be highly resistant to *C. fructicola* in our study (Fig. 1a).

Herein, we identified the comprehensive resistance mechanisms of ‘Seli’ compared with the susceptible variety ‘Cuiguan’ using RNA-seq. DEG analyses further revealed that a higher number of upregulated genes than downregulated genes were identified in ‘Seli’ and ‘Cuiguan’ samples at 6 and 24 h. In particular, most DEGs were upregulated in ‘Seli’ (Fig. 3c). These results are supported by a previous report of resistant tea plants inoculated with *C. fructicola* [15, 28]. GO and KEGG enrichment analyses illustrated that MAPK signaling pathway, response to ROS, phenylpropanoid biosynthesis, flavonoid biosynthesis, and plant hormone signal transduction pathway play crucial roles in pear’s response to anthracnose resistance (Fig. 4).

ROS bursts are one of the earliest responses to pathogen invasion, and many studies have reported that HR and ROS bursts are crucial for resistance to tea anthracnose [15], citrus bacterial canker [29], and rice blast disease [30]. In our study, we observed that a higher number of DEGs were enriched in ROS generation in ‘Seli’, and the H_2O_2 content was significantly higher in ‘Seli’ than in ‘Cuiguan’ after infection with *C. fructicola* (Fig. 2b). These results demonstrated that H_2O_2 is critical for pear anthracnose resistance, and H_2O_2 production may be regulated by PTI signaling. PTI is the first line of defense that inhibits many pathogens, including bacteria, fungi, oomycetes, and viruses [7]. FLS2, a well-characterized PRR, initiates immune signaling and induces an ROS burst through instantaneous heterodimerization with the immune kinase *BAK1* [31–34]. We revealed that the expression of three *BAK1* and one *FLS2* genes was notably upregulated in ‘Seli’ after inoculation with *C. fructicola*, whereas the expression in ‘Cuiguan’ showed no difference (Fig. 5a, Fig. 6a and b), suggesting a role of conferring resistance to the variety. A previous report showed that the conserved effector *NIS1* of *C. orbiculare* could target *BAK1* to suppress both cell death and ROS generation [35]. Similarly, we inferred that the lower expression of *BAK1* was related to anthracnose susceptibility in ‘Cuiguan.’

The MAPK cascade acts as one of the earliest signaling responses to bacterial and pathogenic infections and is located downstream of FLS2 [19, 36]. In this study, we found that the expression of *MPK3* and *MKS1* was notably upregulated in ‘Seli’ after inoculation with *C. fructicola*. Previous studies have reported that *MPK3* is essential for cotton resistance to whitefly, and silencing of *GhMPK3* results in the suppression of MPK–WRKY-mediated hormone pathways and enhancement of whitefly susceptibility [37]. *MPK3* and *MPK6* are positive regulators of *Arabidopsis* defense responses that control ET and JA biosynthesis [38, 39]. Herein, we found that the ET biosynthesis gene *WRKY29* was remarkably upregulated in ‘Seli’ at 6 h after infection with *C. fructicola* (Fig. 5a), we inferred that ET biosynthesis is crucial for cell death and pear anthracnose defense in the early stages. Interestingly, *MKS1* could activate SA-dependent resistance through interaction with *WRKY* transcription factors *WRKY25* and *WRKY33*, and the overexpression of *MKS1* remarkably improved the SA content [40]. This indicated that *MPK3* and *MKS1* play a vital role in pear anthracnose resistance. Calcium, a second messenger, performs crucial roles in plant signaling conduction, including responses to various pathogen stressors [41–43]. Furthermore, we revealed that calcium signaling-related genes, including *CDPK* and *CML*, were remarkably upregulated in ‘Seli’ after *C. fructicola* infection (Fig. 7g and h, Table S2 in Additional File 1).

Phenylpropanoid metabolism, which is derived from multiple pathways, including flavonoid, terpenoid, lignin, and anthocyanin pathways, plays key roles in plant disease resistance [44]. We revealed that the expression of *PAL1*, *4CL*, and *C3'H* in flavonoid metabolites was upregulated in the early stage of *C. fructicola* infection at 6 h (Fig. 5b). This was similar to a previous report on *Stylosanthes* plants wherein infection with *C. gloeosporioides* was managed by upregulating the levels of genes and compounds in the flavonoid biosynthesis pathway [45]. Furthermore, we revealed that *CAD5* and *HCT* in lignin metabolites were only substantial upregulated in 'Seli' after *C. fructicola* inoculation at 6 h (Fig. 5b). Regarding these genes, previous studies have revealed that *CAD5* positively regulated shoot blight resistance in *Bambusa pervariabilis* × *Dendrocalamopsis grandis* by regulating the synthesis of lignin and flavonoids [46], and *HCT* was associated with the synthesis of lignin and the secondary metabolic cell wall [47]. We inferred that these genes in 'Seli' may provide mechanical strength to the cell wall to restrict *C. fructicola* expansion. Previous research has demonstrated that *bHLH137* promotes proanthocyanidin and anthocyanin biosynthesis by regulating the expression of *LAR2* and is involved in the response to *C. gloeosporioides* infection in grapes [48]. Our results revealed that *ANR* and *LAR* related to anthocyanidins synthesis were uniquely upregulated in 'Seli' after *C. fructicola* inoculation at 6 h (Fig. 5b). The function of anthocyanidins in response to *C. fructicola* infection requires further investigation.

Conclusion

Our study revealed that multiple biological processes, including response to ROS, plant hormones, phenylpropanoid, and activation of PTI and ETI, were stimulated in 'Seli' and 'Cuiguan' leaves in response to *C. fructicola* infection. Moreover, our results demonstrated that the activation of specific genes in MAPK, and calcium signaling pathways was highly related to *C. fructicola* resistance in 'Seli' (Fig. 8). These results provide new insights into the regulatory molecular mechanisms of pear anthracnose and highlight the importance of early defense response.

Materials and methods

Ethics approval and consent to participate

The experiments were carried out with the permission of the relevant agencies. Leaves of 'Seli' were obtained from the Shexian Shangfeng township, Huangshan City, Anhui Province, and Gangji Eco-agriculture Experimental Demonstration Base of Anhui Academy of Agricultural Sciences, Hefei City, Anhui Province, China. The 'Cuiguan' leaves were obtained from the germplasm garden of the Anhui Agricultural University (High Tech Agricultural Garden). *C. fructicola* was isolated from 'Dangshansuli' pear leaves. All the experiments were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable.

