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ORIGINAL ARTICLE

Sugarcane *ScOPR1* gene enhances plant disease resistance through the modulation of hormonal signaling pathways

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Abstract

Key message **Transgenic plants stably overexpressing *ScOPR1* gene enhanced disease resistance by increasing the accumulation of JA, SA, and GST, as well as up-regulating the expression of genes related to signaling pathways.**

Abstract 12-Oxo-phytodienoate reductase (OPR) is an oxidoreductase that depends on flavin mononucleotide (FMN) and catalyzes the conversion of 12-oxophytodienoate (12-OPDA) into jasmonic acid (JA). It plays a key role in plant growth and development, and resistance to adverse stresses. In our previous study, we have obtained an *OPR* gene (*ScOPR1*, GenBank Accession Number: MG755745) from sugarcane. This gene showed positive responses to methyl jasmonate (MeJA), salicylic acid (SA), abscisic acid (ABA), and *Sporisorium scitamineum*, suggesting its potential for pathogen resistance. Here, in our study, we observed that *Nicotiana benthamiana* leaves transiently overexpressing *ScOPR1* exhibited weaker disease symptoms, darker 3,3-diaminobenzidine (DAB) staining, higher accumulation of reactive oxygen species (ROS), and higher expression of hypersensitive response (HR) and SA pathway-related genes after inoculation with *Ralstonia solanacearum* and *Fusarium solanacearum* var. *coeruleum*. Furthermore, the transgenic *N. benthamiana* plants stably overexpressing the *ScOPR1* gene showed enhanced resistance to pathogen infection by increasing the accumulation of JA, SA, and glutathione S-transferase (GST), as well as up-regulating genes related to HR, JA, SA, and ROS signaling pathways. Transcriptome analysis revealed that the specific differentially expressed genes (DEGs) in *ScOPR1*-OE were significantly enriched in hormone transduction signaling and plant–pathogen interaction pathways. Finally, a functional mechanism model of the *ScOPR1* gene in response to pathogen infection was depicted. This study provides insights into the molecular mechanism of *ScOPR1* and presents compelling evidence supporting its positive involvement in enhancing plant disease resistance.

Keywords Sugarcane · 12-Oxo-phytodienoic acid reductase · Pathogen infection · Genetic transformation · Resistance mechanism

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Introduction

Sugarcane (*Saccharum* spp.) is the main crop for sugar production in China, contributing to over 85% of the total sugar yield (Ruan et al. 2018; Li and Yang 2015; Dotaniya et al. 2016). It is susceptible to a fungal disease called sugarcane smut, caused by *Sporisorium scitamineum*. The pathogenic mycelium of the smut fungus invades cane shoots and spreads through intercellular filaments, affecting the growing point, resulting in mutations and the production of black whips, which even hinders stem formation in the cane (Rajput et al. 2021; Shamsul et al. 2021; Que et al. 2014). Developing and cultivating sugarcane varieties that are resistant to smut is the primary strategy to combat this disease. Therefore, exploring disease-resistant genes not only provides a genetic resource but also establishes a theoretical foundation for molecular breeding in sugarcane.

Jasmonates (JAs), which include jasmonate acid (JA) and its derivative methyl jasmonate (MeJA), are crucial signaling molecules derived from hydroxyl lipids in plants (Wasternack and Hause 2013; Campos et al. 2014). Generally, the synthesis of JAs occurs in the chloroplast and peroxisome. Within the chloroplast, unsaturated fatty acids are oxygenated by lipoxygenase (LOX) to produce 12-oxo-phytodienoic acid (12-OPDA) through the actions of allene oxide synthase (AOS) and allene oxide cyclase (AOC) (Chini et al. 2018; Mou et al. 2019). In the peroxisome, 12-OPDA is converted into JA by 12-oxo-phytodienoic acid reductase (OPR) and three β-oxidation steps of the carboxylic acid side chain (Chini et al. 2018; Mou et al. 2019). JA is catabolized in the cytoplasm to produce structures like methyl jasmonate (MeJA), jasmonoyl-l-isoleucine (JA-Ile), *cis*-jasmone (CJ), and 12-hydroxyjasmonic acid (12-OH-JA) (Chini et al. 2018; Mou et al. 2019). Research indicates that OPR, a flavin mononucleotide (FMN)-dependent oxidoreductase, catalyzes OPDA into JA precursor, marking the final step of JA synthesis (Mou et al. 2019; Tani et al. 2008; Breithaupt et al. 2006). OPRs are a multiprotein family with two classes, OPR I and OPR II, based on their substrate preference. Notably, OPR II has the ability to convert (9S, 13S)-OPDA into (+)-7-epi-JA precursor, while OPR I has different substrate preferences and may aid in substrate (Schaller et al. 1998; Strassner et al. 2002). It was found that after simultaneous mutation of two *OPR3* homologous genes by CRISPR/Cas9, the mutant showed complete male sterility and the fertility could be easily restored by exogenous MeJA treatment (Cheng et al. 2023). Besides, a meta-analysis of barley transcriptome datasets revealed that *OPR3* was involved in JA biosynthesis (Soltani et al. 2023). Furthermore, *OPR3*-independent JA biosynthesis pathway is ancient and predates the emergence of the *OPR3*-independent pathway (Chini et al. 2023). The first plant *OPR* gene was isolated from *Arabidopsis thaliana* in 1997, and subsequent research has identified numerous *OPR* genes (Schaller and Weiler 1997). Currently, there are 3 *OPRs* in *Arabidopsis* and *Lycopersicon esculentum* (Breithaupt et al. 2006; Schaller and Weiler 1997; Biesgen and Weiler 1999), 5 in *Citrullus lanatus* (Guang et al. 2021), 6 in *Pisum sativum* (Matsui et al. 2004), 8 in *Zea mays* (Zhang et al. 2005), 13 in *Oryza sativa* (Li et al. 2011)**,** and 48 in *Triticum aestivum* (Mou et al. 2019).

In plants, the OPR gene family is extensively involved in regulating growth and development, resistance to pathogen infection, and tolerance to adversity stress, while the specific function varies among different family members (Ponting et al. 2002; Liu et al. 2020; Tan et al. 2013; Pratiwi et al. 2017; Wang et al. 2016). For example, the *Brassica campestris BcOPR3* gene was found to be up-regulated at a higher rate in disease-resistant plants compared to susceptible plants after infection with *Hyaloperonospora parasitica* (Wen et al. 2017), and its expression could be triggered by the stresses of JA, abscisic acid (ABA), and salicylic acid (SA) (Wen et al. 2017). In *Z. mays*, *ZmOPR1* and *ZmOPR2* contributed to defense against several pathogens (Zhang et al. 2005). Moreover, maize *opr2* mutants exhibited differing sensitivity to various pathogens (Huang et al. 2023). In *Gossypium hirsutum*, virus-induced gene silencing (VIGS) revealed that the plants with *GhOPR9* knockout were more susceptible to *Verticillium dahlia* infection (Liu et al. 2020). Similarly, in *Solanum lycopersicum*, silencing of the *SlOPR3* gene resulted in a lower accumulation of OPDA and JA-lle after infection with *Botrytis cinerea*, making the plants more susceptible to this pathogen (Scalschi et al. 2015). Beyond doubt, these findings strongly support the significant role of OPRs in plant responses to pathogen stress.

A *ScOPR1* gene (GenBank Accession Number: MG755745) was identified and characterized in our previous study from the sugarcane cultivar ROC22, and its gene expression was up-regulated by MeJA, SA, and *S. scitamineum* stresses. Here in our study, transient overexpression of *ScOPR1* in *Nicotiana benthamiana* were performed and three T_4 generation stable transgenic lines were selected. The phenotype, vegetative index, SA and JA contents, glutathione S-transferase (GST) enzyme activity, and immune response-associated gene expression were assessed in transgenic plants post-inoculation with two pathogens, *Ralstonia solanacearum* and *Fusarium solanacearum* var. *coeruleum*. Additionally, RNA-Seq in transgenic plants post-inoculation with *F. solani* var. *coeruleum* was conducted. The present study aims to establish a theoretical foundation for genetic engineering by *ScOPR1* gene for smut resistance improvement in sugarcane breeding.

