

StCCR6-Mediated Lignin Biosynthesis Enhances Potato Resistance Against *Ralstonia solanacearum*

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Abstract

Background

Bacterial wilt, caused by *Ralstonia solanacearum*, is one of the most destructive soil-borne diseases worldwide and severely threatens global potato (*Solanum tuberosum*) production. Lignin deposition in the cell wall constitutes a critical physical barrier for plant defense; however, the regulatory mechanisms governing lignin-mediated resistance in potato remain incompletely understood.

Results

Based on previously published temporal transcriptome and metabolome datasets of potato tubers treated with a low dose of deoxynivalenol (DON) across four time points (0, 4, 12, and 48 h), we dissected the defense response. Multi-omics screening revealed that the phenylpropanoid biosynthetic pathway (ko00940) was significantly enriched at 12 h, and *StCCR6* (CCR2), a cinnamoyl-CoA reductase gene, was identified as the most strongly DON-responsive node. Metabolomic profiling further showed that DON treatment drove the accumulation of the lignin monomers coniferyl alcohol and sinapyl alcohol. Functional validation using transgenic lines demonstrated that *StCCR6* overexpression thickened cell walls via accelerated lignin deposition and elevated the activities of ROS-scavenging enzymes (SOD, POD, and CAT), thereby enhancing resistance to *R. solanacearum* in a detached-leaf assay. Conversely, *StCCR6* knockout lines exhibited reduced lignin content, elevated reactive oxygen species and oxidative damage, and increased pathogen susceptibility.

Conclusion

These results establish *StCCR6* as an essential regulator that couples lignin-based physical defense with enzymatic antioxidant scavenging under low-dose DON induction, providing a valuable genetic target for engineering broad-spectrum disease resistance in crops.

1. Introduction

Potato (*Solanum tuberosum* L.) is the world's fourth most important food crop, valued for its high yield potential, environmental adaptability, and rich nutritional profile^[1–2]. It supports both global food security and diverse industrial applications, ranging from food processing to bio-materials^[3]. However, sustainable potato production is severely challenged by a multitude of biotic and abiotic stresses during cultivation, harvest, and storage^[4]. These threats trigger severe yield losses and quality degradation. In particular, the increasing frequency of disease outbreaks represents a major bottleneck for the potato industry and a critical vulnerability in global food security^[4].

Among the bacterial hazards threatening potato cultivation, bacterial wilt, caused by *Ralstonia solanacearum*, stands as a devastating vascular disease^[5, 6]. The pathogen typically invades potato roots through natural wounds formed during lateral root emergence or mechanical damage. Following initial colonization of the root cortex, *R. solanacearum* systematically migrates via the xylem vessels into aerial tissues. Inside the host xylem, the massive accumulation of bacterial extracellular polysaccharides (EPS) and proteins induces vascular blockages, disrupting fluid transport. Although host basal metabolism may temporarily buffer early-stage infection, ongoing pathogen proliferation ultimately triggers irreversible wilting and systemic mortality^[7], making bacterial wilt a catastrophic bottleneck for potato production. During infection, *Fusarium* species, the causative agents of potato dry rot, produce various secondary metabolites, notably deoxynivalenol (DON, or vomitoxin), a prominent type B trichothecene.