Availability of data and materials

The data presented in this study can be found in the article and the supplementary materials, and the generated raw reads have been uploaded to NCBI with genbank accession numbers of PRJNA1078101.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This project was supported by the Key Research Project of Natural Science in Colleges and Universities of the Anhui Provincial Department of Education (2022AHO50926), the China Agriculture Research System (CARS-28-14, CARS-28-39), National Natural Science Foundation of China (32302484), and Anhui Agricultural University Talent Project (rc322105 and K2132002).

Authors' contributions

XT: Conceptualization, Formal analysis, Methodology, Visualization, Validation, Writing– review & editing. FL: Conduced the experiment, Data curation, Formal analysis, Methodology, Writing– review & editing. ZX, YW, GH, KC and RY: Formal analysis, Methodology, Writing– review & editing. WS, LX, GG and WW: Formal analysis, Methodology. LL, LL, ZY, WH and XG: Formal analysis, Writing– review & editing. DW: Conceptualization, Project administration, Validation, Writing– review & editing. BJ: Conceptualization, Investigation, Methodology, Project administration, Validation, Writing– review & editing. All authors read and approved the manuscript.

Acknowledgments

We would like to thank the stationmaster of Wenming Wang from Sation of Science and Technology of Shexian, and professor Zhenghui Gao from Anhui Academy of Agricultural Science for providing us with materials of ‘Seli’.

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References

1. Wu LQ, Zhu LW, Heng W, Ye ZF, Liu G, Shi SX. Identification of Dangshan pear anthracnose pathogen and screening fungicides against it. Sci Ag Sinica. 2010;43(18):3750-3758.
2. Zhang PF, Zhai LF, Zhang XK, Huang XZ, Hong N, Xu WX, Wang GP. Characterization of *Colletotrichum fructicola*, a new causal agent of leaf black spot disease of sandy pear (*Pyrus pyrifolia*). Eur J Plant Pathol. 2015;143(4):651-662.
3. Fu M, Crous PW, Bai Q, Zhang PF, Xiang J, Guo YS, Zhao FF, Yang MM, Hong N, Xu WX, Wang GP. *Colletotrichum* species associated with anthracnose of *Pyrus* spp. in China. Persoonia. 2019;42:1-35.
4. Jones JDG, Dangl JL. The plant immune system. Nature. 2006;444(7117):323-329.
5. Boller T, Felix G. A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annu Rev Plant Biol. 2009;60:379-406
6. Stotz HU, Mitrousia GK, de Wit PJ, Fitt BD. Effector-triggered defence against apoplastic fungal pathogens. Trends Plant Sci. 2014;19(8):491-500.
7. Bigeard J, Colcombet J, Hirt H. Signaling Mechanisms in Pattern-Triggered Immunity (PTI). Mol Plant. 2015;8(4):521-539.
8. Xu J, Zhang S. Mitogen-activated protein kinase cascades in signaling plant growth and development. Trends Plant Sci. 2015;20(1):56-64.
9. Nürnberger T, Brunner F, Kemmerling B, Piater L. Innate immunity in plants and animals: striking similarities and obvious differences. Immunol Rev. 2004;198(1):249-266.
10. Meng X, Zhang S. MAPK cascades in plant disease resistance signaling. Annu Rev Phytopathol. 2013;51:245-266.
11. Zhang J, Zhou JM. Plant immunity triggered by microbial molecular signatures. Mol Plant. 2010;3(5):783-93.
12. Greenberg JT, Yao N. The role and regulation of programmed cell death in plant-pathogen interactions. Cell Microbiol. 2004;6(3):201-211.
13. Denance N, Sanchez-Vallet A, Goffner D, Molina A. Disease resistance or growth: the role of plant hormones in balancing immune responses and fitness costs. Front Plant Sci. 2013;4:155.
14. Yang DL, Yang Y, He Z. Roles of plant hormones and their interplay in rice immunity. Mol Plant. 2013;6(3):675-685.
15. Wang YC, Hao XY, Lu QH, Wang L, Qian WJ, Li N, Ding CQ, Wang XC, Yang YJ. Transcriptional analysis and histochemistry reveal that hypersensitive cell death and H_2O_2 have crucial roles in the resistance of tea plant (*Camellia sinensis* (L.) O. *Kuntze*) to anthracnose. Hortic Res. 2018;5:18.
16. Zhu LM, Yang QH, Yu XM, Fu XJ, Jin HX, Yuan FJ. Transcriptomic and metabolomic analyses reveal a potential mechanism to improve soybean resistance to anthracnose. Front Plant Sci. 2022;13:850829.