Materials and methods

Bioinformatics analysis of *ScOPR1*

The conserved domain prediction of the ScOPR1 protein was conducted using the NCBI (https://www.ncbi.nlm.nih.gov/cdd). The promoter sequence (2000 bp upstream) of two *ScOPR1* homologous genes, *SsPON.05G0025620-1B* and *Sh_227A23_contig-1_t000020,* were extracted from *S. spontaneum* (Zhang et al. 2018) and sugarcane cultivar R570 genomes (Garsmeur et al. 2018), respectively. The PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) was used to predict the *cis*-regulatory elements (CREs) and the TBtools was used for visualization (Chen et al. 2020).

Transient overexpression of *ScOPR1* in *Nicotiana benthamiana*

Referred to our previous study (Sun et al. 2020), an *OPR* gene was screened from the sugarcane transcriptome unigene library constructed by our group, and a full-length cDNA sequence, named *ScOPR1* (GenBank Accession Number: MG755745), was amplified from ROC22 buds inoculated with smut pathogen for 48 h using RT-PCR. The recombinant vector pEarleyGate 203-*ScOPR1* (*35S::ScOPR1*) and the control vector (*35S::00*) were produced using the Gateway technique. They were then transiently overexpressed in *N. benthamiana* leaves via the *Agrobacterium*-mediated delivery (Choi et al. 2012; Wang et al. 2020). Subsequently, *R. solanacearum* and *F. solani* var. *coeruleum* were inoculated into the leaves of 6-week-old *N. benthamiana* that transiently overexpressed *35S::ScOPR1* and *35S::00* for 1 d, respectively. Then, the phenotypic changes were tracked and photographed (Dang et al. 2013). Post inoculation with pathogen for 1 d and 6 d, the *N. benthamiana* leaves were collected for 3,3′-diaminobenzidine tetrahydrochloride (DAB) staining to measure the accumulated hydrogen peroxide (H_2O_2) content (Choi et al. 2012; Sohn et al. 2007; Wang et al. 2020; Wu et al. 2023). The expression of the *ScOPR1* gene in transiently *N. benthamiana* plants was analyzed through reverse transcription PCR (RT-PCR) with the primers *ScOPR1*-gate-F/R (Table S1). The expression levels of five immune-related marker genes, consisting of two hypersensitive response (HR) genes (*NbHSR201* and *NbHSR515*) and three SA-related genes (*NbPR2*, *NbPR3*, and *NbPR1-a/c*) (Wang et al. 2023a, b), were analyzed using real-time quantitative PCR (RT-qPCR), respectively (Table S1). Data normalization to the expression level of *NbEF-1a* (Brogue et al. 1991; Zhang et al. 2019; Wu et al. 2023). All treatments were performed with three biological replicates. The relative expression levels were determined utilizing the $2^{-\Delta\Delta CT}$ approach (Livak and Schmittgen 2001), and the statistical analysis, including significance ($P<0.05$) and standard error, was conducted using DPS 7.05 with Duncan's new multiple range test.

Generation of transgenic *N. benthamiana* plants overexpressing *ScOPR1* gene and the evaluation of its disease resistance

Agrobacterium tumefaciens GV3101 cells harboring pEarleyGate 203-*ScOPR1* were delivered into *N. benthamiana* utilizing the leaf-disk methodology, followed by the screening of transgenic plant materials in a subculture medium (4.4 g/L MS, 8 g/L agar, pH = 5.8) containing 0.01% basta (Burow et al. 1990). Positive transgenic *N. benthamiana* lines were screened by RT-PCR using primers *ScOPR1*-gate-F/R (Table S1). Subsequently, three T4 generation transgenic *N. benthamiana* plants were generated, referred to as ScOPR1-OE1, ScOPR1-OE2, and ScOPR1-OE3. The pathogens of *R. solanacearum* and *F. solani* var. *coeruleum* were inoculated into the leaves of *ScOPR1*-OE and wild-type (WT) plants with three biological replicates, respectively. All the subjected materials were grown at 28 °C under a light/dark cycle of 16 h/8 h and 75% relative humidity. The phenotypic changes of the leaves were tracked and observed. Besides, GST activity, as well as SA and JA contents were evaluated using ELISA kits (Shanghai Enzyme-linked Biotechnology, China) at 0 d and 2 d post-inoculation with pathogens, following the manufacturer's instructions. Furthermore, the expression levels of eight immune-related marker genes, including HR marker genes *NbHSR201* and *NbHSR515*, SA-related genes *NbPR2* and *NbNPR1*, JA-related genes *NbLOX1* and *NbDEF1* (Torres 2010), and reactive oxygen species (ROS)-related genes *NbGST1* and *NbAPX* (Lai et al. 2013) (Table S1) were analyzed by RT-qPCR using *NbEF-1α* as an internal reference gene (Brogue et al. 1991; Zhang et al. 2019; Wu et al. 2023).

RNA sequencing and data analysis

The *N. benthamiana* leaf samples after inoculation with *F. solani* var. *coeruleum* at the beginning (0 d, control, CK) and 2 d (treatment, T), resulting in four sample sets (WT-CK, *ScOPR1*-CK, WT-T, and *ScOPR1*-T) with three biological replicates, were collected for RNA-Seq. Then, total RNA extraction, cDNA library construction, Illumina sequencing, data analysis, reference *N. benthamiana* genome mapping, differentially expressed genes (DEGs) identification (fold change ≥ 2 and *P*-value < 0.05) and DEGs annotation were referred to our previous studies (Wu et al. 2022a, 2022b; Wang et al. 2023a, b; Sun et al. 2023). Seven candidates DEGs including

NPR1 (*Niben101Scf19043g00002*), *DELLA* (*Niben-101Scf15437g02006*), *HDT1* (*Niben101Scf09416g05012*), *CPK28* (*Niben101Scf05805g02006*), *CTL1* (*Niben-101Scf03036g03023*), *BKI1* (*Niben101Scf03420g01001*), and *MPK4* (*Niben101Scf07241g00013*) were randomly screened for RT-qPCR validation.

Results

Sequence characteristics of sugarcane *ScOPR1*

As depicted in Fig. 1A, the sugarcane *ScOPR1* encoded 371 amino acids (AA) and contained a conserved OYE_like_FMN domain from 10 to 349 AA. This gene showed 99.19% and 94.34% similarity with the homologous gene in *S. spontaneum* (*SsPON.05G0025620-1B*) and sugarcane cultivar R570 (*Sh_227A23_contig-1_t000020*) (Fig. S1). Besides, both genes contained *cis*-regulatory elements related to growth and development, light response, and hormone response, with the unique presence of stress-responsive elements, while *Sh_227A23_contig-1_t000020* specifically contained stress-responsive elements (Fig. 1B), suggesting a potential involvement of *ScOPR1* gene in various aspects of plant growth and response to environmental stresses. Meanwhile, the expression levels of the *ScOPR1* gene were increased under SA, MeJA, and *S. scitamineum* stresses. Moreover, compared with the control, its expression