Emerging evidence indicates that low concentrations of such pathogen-derived toxins can act as potent elicitors or signaling molecules that trigger endogenous plant defense mechanisms and induce systemic resistance^[8–11]. This toxin-mediated priming effect is highly conserved across diverse crops. For example, sub-lethal doses of DON effectively prime defense responses in wheat and rice^[8–9], while low concentrations of *Bipolaris maydis* toxins enhance broad-spectrum adaptability and peroxidase (POD) activity in maize^[12]. Crucially, low-dose DON has been shown to suppress dry rot in potato tubers by activating key enzymes within the phenylpropanoid biosynthetic pathway^[13, 14], highlighting its potential to stimulate host structural defenses. Exogenous DON treatment has been shown to effectively restrict disease expansion in leaves by boosting host antioxidant defenses and structural reinforcement, evidenced by elevated SOD activity, decreased MDA content, and massive lignin deposition^[4, 13, 15, 16]. In potato, targeted DON priming prior to *R. solanacearum* inoculation universally promotes structural lignin accumulation and mitigates bacterial wilt severity^[13]. Given that cinnamoyl-CoA reductase (CCR) is the fundamental rate-limiting enzyme in the phenylpropanoid pathway, catalyzing the conversion of hydroxycinnamoyl-CoA esters into aromatic aldehyde precursors^[17, 18], its activity directly determines the rate and scale of cell wall lignification during stress. Therefore, CCR family members represent compelling candidates for uncovering core regulators of plant immunity. Consistently, genetic studies indicate that CCR homologs are core regulators of structural resistance across diverse plant taxa^[19–23]. While the loss of CCR function severely compromises cell wall lignification, leading to vascular abnormalities and stunted growth^[19–20, 24, 25], its overexpression enhances disease resistance^[21–22, 26, 27]. For instance, elevated CCR transcriptional levels in *O. sativa*, *A. thaliana*, and *C. moschata* prime robust physical barriers that thwart pathogen expansion^[21–22]. In resistant hosts, CCR gene expression is often rapidly induced upon pathogen challenge, showing a strong positive correlation with the activation of active immune responses^[22]. Building on this conserved role, the present study focuses on *StCCR6*, a CCR family member identified among DON-responsive potato genes, to clarify its contribution to lignified cell wall defense against *Ralstonia solanacearum*.

Beyond biotic defense, CCR genes function as multi-responsive hubs integrating various abiotic stress signaling pathways. For instance, CCR homologs from *Toona sinensis*, *Apium graveolens*, *Glycine max*, and *Oryza sativa* are strongly induced by temperature fluctuations, drought, salinity, and ultraviolet irradiation^[28–29]. Furthermore, the transcriptional regulation of CCR by exogenous phytohormones exhibits pronounced species-specific and member-specific variations, as evidenced by the antagonistic or differential responses to abscisic acid (ABA) and gibberellin^[30–31]. Concurrently, different members within the CCR family display strict transcriptional selectivity under pathogen challenge. For example, upon rust pathogen infection in switchgrass, PvCCR2 is specifically upregulated in leaves whereas PvCCR1 remains unresponsive^[32]. Similarly, CCR expression in Brassica species exhibits distinct oscillatory patterns during *Xanthomonas campestris* invasion^[33]. This pervasive functional specialization highlights the necessity of identifying specific CCR variants tailored to distinct immune responses.

In the present study, we utilized multi-omics profiling of DON-treated potato tubers to dissect the metabolic networks mediating induced resistance. By employing weighted gene co-expression network analysis (WGCNA), we successfully identified^[34] *StCCR6* as a core hub gene within the lignin biosynthetic pathway. Following genome-wide and tissue-specific transcriptional characterization of the potato CCR family, the biological function of *StCCR6* was validated through stable transgenic lines. Physiological, biochemical, and pathological assays revealed that *StCCR6* expression significantly fortifies structural defenses and antioxidant capacity, thereby conferring robust resistance to *Ralstonia solanacearum*. Overall, this work uncovers the pivotal role of *StCCR6* in potato immunity, laying a solid theoretical and practical foundation for engineering sustainable disease resistance in crops.^[35]

2. Materials and methods

2.1 Analysis of Transcriptome and Metabolome in Potato Disease Resistance Induced by Deoxynivalenol

2.1.1 Plant materials and deoxynivalenol (DON) treatment

The tubers were treated with deoxynivalenol (DON) at a concentration of 5 ng·mL⁻¹. Tuber samples were collected at 0, 4, 12, and 48 h post-treatment, designated as DON 0h, DON 4h, DON 12h, and DON 48h, respectively... All collected samples were immediately frozen in liquid nitrogen and stored at -80 °C until further analysis. Three independent biological replicates were performed for each time point.