17. Fu M, Bai Q, Zhang H, Guo YS, Peng YH, Zhang PF, Shen L, Hong N, Xu WX, Wang GP. Transcriptome analysis of the molecular patterns of pear plants infected by two *Colletotrichum fructicola* pathogenic strains causing contrasting sets of leaf symptoms. Front Plant Sci. 2022;13:761133.
18. Shan YF, Li MY, Wang RZ, Li XG, Lin J, Li JM, Zhao KJ, Wu J. Evaluation of the early defoliation trait and identification of resistance genes through a comprehensive transcriptome analysis in pears. J Integr Agr. 2023;22(1):120-138.
19. Pitzschke A, Schikora A, Hirt H. MAPK cascade signalling networks in plant defence. Curr Opin Plant Biol. 2009;12(4):421-426.
20. Sudheeran PK, Sela N, Carmeli-Weissberg M, Ovadia R, Panda S, Feygenberg O, Maurer D, Oren-Shamir M, Aharoni A, Alkan N. Induced defense response in red mango fruit against *Colletotrichum gloeosporioides*. Hortic Res. 2021;8(1):17.
21. Singh KB, Foley RC, Oñate-Sánchez L. Transcription factors in plant defense and stress responses. Curr Opin Plant Biol. 2002;5(5):430-436.
22. Farmer EE, Goossens A. Jasmonates: what ALLENE OXIDE SYNTHASE does for plants. J Exp Bot. 2019;70(13):3373-3378.
23. Lima NB, de A. Batista MV, De Morais MA, Barbosa MAG, Michereff SJ, Hyde KD, P.S.Câmara M. Five *Colletotrichum* species are responsible for mango anthracnose in northeastern Brazil. Fungal Divers. 2013;61(1):75-88.
24. Huang R, Sun WX, Wang L, Li QL, Huang SP, Tang LH, Guo TX, Mo JY, Hsiang T. Identification and characterization of *Colletotrichum* species associated with anthracnose disease of banana. Plant Pathol. 2021;70(8):1827-1837.
25. Huang F, Chen GQ, Hou X, Fu YS, Cai L, Hyde KD, Li HY. *Colletotrichum* species associated with cultivated citrus in China. Fungal Divers. 2013;61(1):61-74.
26. Shang SP, Wang B, Zhang S, Liu GL, Liang XF, Zhang R, Gleason ML, Sun GY. A novel effector CfEC92 of *Colletotrichum fructicola* contributes to glomerella leaf spot virulence by suppressing plant defences at the early infection phase. Mol Plant Pathol. 2020;21(7):936-950.
27. Sun J, Chen CX, Chao G, Yin H, Qi KJ, Zhang SL, Wu J. The anthracnose-resistance identification and screening of pear cultivar resourses. Int J Fruit Sci. 2016;33:184-195.
28. Chang J, Wang KL, Zhang CC, Han XJ, Zhang XD, Ren HD, Yao XH. Transcriptome analysis of resistant and susceptible pecan (*Carya illinoinensis*) reveals the mechanism of resistance to black spot disease (*Colletotrichum fioriniae*). J Agric Food Chem. 2023;71(14):5812-5822.
29. Li Q, Qin XJ, Qi JJ, Dou WF, Dunand C, Chen SC, He YR. CsPrx25, a class III peroxidase in *Citrus sinensis*, confers resistance to citrus bacterial canker through the maintenance of ROS homeostasis and cell wall lignification. Horticul Res. 2020;7(1).
30. Li Y, Cao XL, Zhu Y, Yang XM, Zhang KN, Xiao ZY, Wang H, Zhang JH, Zhang LL, Li GB, Zheng YP, Fan J, Wang J, Chen XQ, Wu XJ, Zhao JQ, Dong OX, Chen XW, Chern M, Wang WM. Osa-miR398b boosts H_2O_2 production and rice blast disease-resistance via multiple superoxide dismutases. New Phytol. 2019;222(3):1507-1522.

31. Chinchilla D, Zipfel C, Robatzek S, Kemmerling B, Nürnberger T, Jones JD, Felix G, Boller T. A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. Nature. 2007;448(7152):497-500.
32. Roux M, Schwessinger B, Albrecht C, Chinchilla D, Jones A, Holton N, Malinovsky FG, Tör M, de Vries S, Zipfel C. The *Arabidopsis* leucine-rich repeat receptor-like kinases BAK1/SERK3 and BKK1/SERK4 are required for innate immunity to hemibiotrophic and biotrophic pathogens. Plant Cell. 2011;23(6):2440-2455.
33. Li L, Li M, Yu L, Zhou Z, Liang X, Liu Z, Cai G, Gao L, Zhang X, Wang Y, Chen S, Zhou JM. The FLS2-associated kinase BIK1 directly phosphorylates the NADPH oxidase RbohD to control plant immunity. Cell Host Microbe. 2014;15(3):329-338.
34. Irieda H, Inoue Y, Mori M, Yamada K, Oshikawa Y, Saitoh H, Uemura A, Terauchi R, Kitakura S, Kosaka A, Singkaravanit-Ogawa S, Takano Y. Conserved fungal effector suppresses PAMP-triggered immunity by targeting plant immune kinases. Proc Natl Acad Sci U S A. 2019;116(2):496-505.
35. Li B, Ferreira MA, Huang M, Camargos LF, Yu X, Teixeira RM, Carpinetti PA, Mendes GC, Gouveia-Mageste BC, Liu C, Pontes CSL, Brustolini OJB, Martins LGC, Melo BP, Duarte CEM, Shan L, He P, Fontes EPB. The receptor-like kinase NIK1 targets FLS2/BAK1 immune complex and inversely modulates antiviral and antibacterial immunity. Nat Commun. 2019;10(1):4996.
36. Pedley KF, Martin GB. Role of mitogen-activated protein kinases in plant immunity. Curr Opin Plant Biol. 2005;8(5):541-547.
37. Li JY, Zhu LZ, Hull JJ, Liang SJ, Daniell H, Jin SX, Zhang XL. Transcriptome analysis reveals a comprehensive insect resistance response mechanism in cotton to infestation by the phloem feeding insect *Bemisia tabaci* (whitefly). Plant Biotechnol J. 2016;14(10):1956-1975.
38. Tena G, Boudsocq M, Sheen J. Protein kinase signaling networks in plant innate immunity. Curr Opin Plant Biol. 2011;14(5):519-529.
39. Schweighofer A, Meskiene I. Regulation of stress hormones jasmonates and ethylene by MAPK pathways in plants. Mol Biosyst. 2008;4(8):799-803.
40. Andreasson E, Jenkins T, Brodersen P, Thorgrimsen S, Petersen NH, Zhu SJ, Qiu JL, Micheelsen P, Rocher A, Petersen M, Newman MA, Nielsen HB, Hirt H, Somssich I, Mattsson O, Mundy J. The MAP kinase substrate MKS1 is a regulator of plant defense responses. EMBO J. 2005;24(14):2579-2589.
41. Saand MA, Xu YP, Li W, Wang JP, Cai XZ. Cyclic nucleotide gated channel gene family in tomato: genome-wide identification and functional analyses in disease resistance. Front Plant Sci. 2015;6:303.
42. Bundo M, Coca M. Calcium-dependent protein kinase OsCPK10 mediates both drought tolerance and blast disease resistance in rice plants. J Exp Bot. 2017;68(11):2963-2975.
43. Jiang Y, Ding P. Calcium signaling in plant immunity: a spatiotemporally controlled symphony. Trends Plant Sci. 2023;28(1):74-89.
44. Dong NQ, Lin HX. Contribution of phenylpropanoid metabolism to plant development and plant-environment interactions. J Integr Plant Biol. 2021;63 (1):180-209.