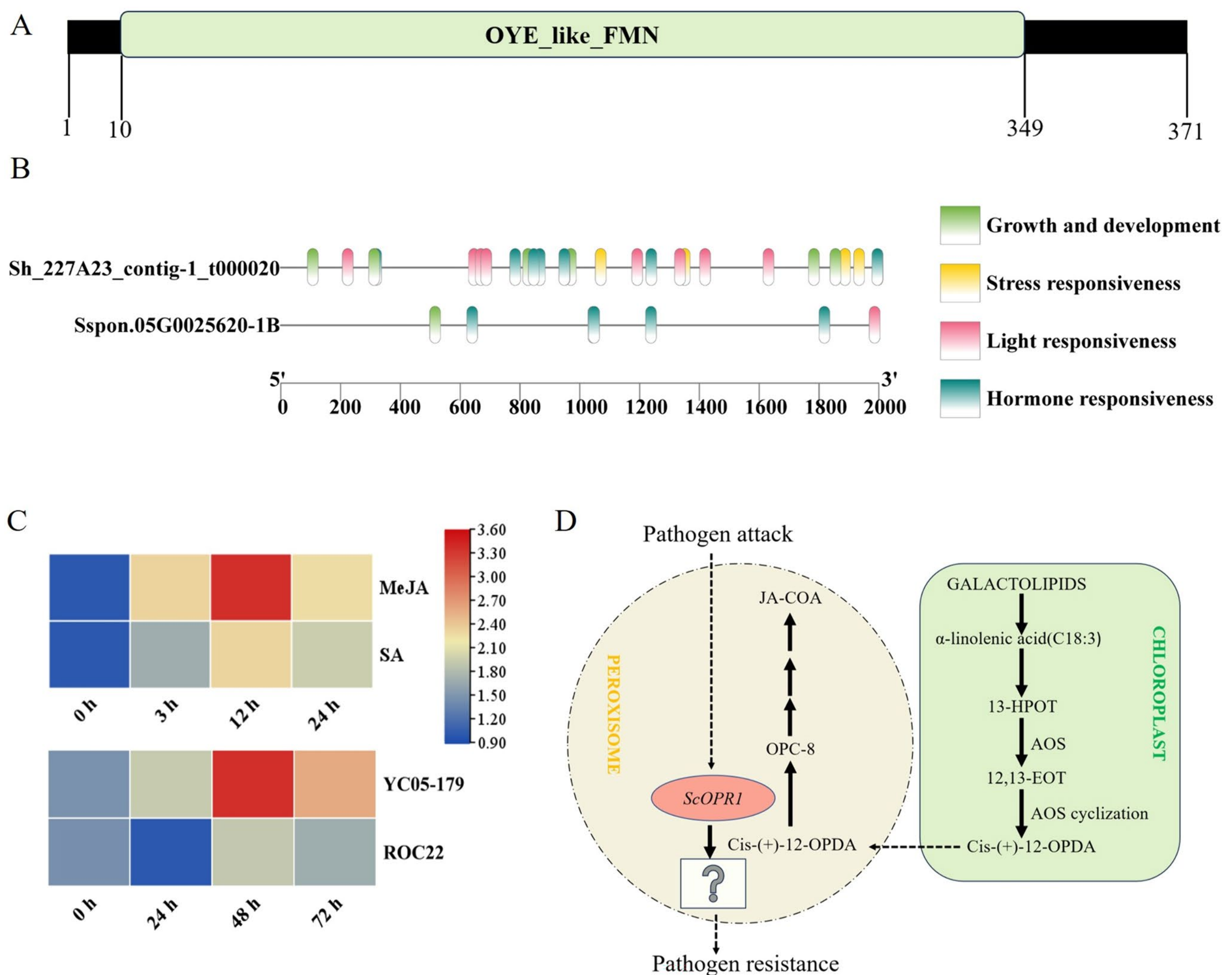


Fig. 1 Characterization of *ScOPR1* gene in sugarcane. **A** Conserved domains of ScOPR1 protein. **B** *Cis*-regulatory element (CREs) analysis of the *ScOPR1* homologous gene *SsPON.05G0025620-1B* in *S. spontaneum* and *Sh_227A23_contig-1_t000020* in R570. Different color boxes corresponded to different CREs. **C** Expression patterns of *ScOPR1* in sugarcane under MeJA, SA, and *S. scitamineum* stresses. Color bars represent the normalized values ($\log_2$ Relative exprssion), ranging from blue (low expressionlevel) to red (high expression level). **D** Jasmonate biosynthetic pathway

was up-regulated and reached a peak at 48 h with 2.62-fold compared to the control (0 h) in the smut-resistance variety YC05-179, but down-regulated at 24 h in the susceptible variety ROC22 (Fig. 1C) (Sun et al. 2018). These results suggested a role of the *ScOPR1* gene in conferring resistance to *S. scitamineum* through JA and SA biosynthesis pathways in sugarcane (Fig. 1D).

Transient overexpression of *ScOPR1* led to an enhancement in the disease resistance

As shown in Fig. 2A, the *ScOPR1* gene was successfully transiently overexpressed in *N. benthamiana*. Following inoculation with *R. solanacearum* for 1 d, the disease symptoms and DAB staining color had no significant difference between *35S::ScOPR1* and the control (*35S::00*) (Fig. 2B). However, after 6 d, the symptoms in the leaves of *35S::00* were more severe compared to *35S::ScOPR1*. Furthermore, the DAB staining color of *35S::ScOPR1* was darker than the control, indicating a significantly higher H_2O_2 content in the *35S::ScOPR1* plants (Fig. 2B). Furthermore, the expression levels of genes related to HR and SA pathways were significantly increased in *35S::ScOPR1* plants after infected with *R. solanacearum* compared to the control. Especially, 6 days after injection with *R. solanacearum*, the expression levels of *NbHSR201*, *NbHSR515*, *NbPR-1a/c*, and *NbPR2* were 4.40-, 9.41-, 46.76-, and 11.56-fold higher than the control, respectively (Fig. 2C). Similarly, there was no significant difference in phenotypes between *35S::ScOPR1* and *35S::00* after inoculation with *F. solanacearum* var. *coeruleum*, while a high content of H_2O_2 accumulated in the *35S::ScOPR1* plants (Fig. 2D). Besides, the expression of HR marker and SA-related genes were significantly up-regulated in *35S::ScOPR1* leaves at 1 d or 6 d, with the