2.1.2 Acquisition and re-analysis of transcriptomic and metabolomic datasets

The DON-treated tuber samples prepared as described in Section 2.1.1 were derived from our previously published dataset^[13] and are publicly available in the NCBI Sequence Read Archive (SRA) database

under the BioProject accession number PRJNA943451 (<https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA943451>); these data were re-analyzed here to investigate StCCR6-mediated phenylpropanoid biosynthesis. Transcriptomic sequencing and metabolomic profiling were both performed by Wuhan Metware Biotechnology Co., Ltd. (Wuhan, China).

2.1.3 Screening of Differentially Expressed Genes (DEGs) and Differentially Accumulated Metabolites (DAMs)

For transcriptome analysis, differentially expressed genes (DEGs) were identified using the DESeq2 package v1.38.0 in R, with thresholds of $|\log_2 \text{ fold change}| \geq 1$ and an adjusted P value < 0.05 . For metabolome analysis, differentially accumulated metabolites (DAMs) were screened using OPLS-DA (VIP > 1) combined with $|\log_2 \text{ fold change}| \geq 1$ and a two-tailed t-test P value < 0.05 . Hierarchical clustering analysis and visualization of the DEGs and DAMs were conducted using the pheatmap package in R to generate expression heatmaps.

2.1.4 Functional Enrichment and Annotation of Genes and Metabolites

All protein sequences in the Atlantic potato genome were functionally annotated using the online tool eggNOG (available at <http://eggnog5.embl.de/app/emapper>). GO annotation and KEGG enrichment analysis of the screened differentially expressed genes (DEGs) and differentially accumulated metabolites (DAMs) were performed with TBtools. Bar charts and enrichment bubble charts were generated using the ggplot2 package.

2.1.5 Integrated Analysis of Potato Transcriptome and Metabolome

To investigate the relationship between genes and metabolites across different DON treatment time points a weighted gene co-expression network analysis WGCNA was performed using the WGCNA package v1.71 in R v4.2.1. A gene correlation matrix was constructed based on Pearson correlation coefficients with a threshold of 0.8 and converted into a signed network. The correlation matrix was subsequently transformed into an adjacency matrix using an appropriately selected soft thresholding power. Co expression modules were constructed using the blockwiseModules function followed by module detection and the merging of highly similar modules. Module eigengenes MEs were calculated to evaluate module trait relationships by analyzing the correlation between the ME matrix and the trait matrix representing different DON treatment durations.

To identify hub genes and construct regulatory networks nodes with a Pearson correlation coefficient $R > 0.9$ and $P \text{ value} < 0.05$ were selected. The key gene networks were calculated and visualized using Cytoscape software v3.7.2. Additionally correlation analysis between specific DEGs and DAMs was performed and visualized using the ggcov package in R.

A Pearson correlation analysis was conducted between the expression of 20 StCCR family members and the contents of the two lignin monomers coniferyl alcohol and sinapyl alcohol across the eight conditions (0, 4, 12, and 48 h under CK and DON). The significance of correlations was assessed by a two-tailed t-test

A co-variation analysis was performed between phenylpropanoid pathway genes and metabolites. Genes and metabolites were assigned to nine quadrants according to the direction and significance of their $\log_2$ fold changes at each DON time point versus CK 0 h, and the proportion of gene–metabolite pairs in each quadrant was calculated.

Transcription factors (TFs) were identified from the eggNOG functional annotation (Section 2.1.4). TFs showing strong correlation with StCCR6 and the target phenylpropanoid metabolites ($|PCC| > 0.9$, $P < 0.05$) were selected to construct the network in Cytoscape.