45. Jiang LY, Wu PP, Yang LY, Liu C, Guo PF, Wang H, Wang SC, Xu FP, Zhuang QW, Tong XZ, Liu PD,Luo LJ. Transcriptomics and metabolomics reveal the induction of flavonoid biosynthesis pathway in the interaction of *Stylosanthes-Colletotrichum gloeosporioides*. Genomics. 2021;113(4):2702-2716.
46. Luo FY, Yan P, Xie LL, Li SY, Zhu TH, Han S, Lin TT, Li SJ. Molecular mechanisms of phenylpropane-synthesis-related genes regulating the shoot blight resistance of *Bambusa pervariabilis* x *Dendrocalamopsis grandis*. Int J Mol Sci. 2022;23(12).
47. Zheng K, Cai YS, Qu YY, Teng L, Wang CY, Gao J, Chen QJ. Effect of the HCT gene on lignin synthesis and fiber development in *Gossypium barbadense*. Plant Sci. 2024;338.
48. Yu D, Wei W, Fan ZQ, Chen JY, You YL, Huang WD, Zhang JC. VabHLH137 promotes proanthocyanidin and anthocyanin biosynthesis and enhances resistance to *Colletotrichum gloeosporioides* in grapevine. Hortic Res. 2023;10(2):uhac261.
49. Salotti I, Liang YJ, Ji T, Rossi V. Development of a model for *Colletotrichum* diseases with calibration for phylogenetic clades on different host plants. Front Plant Sci. 2023;14:1069092.
50. Gao Y, Yang Q, Yan X, Wu X, Yang F, Li J, Wei J, Ni J, Ahmad M, Bai S, Teng YW. High-quality genome assembly of 'Cuiguan' pear (*Pyrus pyrifolia*) as a reference genome for identifying regulatory genes and epigenetic modifications responsible for bud dormancy. Hortic Res. 2021;8(1):197.
51. Kim D, Paggi JM, Park C, Bennett C, Salzberg SL. Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. Nat Biotechnol. 2019;37(8):907-915.
52. Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, Pimentel H, Salzberg SL, Rinn JL, Pachter L. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat Protoc. 2012;7(3):562-578.
53. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics. 2008;9(1):559.
54. Otasek D, Morris JH, Bouças J, Pico AR, Demchak B. Cytoscape Automation: empowering workflow-based network analysis. Genome Biol. 2019;20(1):185.

Figures

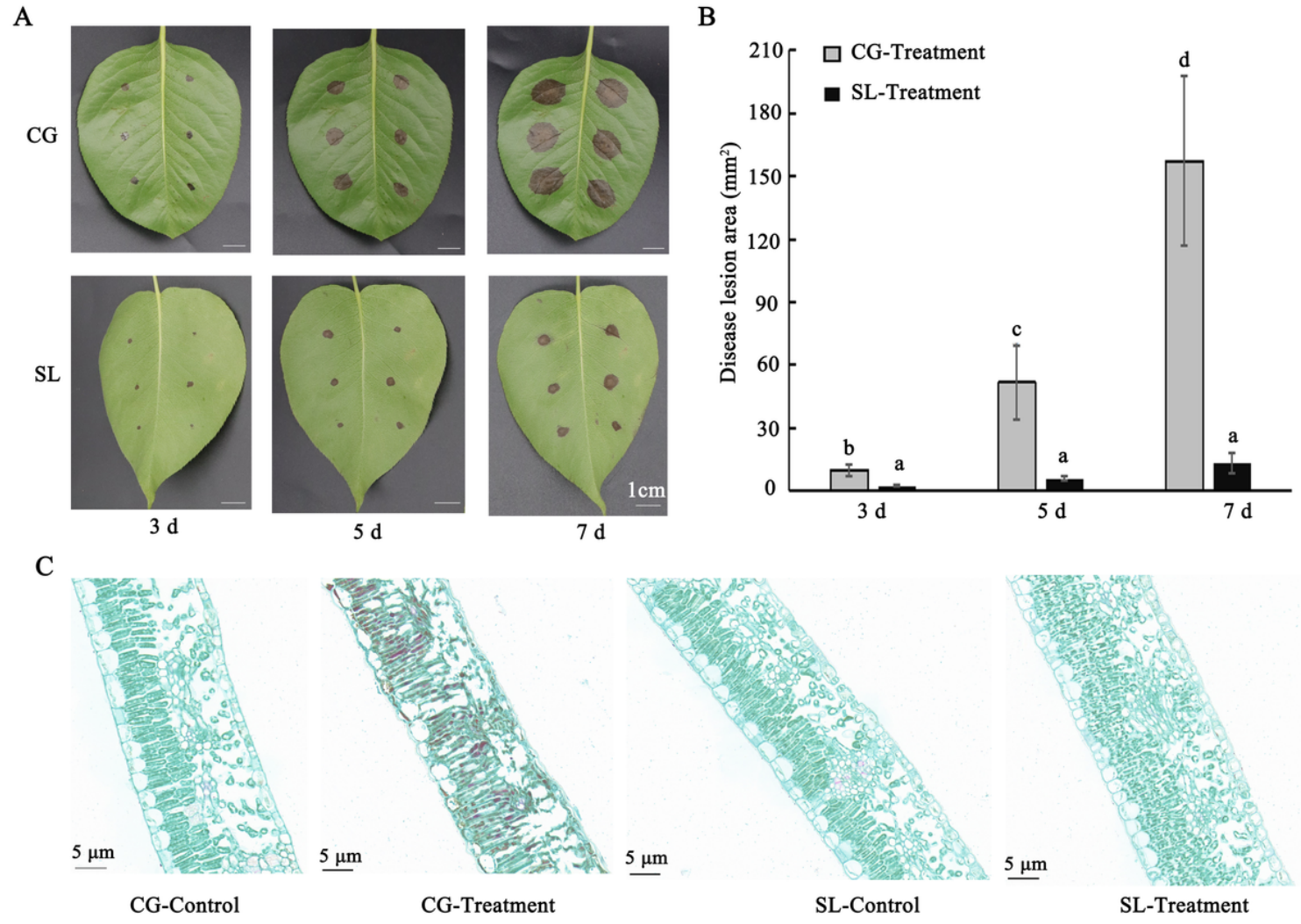

Figure 1

Pear varieties of 'Seli' (SL) and 'Cuiguan' (CG) were inoculated with *Colletotrichum fructicola* (1×10^8 CFU/mL). **(a)** Phenotypes of 'Seli' and 'Cuiguan' leaves at 3, 5, and 7 days after inoculation. Scale bar = 1 cm. **(b)** Disease lesion area of 'Seli' and 'Cuiguan' leaves at 3, 5, and 7 days after inoculation. **(c)** Leaf internal anatomy structure at 0 and 5 days after inoculation. Scale bar = 5 μm.