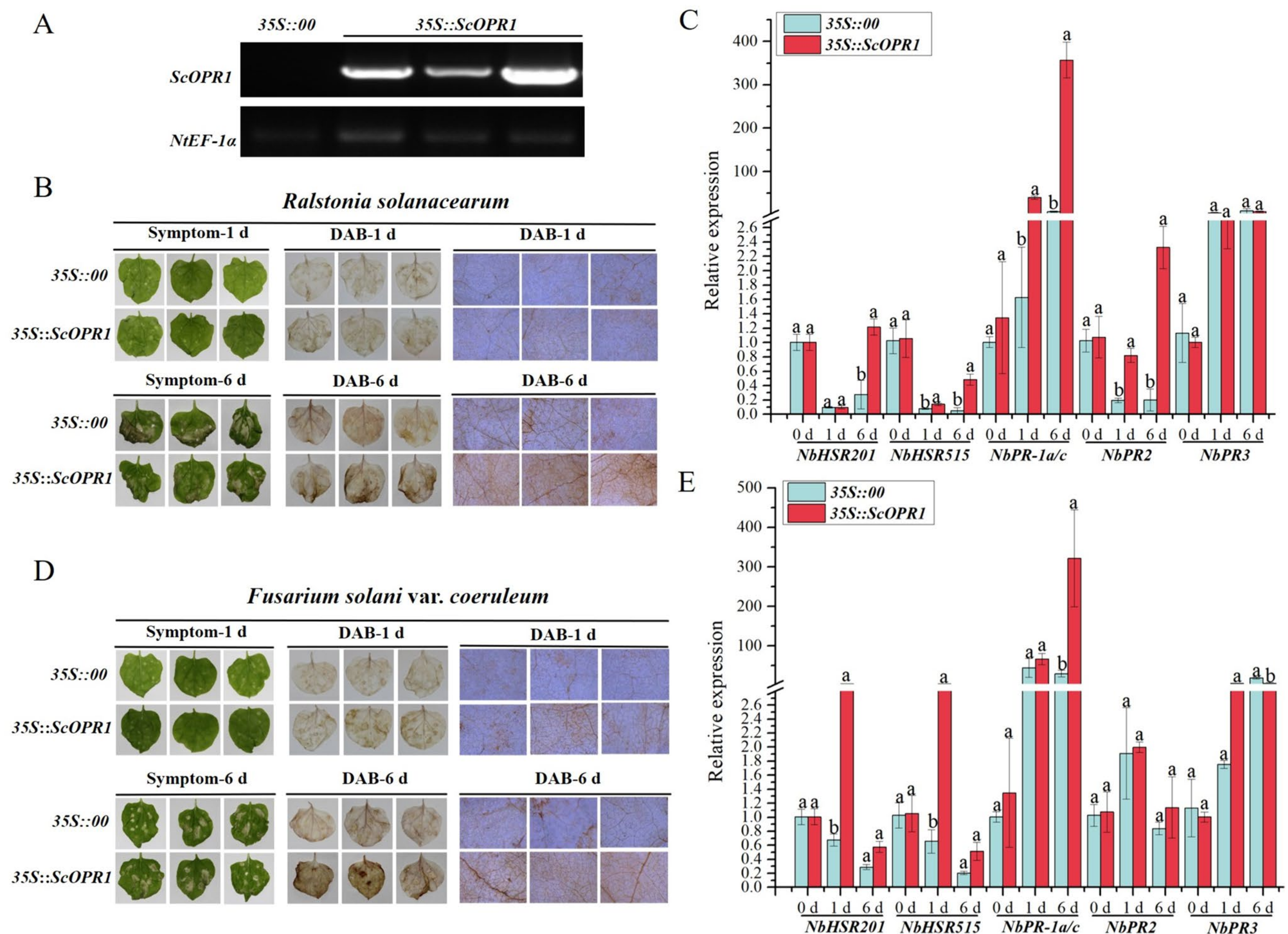
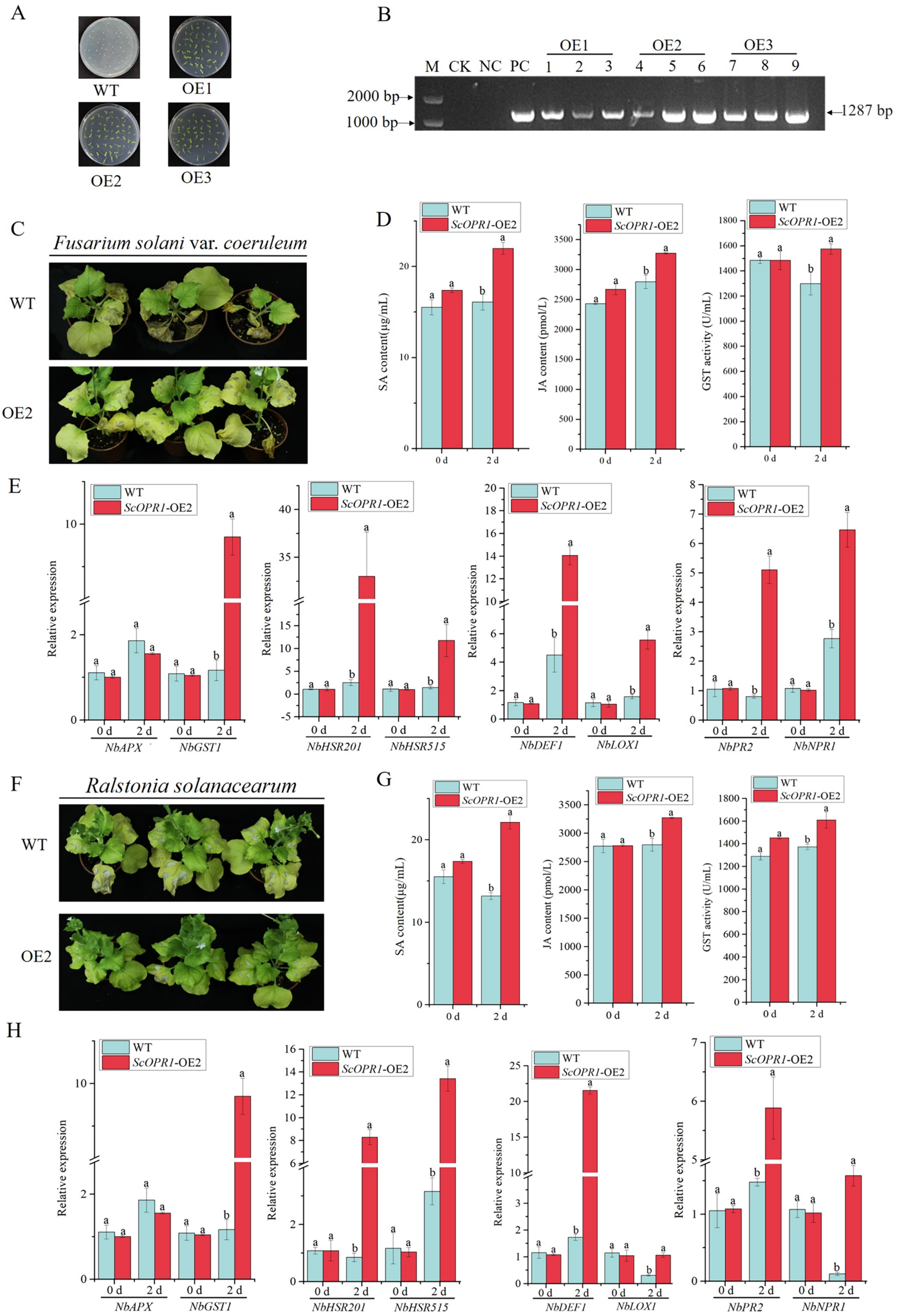


Fig. 2 Transient overexpression of the *ScOPR1* gene in *N. benthamiana*. **A** RT-PCR results of *ScOPR1* in *N. benthamiana* leaves after transient overexpression for 1 d. *35S::ScOPR1*, pEarleyGate 203-*ScOPR1*; *35S::00*, the empty vector pEarleyGate 203. **B**, **D** Phenotype and DAB staining of *N. benthamiana* leaves transiently overexpressing *35S::ScOPR1* and *35S::00* after inoculation with *R. solanacearum* and *F. solani* var. *coeruleum* for 1 d and 6 d. **C**, **E** The expression levels of HR marker and SA-related genes in *N. benthamiana* leaves following inoculation with *R. solanacearum* and *F. solani* var. *coeruleum* at 1 d and 6 d. All data points represent means ± standard error ($n = 3$). The significant differences are represented by different letters


A
WT
OE1
OE2
OE3
B
M CK NC PC
OE1 OE2 OE3
1 2 3 4 5 6 7 8 9
2000 bp
1000 bp
1287 bp
C
Fusarium solani var. coeruleum
WT
OE2
D
WT
ScOPR1-OE2
SA content(μg/mL)
JA content (pmol/L)
GST activity (U/mL)
0 d
2 d
E
Relative expression
NbAPX
NbGST1
NbHSR201
NbHSR515
NbDEF1
NbLOX1
NbPR2
NbNPR1
F
Ralstonia solanacearum
WT
OE2
G
H

◂**Fig. 3** Disease resistance evaluation of T_4 generation of transgenic *N. benthamiana* overexpressing the *ScOPR1* gene. **A** T_4 transgenic *N. benthamiana* seeds on MS plates with herbicides. WT, wild-type *N. benthamiana*; OE1–OE3, three *ScOPR1* transgenic *N. benthamiana* lines. **B** RT-PCR detection of T_4 generation transgenic *N. benthamiana* plants. M, 2000 bp DNA marker; CK, blank control; NC, negative control; PC, positive control. **C**, **F** Phenotypes of transgenic *N. benthamiana* after inoculation with *F. solani* var. *coeruleum* 23 d and *R. solanacearum* 15 d. **D**, **G** Determination of SA and JA contents, and GST activity in transgenic *N. benthamiana* after inoculation with *F. solani* var. *coeruleum* and *R. solanacearum* for 0 d and 2 d. **E**, **H** Expression pattern of ROS-, HR-, JA- and SA-related genes in transgenic *N. benthamiana* after inoculation with *R. solanacearum* and *F. solani* var. *coeruleum* for 0 d and 2 d. All data points were means ± standard error ($n = 3$). Significant differences are calculated by Duncan's new multiple range test (P-value < 0.05) and represented by different letters

NbPR-1a/c gene showed the 10.83-fold higher than *35S::00* (Fig. 2E).

Stable overexpression of *ScOPR1* positively regulated the defense response against pathogen infection

Totally, three T_4 lines of *ScOPR1* genetically modified *N. benthamiana* plants were successfully acquired and verified by RT-PCR (Fig. 3A, B). After inoculation with *F. solani* var. *coeruleum* 23 d and *R. solanacearum* 16 d, the WT leaves showed more obvious disease spots and yellowing than that of the transgenic plants (Fig. 3C, F). Compared to the control, the contents of JA and SA, and the activity of GST in *ScOPR1*-OE2 plants were significantly increased post infection with both two pathogens (Fig. 3D, G). In addition, the expression levels of ROS-, HR-, JA- and SA-related genes were also up-regulated in the transgenic plants after challenging with pathogens (Fig. 3E, H). These results indicated that the stably overexpression of the *ScOPR1* gene could enhance the disease resistance of *N. benthamiana* to pathogen infection by promoting the expression of several genes involved in HR, JA, SA, and ROS signaling pathways.

Transcriptome difference between *ScOPR1* overexpressing transgenic lines and WT plants in the process of disease response

Since the WT-CK1 dataset showed a weak correlation with the other biological replicates (Fig. 4A), it was excluded from further analysis. A total of 98.64 GB of high-quality data was obtained, with Q30 above 93% and GC content exceeding 41%, indicating that the sequencing quality of these libraries was excellent and suitable for further analysis (Table S2). Additionally, a total of 2667 (1033 up- and 1634 down-regulated) and 187 DEGs (118 up- and 69 down-regulated) were found in the treatment (*ScOPR1*-CK_vs_*ScOPR1*-T) and the control group (WT-CK_vs_WT-T), respectively (Fig. 4B, Tables S3, S4). There were 20 common up-regulated and 29 common down-regulated DEGs in both groups. In addition, the control group had 98 specific up-regulated and 40 specific down-regulated DEGs, while the treatment group had 1013 specific up-regulated and 1605 specific down-regulated DEGs (Fig. 4C). GO enrichment showed that the specific DEGs of *ScOPR1*-CK_vs_*ScOPR1*-T were enriched in the JA signaling pathway (GO: 2,000,022), plant-type HR (GO: 0010363), SA metabolic process (GO: 0010337), defense response to fungus (GO: 1,900,150), immune response (GO: 0050776), and response to ABA (GO: 0009737) (Fig. 4D, Table S5). KEGG pathway enrichment indicated that the DEGs specific to treatment group primarily participated in plant–pathogen interaction (ko04626) and several metabolic pathways (ko00860, ko00780, ko00500, ko00520, ko00564, and ko00591) (Fig. 4E, Table S6). These results demonstrated that *ScOPR1* transgenic plants could activate more abundant DEGs in biological processes and metabolic pathways in defense against pathogen infection.

ScOPR1 expression-mediated several signaling pathways in the defense response to pathogen infection

According to the results of KEGG enrichment, three disease resistance pathways including plant hormone signal transduction, MAPK signaling pathway-plant, and plant–pathogen interaction were selected to make a straightforward molecular network. Notably, three WT-CK_vs_WT-T special regulated DEGs, namely *PP2CA*, *EIN2*, and *MTB1* were up-regulated (Fig. 5A, Table S7). Besides, 11 regulated DEGs (*LECRK2*, *SCL15*, *MMK2*, *MMK2*, *MMK2*, *PYL4*, *JAR6*, *NPR1*, *PR1*, *CAT1*, and *CAT3*) specific to *ScOPR1*-CK_vs_*ScOPR1*-T were also up-regulated (Fig. 5A, Table S7). While 16 regulated DEGs specific to *ScOPR1*-CK_vs_*ScOPR1*-T, including *SD31*, *FLS2*, *XA21*, *At3g47570*, *NLP2*, *NSP2*, *CIGR1*, *SCL23*, *SCL3*, *PAT1*, *TIFY10B*, *MAKR1*, *WRKY33*, *At1g67720*, and *RBOHA*, were down-regulated (Fig. 5A, Table S7). Additionally, six common regulated DEGs (*CPK32*, *CHI14*, *CTR1*, *GID1B*, *LRR1*, and *At2g23950*) and eight common regulated DEGs (*CPK32*, *GID1B*, *CXE11*, *SD25*, *LRK10*, and *PR5K*) were up-regulated in the WT-CK_vs_WT-T and *ScOPR1*-CK_vs_*ScOPR1*-T group, respectively. However, there were night common regulated DEGs consist of *CPK32*, *CPK28*, *CPK1*, *CTL1*, *STY46*, *At1g07650*, *IRK*, *CRK33*, and *LECRK1*, were down-regulated in *ScOPR1*-CK_vs_*ScOPR1*-T group (Fig. 5A, Table S7). Interestingly, the regulatory mechanisms were different in WT and *ScOPR1*-OE during the