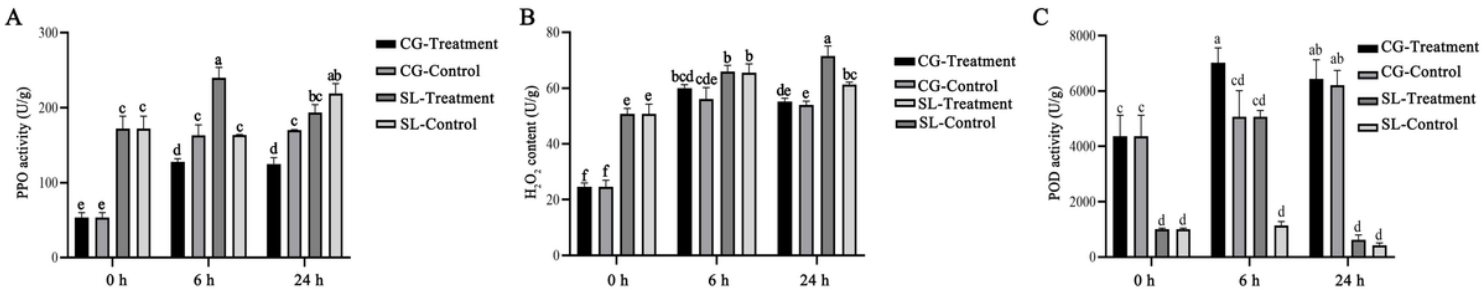

Figure 2

Enzyme activity of ‘Seli’ (SL) and ‘Cuiguan’ (CG) leaves after inoculation with *Colletotrichum fructicola* (treatment) and sterile water (control). **(a-c)** The leaf enzyme activity of polyphenol oxidase (PPO), H_2O_2, and peroxidase (POD) at 0, 6, and 24 h after inoculation. Data from three independent replicates are expressed as the mean ± standard deviation. Different letters above the bars represent significant differences according to Duncan’s multiple range test (P = 0.05; n = 3).

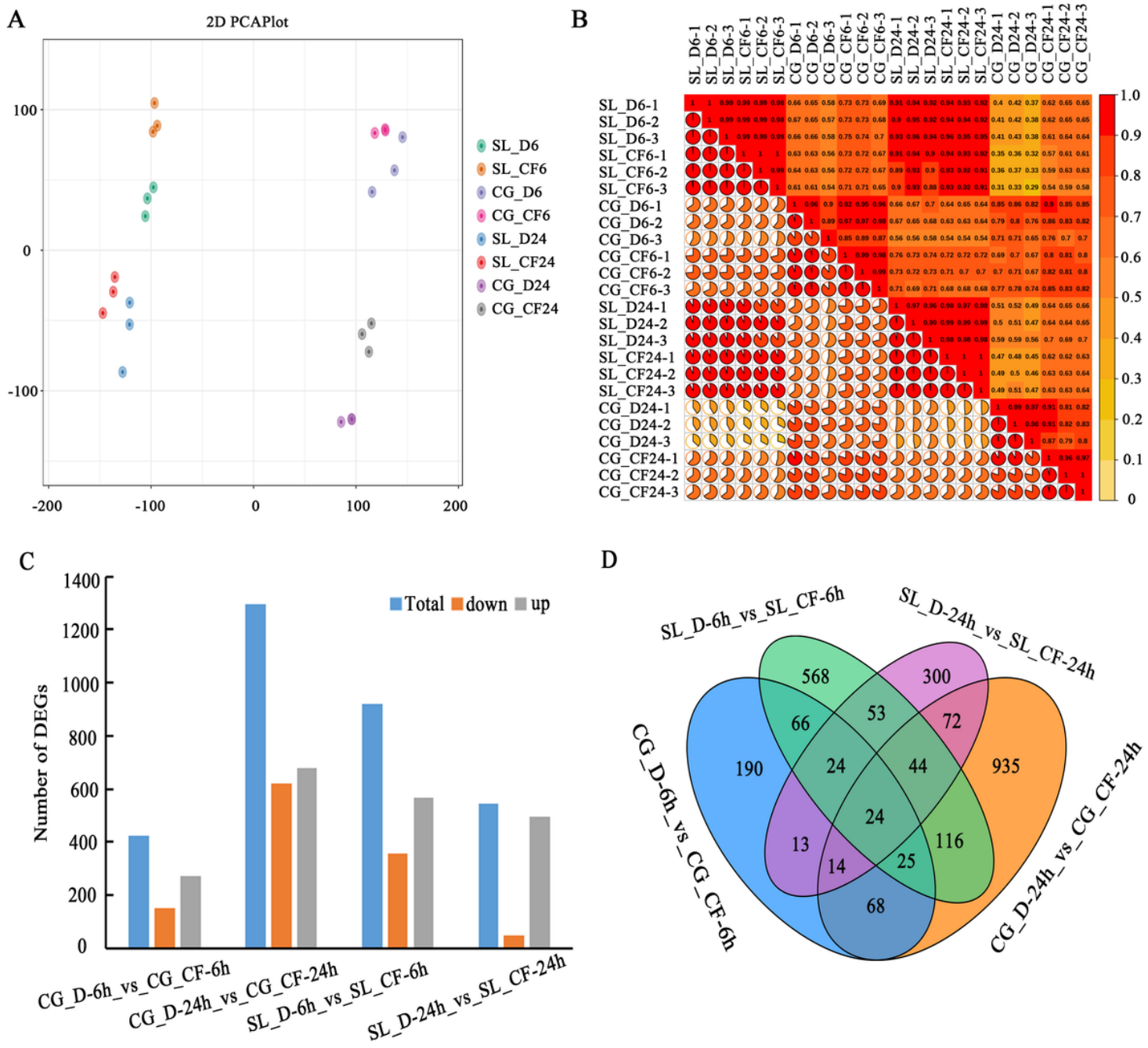

Figure 3

Statistical analysis of differentially expressed genes (DEGs) in response to *Colletotrichum fructicola* inoculation at 6 and 24 h. **(a)**Principal component 1 (32.77% of the variance) and principal component 2 (13.34% of the variance). **(b)** Pearson’s correlation coefficient testing in ‘Seli’ and ‘Cuiguan’ sample

datasets. **(c)** The overall upregulated and downregulated DEGs in 'Seli' and 'Cuiguan' post-infection. **(d)** Venn diagram showing the transcript distribution in 'Seli' and 'Cuiguan' samples inoculated with *C. fructicola* at 6 and 24 h. SL_CF and CG_CF indicated that 'Seli' and 'Cuiguan' leaves were inoculated with *C. fructicola*, respectively, and SL_D and CG_D indicated that 'Seli' and 'Cuiguan' leaves were inoculated with sterile water, respectively. Duration (hours) of *C. fructicola* inoculation is indicated by the number following 'Seli' or 'CuiGuan' names, e.g., SL_CF-6 h indicates 6 h of *C. fructicola* inoculation in 'Seli', whereas SL_D-6 h indicates 6 h of sterile water inoculation in 'Seli'.