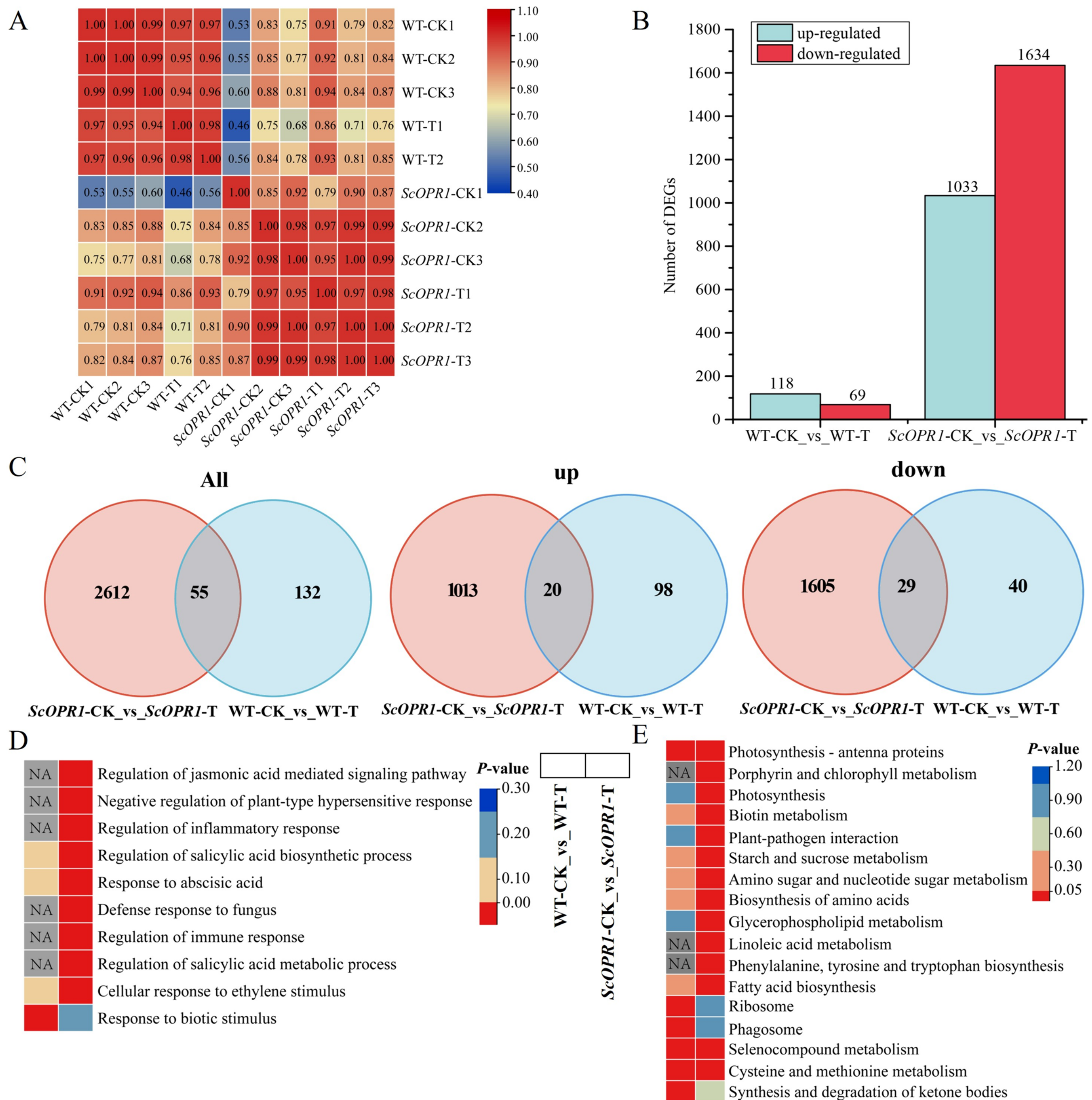


Fig. 4 Transcriptome variances between *ScOPR1*-overexpressing transgenic lines and wild-type plants during the process of disease response. **A** The correlation heat map. WT-CK, WT-T, *ScOPR1*-CK, and *ScOPR1*-T represent the wild-type *N. benthamiana* and transgenic *N. benthamiana* overexpressing *ScOPR1* after inoculation with *Fusarium solani* var. *coeruleum* for 0 d (CK) and 2 d (T), respectively. **B**, **C** The number of DEGs in WT-CK_vs_WT-T and *ScOPR1*-CK_vs_*ScOPR1*-T. **D**, **E** GO and KEGG enrichment of specific DEGs in WT-CK_vs_WT-T and *ScOPR1*-CK_vs_*ScOPR1*-T

resistance against pathogen infection. Furthermore, seven DEGs (*NPR1*, *DELLA*, *HDT1*, *CPK28*, *CTL1*, *BKI1*, and *MPK4*) involved in the MAPK signaling, plant–pathogen interaction, and plant hormone signal transduction pathways were randomly selected and verified by RT-qPCR (Fig. 5B, C). It was obvious that the relative expression trend of these seven genes was consistent with ($R^2 = 0.997$) the expression trend of $\log_2$ (fold change) in the transcriptome (Fig. 5B, C, and Fig. S2).

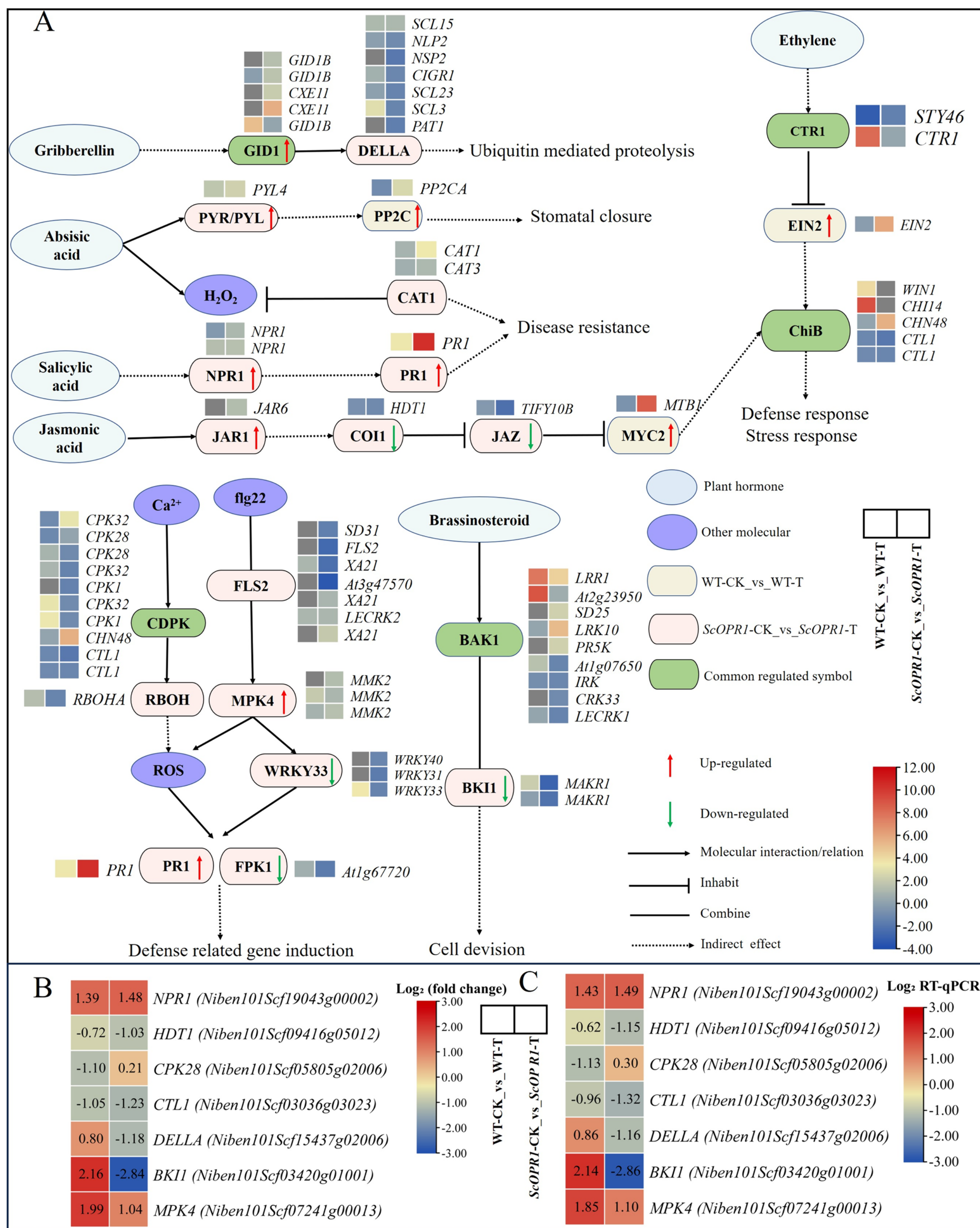


Fig. 5 Expression patterns of DEGs in disease resistance-related pathways. **A** Expression patterns of DEGs uniquely or common regulated in the *ScOPR1*-CK_vs_*ScOPR1*-T or the WT-CK_vs_WT-T group. **B**, **C** Log_2 (fold change) values and relative expression levels of seven key genes in WT and *ScOPR1* transgenic *N. benthamiana* inoculated with *F. solani* var. *coeruleum* for 2 d

Transcription factors and protein kinases played an important role in disease resistance

As reported, transcription factors (TFs) and protein kinases (PKs) played an important role in plant resistance to the pathogen (Sun et al. 2023). A total of 147 TFs and 126 PKs from the specifically regulated DEGs in *ScOPR1*-CK_vs_*ScOPR1*-T, were predicted (Fig. 6A, Tables S8, S9). These 126 PKs (45 up- and 81 down-regulated) were mainly enriched in the CAMK_CDPK, RLK-Pelle_DLSV,

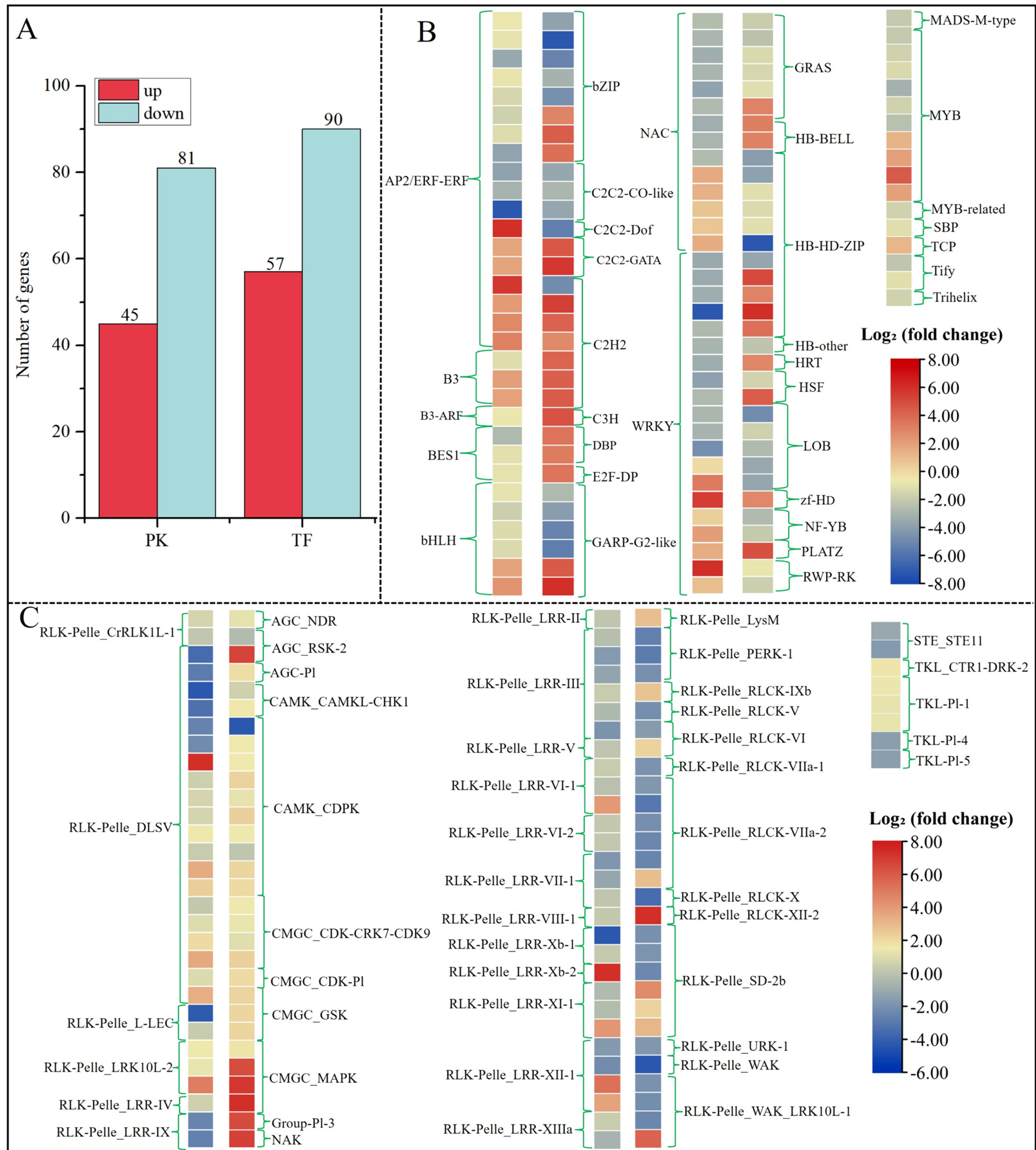


Fig. 6 Expression patterns of TFs and PKs in transgenic lines overexpressing *ScOPR1* were closely related to enhanced disease resistance. **A** The number counts of TFs and PKs. **B**, **C** Log_2 (fold change) variance of TFs and PKs in *ScOPR1*-CK_vs_*ScOPR1*-T group

RLK-Pelle_LRR-III, RLK-Pelle_LRR-XI-1, RLK-Pelle_RLCK-VIIa-2, and RLK-Pelle_SD-2b families (Fig. 6C, Table S8), with the fact that CAMK_CDPK was mainly acted on regulating plant growth and development through a series of cascading signaling processes (Harmon et al. 2001). Notably, RLK-Pelle was abundant in plants and the RLK-Pelle_DLSV, RLK-Pelle_RLCK-VIIa-2, and RLK-Pelle_SD-2b families were closely related to the plant immune system, involving plant protection from pathogen attack. Interestingly, 147 TFs (57 up- and 90 down-regulated) were closely related to ABA signaling (bZIP and NAC), JA signaling (bHLH), and ethylene (ET) signaling (AP2/ERF) pathways (Fig. 6B, Table S9).

Discussion

Till now, an increasing number of *OPR* genes have been discovered in various plants due to their significant roles in response to biotic stress (Matsui et al. 2004; Zhang et al. 2005; Nie et al. 2022). According to the results of promoter analysis, the *ScOPR1* gene was involved in plant growth and development, as well as response to both biotic and abiotic stresses. Meanwhile, the expression of *ScOPR1* gene was not only triggered by the phytohormone signaling molecules MeJA and SA but also could actively respond to *S. scitamineum* stress (Sun et al. 2018), suggesting that *ScOPR1* participated in the response to pathogen invasion in sugarcane. Similarly, two maize *OPR* genes Z*mOPR1* and *ZmOPR2*, seemed to be involved in defense mechanisms against *C. carbonum*, *C. heterostrophus*, and *F. verticillioides* (Zhang et al. 2005). Likewise, the mutation of *ZmOPR2* resulted in decreased resistance to corn smut (Zhang et al. 2005). In the present study, the temporary overexpression of *ScOPR1* increased the resistance of *N. benthamiana* to *F. solani* var. *coeruleum* and *R. solanacearum* (Fig. 2B, D) by up-regulating HR- and SA-related genes (Fig. 2C, E), indicating its positive role in plant disease resistance. Notably, this fact could also be confirmed by the stable overexpression of *ScOPR1* in transgenic *N. benthamiana* (Fig. 3).

Previous studies have found that it is important to regulate the concentration of ROS at an appropriate level for normal plant growth (Sofo et al. 2015). As an indicator of ROS, H_2O_2 can rapidly react with DAB under the catalysis of peroxidase to form brown compounds, thereby positioning H_2O_2 in plant tissues (Mittler et al. 1998). In our study, the DAB staining in the leaves of transgenic tobacco plants overexpressing *ScOPR1* was darker compared to the control when they were subjected to pathogen inoculation for 6 days (Fig. 2B, D). Furthermore, we observed an increase in ROS metabolism, including H_2O_2 accumulation, in *ScOPR1*-OE2 plants after inoculation with *F. solani* var. *coeruleum* for 2 d (Fig. 5A). When plants are attack by pathogens, those genes related to ROS scavenging systems, such as *CAT*, *GST*, and *APX*, play a crucial role in plant disease resistance (Kumar 2014; Boatwright and Pajerowska-Mukhtar 2013; Chan and Lam 2014; Zhang et al. 2016). Likewise, the contents of GST and CAT enzyme of *ScOPR1*-OE2 were significantly higher after inoculation with pathogens compared to the control (Figs. 3D, G, 5A). It can be reasonably deduced that overexpression of *ScOPR1* could activate the ROS signaling pathway during the response of plant to exogenous pathogens. Thordal-Christensen et al. (1997) speculated that ROS was involved in the HR pathway, which is a defense mechanism of plants against pathogen infection in the host-parasite incompatibility relationship. Here in our study, under pathogen stresses, the expression of HR marker genes (*NbHSR515* and *NbHSR201*) was significantly up-regulated in *ScOPR1*-OE2 plants compared to the control (Fig. 3E, H), indicating that *N. benthamiana* plants overexpressing *ScOPR1* could facilitate the occurrence of HR.