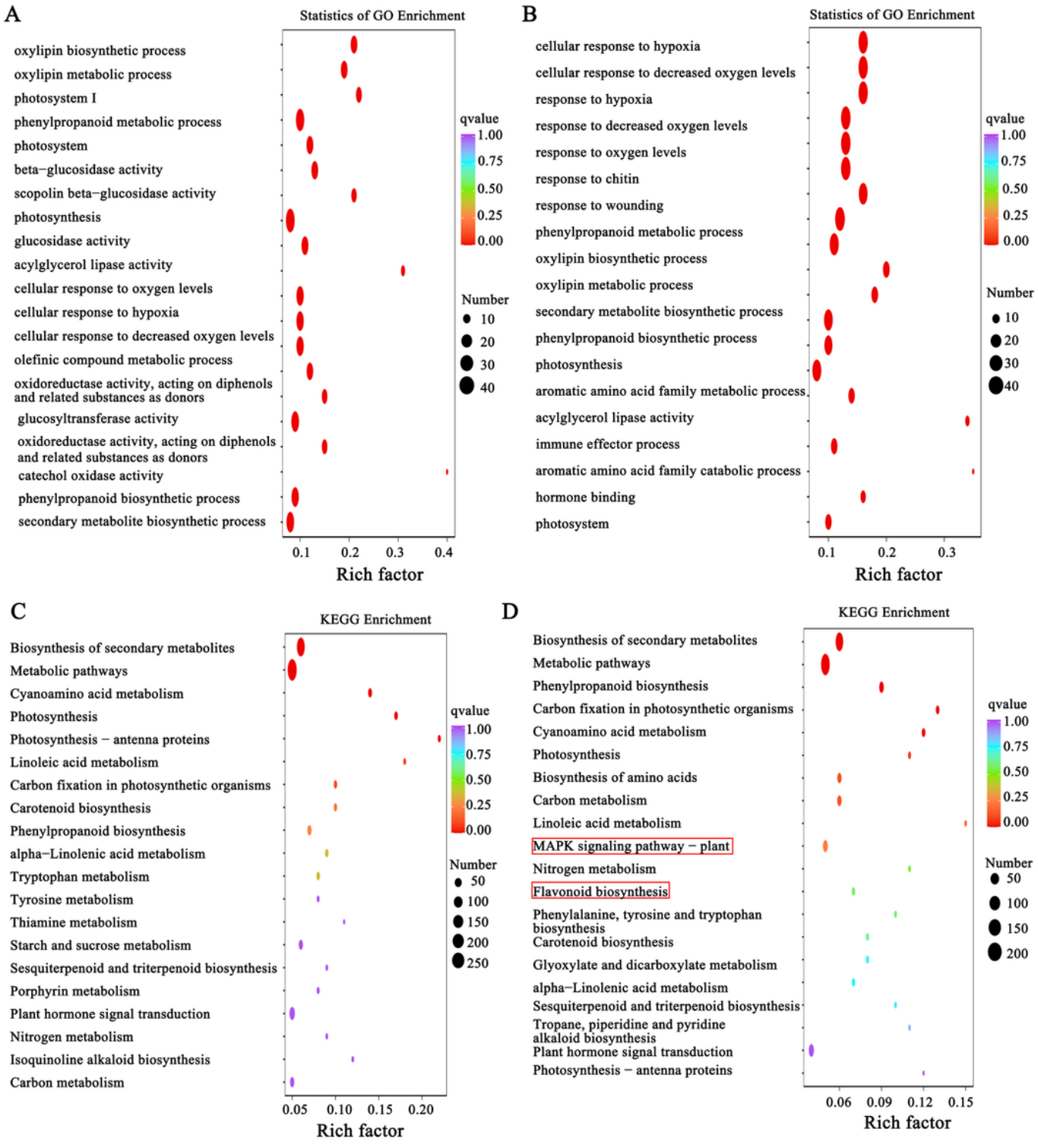

Figure 4

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment pathways of differentially expressed genes (DEGs) in ‘Seli’ (SL) and ‘Cuiguan’ (CG) at 6 and 24 h after inoculation with *C. fructicola*. **(a)** GO enrichment of CG-D vs. CG-CF. **(b)** GO enrichment of SL-D vs. SL-CF. **(c)** KEGG enrichment of CG-D vs. CG-CF. **(d)** KEGG enrichment of SL-D vs. SL-CF. Treatment: D, sterile water; CF, *Colletotrichum fructicola.*

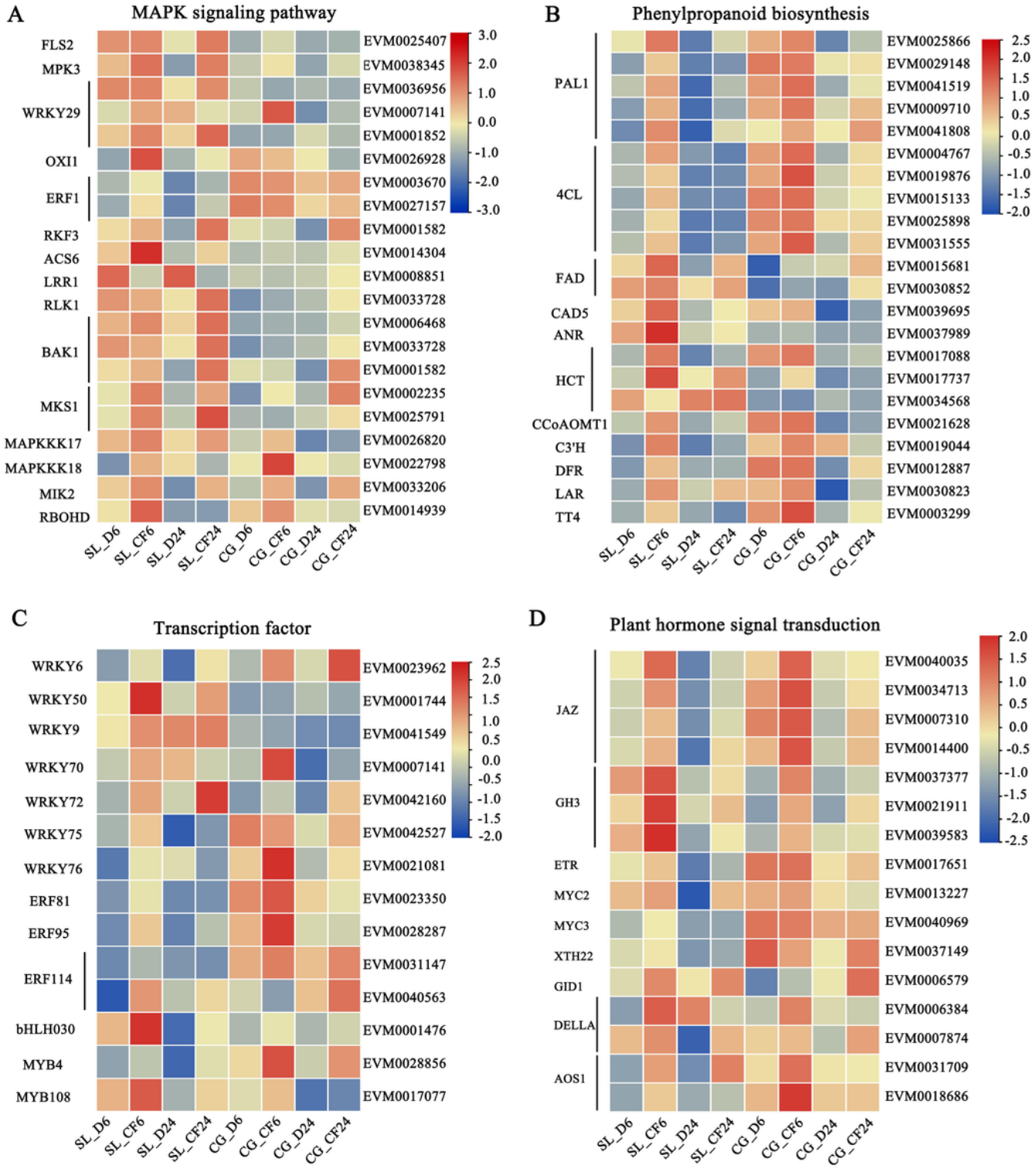

Figure 5

Heatmap analyses of the differentially expressed genes (DEGs) linked to disease resistance in ‘Seli’ (SL) and ‘Cuiguan’ (CG) leaves after *Colletotrichum fructicola* infection at 6 and 24 h. **(a)** Heatmap of DEGs involved in the MAPK signaling pathway. **(b)** Heatmap of DEGs involved in phenylpropanoid biosynthesis. **(c)** Heatmap of transcription factors. **(d)**Heatmap of DEGs involved in plant hormone signal transduction. Treatment: D, sterile water; CF, *C. fructicola*