Lipid metabolism is closely related to the synthesis and transport of JA and SA, and OPR3 is a crucial enzyme in JA synthesis (Mou et al. 2019; Tani et al. 2008; Breithaupt et al. 2006). Recent studies demonstrated that plant *OPR* genes were involved in various defense signaling pathways (Zhang et al. 2005; Sun et al. 2018). In *A. thaliana*, *OPR3* mutants *ddel* and *opr3* both lacked the function of synthesizing JA (Tan et al. 2013). When stimulated by SA, JA, and ET, the expression levels of *ClOPR2* and *ClOPR4* were notably increased in watermelon (Guang et al. 2021). In cotton, *GhOPR9* was identified as a regulator of JA pathway-related gene expression during *Verticillium wilt* infection, highlighting its crucial role in cotton's resistance to *V. wilt* (Liu et al. 2020). The antagonistic relationship between SA and JA in biotrophic and hemibiotrophic pathogen resistance has been extensively documented (Kumar 2014; Boatwright and Pajerowska-Mukhtar 2013). Huang et al. (2023) discovered that SA could counteract JA by utilizing *ZmOPR2* to inhibit JA biosynthesis during plant–pathogen interactions in maize. In the present study, the expression levels of SA- and JA-related genes in *ScOPR1*-OE2 were markedly elevated compared to the control group (Fig. 3E, H). Besides, the enzyme activity assay revealed an increase in the contents of JA and SA (Fig. 3D, G). Overall, the results suggested that transgenic overexpression of *ScOPR1* could enhance resistance to external pathogen infection by up-regulating genes associated with the JA and SA pathways. However, it is still unclear why SA and JA do not act antagonistically in pathogen resistance in transgenic *ScOPR1*-OE2. It is thus hypothesized that the exact in vivo substrates and end products of OPR1 enzyme action are still unknown, warranting further research to elucidate the underlying mechanism. Nonetheless, RNA-seq results showed that DEGs related to SA signaling (*NPR1* and *PR1*) were up-regulated, and the JA pathway was also activated, as evidenced by the

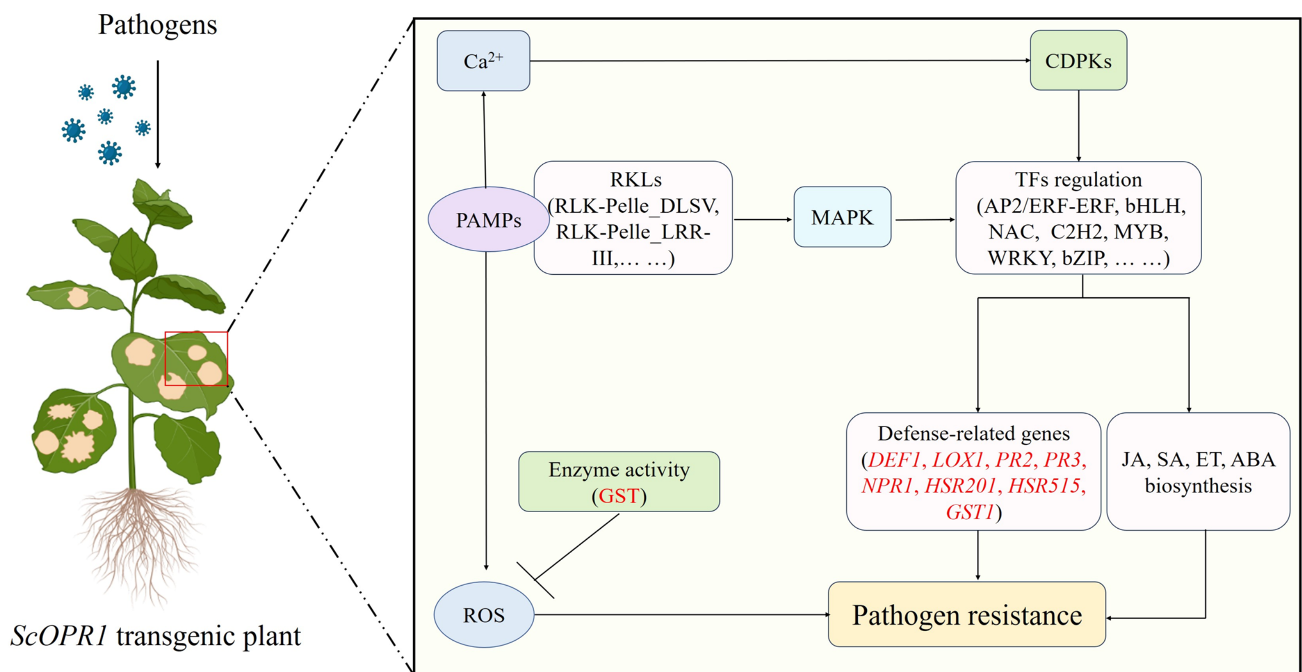


Fig. 7 A functional model of *ScOPR1* overexpression-mediated defense response of transgenic plants to pathogen infection. *PAPMs* pathogen-associated molecular proteins; *CDPKs* calcium-dependent protein kinases; *RKLs* receptor-like kinases; *MAPK* mitogen-activated protein kinase; *JA* jasmonic acid; *ABA* abscisic acid; *ROS* reactive oxygen species; *ET* ethylene; *TFs* transcription factors; *ScOPR1* overexpressing transgenic lines, respectively

down-regulation of *JAZ* and the up-regulation of both *MYC2* and *JAR1* (Fig. 5A), suggesting a synergistic relationship between the JA and SA signaling pathways.

The *OPR3* gene expression can be triggered by various stimuli, including touch, wind, wounding, UV-light, and brassinosteroids (BRs) (Schaller et al. 2000). Brassinosteroids are a type of steroid hormone that plays a significant role in plant growth, development, and response to stress (Wang et al. 2022). When plants are under stimuli, BRs bind and activate *BRI1* and *BAK1*, and the activated *BRI1* can further transmit signals by phosphorylating different substrates (Wang et al. 2022). Similarly, our transcriptome analysis confirmed that BRs participated in disease resistance by activating *BRI1* and *BAK1* (Fig. 5A). Studies have shown that flg22, a flagellin epitope and PAMP, weakens the hypersensitive cell death, resistance, and biomass reduction induced by *Pseudomonas syringae* (Pst) *AvrRpt2* in *Arabidopsis* (Wang et al. 2023a, b). It attaches to the receptor-like kinase FLS2, initiating the influx of Ca^{2+} across the plasma membrane (PM) (Chi et al. 2021). It is widely acknowledged that the FLS2 receptor and ROS burst exhibit sensitivity adaptation upon flg22 stimulation, which is referred to as desensitization and resensitization, to prevent excessive responses to pathogen infection (Chi et al. 2021). In this study, we demonstrated that flg22 bound to FLS2, resulting in the influx of Ca^{2+} into the PM. CDPK, serving as a Ca^{2+} receptor, gets activated, leading to the expression of ROS burst and disease-related factors such as *PR1*, *WRKY33*, and *FPK1*, all of which together contribute to plant resistance against pathogen infection (Fig. 5A). Recent study has shown that the ET and JA signaling pathways, along with MPK3/MPK6 signaling pathway, synergistically stimulate camalexin synthesis to enhance plant disease resistance (Zhou et al. 2022). Furthermore, we observed that following inoculation with *F. solanacearum* var. *coeruleum*, both ET and JA signals were activated and contributed to disease resistance (Fig. 5A). These results suggested that *ScOPR1* functions in enhancing plant resistance against pathogen infection by coordinating the activation of BRs, Ca^{2+}, MAPK, and ET signaling pathways.

Conclusions

By integrating phenotypic observations, DAB staining, physiological and biochemical changes, immune-related gene expression, and RNA-seq analysis, our study revealed that the *ScOPR1* overexpression in *N. benthamiana* plants post-pathogen infection facilitated the interaction between pathogen-associated molecular proteins (PAMPs) and RLK proteins, which activated the MAPK cascade signaling pathway. This activation then induced the expression of AP2/ERF-ERF, bHLH, NAC, C2H2, MYB, bZIP, and WRKY transcription factors and *DEF1*, *LOX1*, *PR2*, *NPR1*, and

GST1 defense-related genes involved in JA, SA, ET, and ABA pathways, thereby increasing the disease resistance of tobacco to pathogens. At the same time, the binding of PAMPs to RLK triggered a release of Ca^{2+} and activation of CDPKs calcium receptor proteins. Furthermore, pathogen infection resulted in the production of ROS, which to some extent induced an immune response known as HR in the plant itself, ultimately leading to increased resistance. Finally, a functional mechanism model of *ScOPR1* overexpression-mediated defense response of transgenic plants to pathogen infection was depicted (Fig. 7). This study offered valuable insights into the role of the *ScOPR1* gene in conferring pathogen resistance and highlighted its molecular mechanisms in sugarcane.

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Data availability Data will be made available on request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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