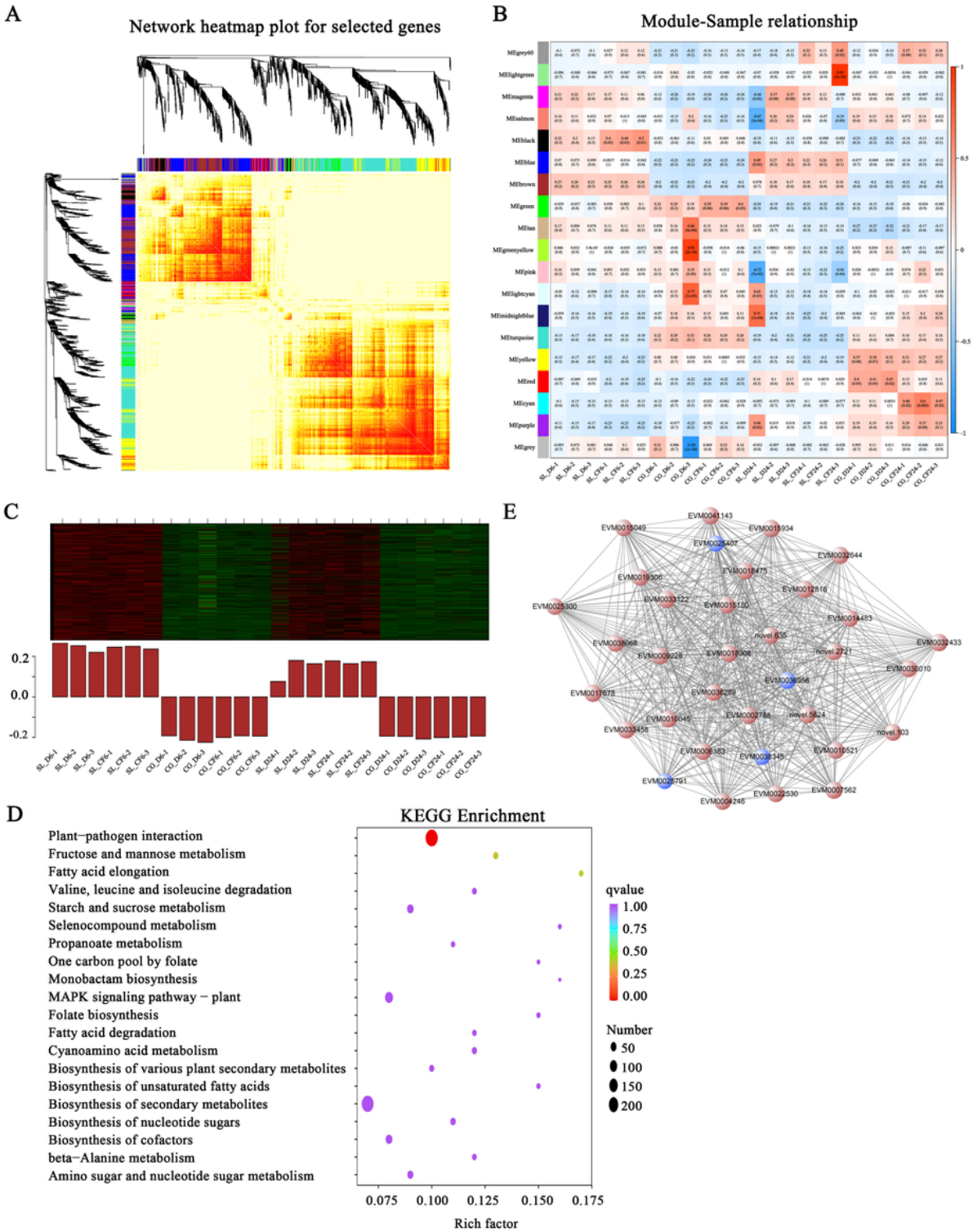

Figure 6

Weighted gene coexpression network. **(a)**Heatmap of the gene coexpression modules. Different modules are indicated by different colors. **(b)** Correlation between the samples and modules. The correlation coefficient (top) and P-value (bottom) are indicated in each rectangle. The colored bar represents the correlation level between the samples and modules. **(c)** Representative modules identified in the heatmap. **(d)**Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment of differentially expressed genes (DEGs) in the brown module following *Colletotrichum fructicola* infection in 'Seli' and 'Cuiguan' leaves. **(e)** Protein–protein interaction network based on the hub genes (blue nodes) from the brown module.

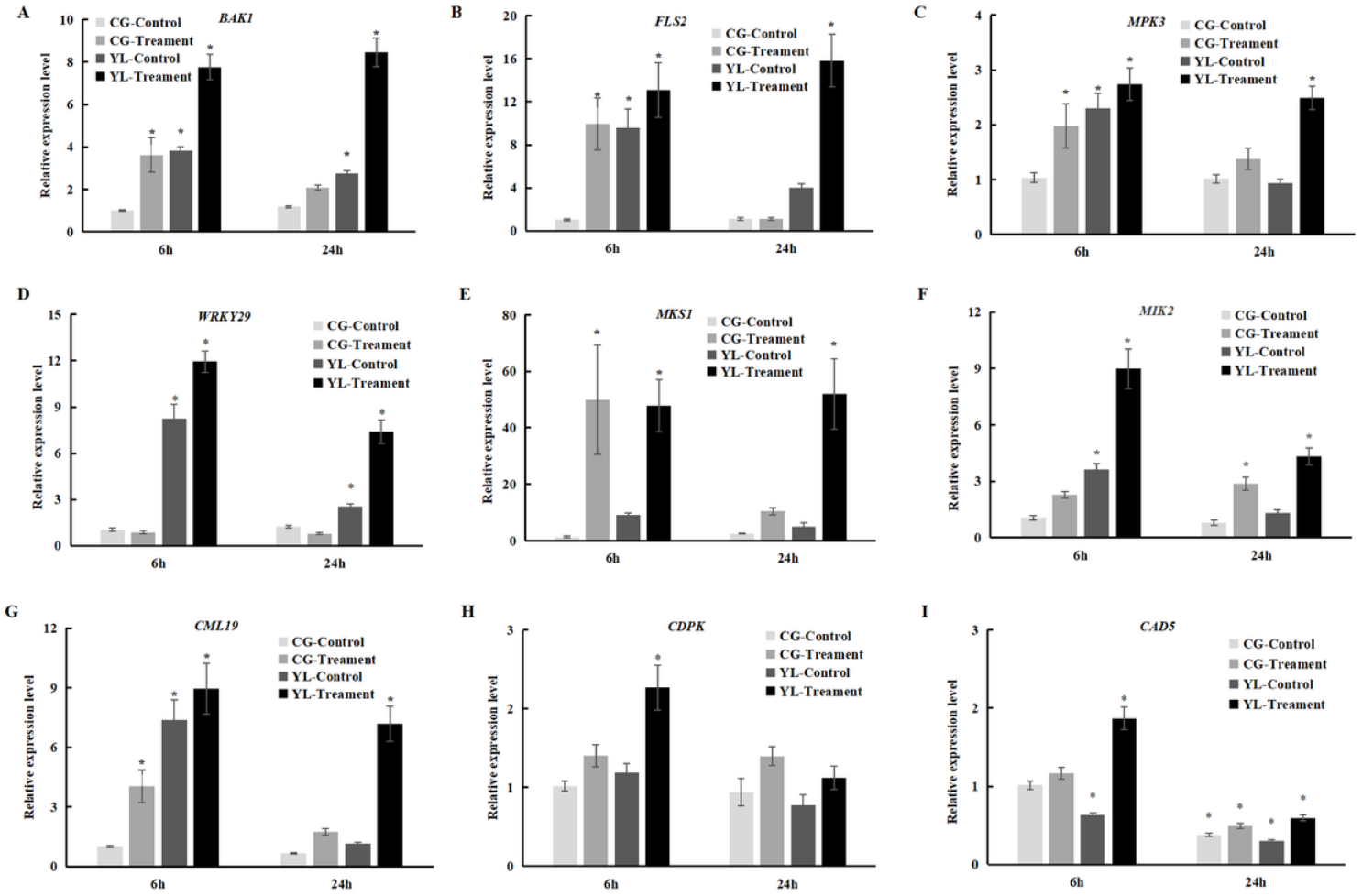

Figure 7

qRT–PCR validation of the selected differentially expressed genes (DEGs) in 'Seli' and 'Cuiguan' leaves after *Colletotrichum fructicola* inoculation. **(a-f)** Nine genes were evaluated: BRI1-associated receptor kinase 1 (*BAK1*), LRR receptor-like kinase (*FLS2*), mitogen-activated protein kinase 3 (*MPK3*), WRKY-type transcription factor 29 (*WRKY29*), MAP kinase substrate 1 (*MKS1*), male discoverer 1-interacting receptor-like kinase 2 (*MIK2*), calmodulin-like protein 19 (*CML19*), calcium-dependent protein kinase (*CDPK*), and cinnamyl alcohol dehydrogenase 5 (*CAD5*). The treatment group was inoculated with *C. fructicola*, and

the control group was inoculated with sterile water at 6 and 24 h. Gene expression levels were normalized to those of the ‘Cuiguan’ control group at 6 h post-inoculation. The relative expression level was calculated using the $2^{-\Delta\Delta Ct}$ method with actin as the reference gene. Error bars indicate the standard deviation of three independent repetitions.

Supplementary Files

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