2.2 Molecular Cloning and Bioinformatics Analysis of the StCCR6 Gene

Based on the sequence of the StCCR6 gene, gene specific cloning primers were designed using SnapGene software (Table S1). Using the synthesized cDNA as a template the coding sequence of StCCR6 was amplified via polymerase chain reaction PCR. The amplified PCR fragments were subsequently purified and ligated into the T3 cloning vector to construct the recombinant plasmid vector designated as T3-StCCR6.

2.3 Overexpression and Gene Editing of the StCCR6 Gene

2.3.1 Construction of the Overexpression Vector

The restriction enzyme sites BamHI and SmaI were selected to design specific primers for vector construction (Table S2). Using the T3-StCCR6 plasmid as a template PCR amplification was performed to obtain the target fragment containing the BamHI and SmaI restriction sites. The pBI121 expression vector was digested with the same restriction enzymes and ligated with the purified target fragment. The resulting recombinant overexpression vector was designated as pBI121-StCCR6 (Fig. S1). Subsequently the recombinant plasmid was transformed into *Agrobacterium tumefaciens* strain GV3101 using the freeze thaw method.

2.3.2 Construction of CRISPR/Cas9-Editing Vector

The CRISPR/Cas9 vector pP1C.4 was digested with restriction enzymes EcoRI and XbaI. The sgRNA cassette fragments and other dsDNA fragments for vector assembly were amplified by PCR (Fig. S3). The target sites of the sgRNAs were verified by PCR (Fig. S2). The resulting sgRNA cassette fragment was ligated into the linearized pP1C.4 vector using a homologous recombinase. The recombinant CRISPR/Cas9 product was transformed into *Escherichia coli* (DH5 α) competent cells. Positive colonies were identified via colony PCR and the target plasmids were extracted after confirmation. The extracted

plasmids were further verified by restriction digestion with XbaI (Fig. S4). Two key elements within the vector were examined by PCR using the primer pairs Hygjc2 F/R and RTCas9 F/R (Table S3). The verified recombinant knockout vector was designated as pP1C.4-StCCR6 and subsequently transformed into *Agrobacterium tumefaciens* strain GV3101 and stored at -80°C .

2.3.3 Genetic transformation, regeneration, and identification of transgenic and gene-edited lines

For potato transformation *Agrobacterium* strains harboring the overexpression or knockout vectors were activated and cultured. Pre cultured potato tuber discs were inoculated with the *Agrobacterium* suspension and then transferred to MS co cultivation medium supplemented with $1\text{ mg}\cdot\text{L}^{-1}$ 6-BA, $1.5\text{ mg}\cdot\text{L}^{-1}$ IAA, and $50\text{ }\mu\text{mol}\cdot\text{L}^{-1}$ AS for 2 days in the dark. After co cultivation the tuber discs were washed with sterile water containing $150\text{ mg}\cdot\text{L}^{-1}$ Tim. The discs were then placed on differentiation medium consisting of MS basal medium, $1.0\text{ mg}\cdot\text{L}^{-1}$ 6-BA, $1.0\text{ mg}\cdot\text{L}^{-1}$ IAA, $1.0\text{ mg}\cdot\text{L}^{-1}$ ZT, $50\text{ mg}\cdot\text{L}^{-1}$ Kan, and $400\text{ mg}\cdot\text{L}^{-1}$ Cef. Regenerated shoots reaching 1 to 2 cm in height were excised and transferred to root induction medium containing MS basal medium, $50\text{ mg}\cdot\text{L}^{-1}$ CCC, $75\text{ mg}\cdot\text{L}^{-1}$ Kan, and $250\text{ mg}\cdot\text{L}^{-1}$ Cef for rooting selection. The genetic transformation of gene edited potato lines followed the same procedure. (Fig. S5)

To verify the overexpression lines genomic DNA extracted from transgenic and wild type potato plants was used as a template for PCR amplification with GUS gene specific primers using a direct PCR kit (Table S4). The PCR products were evaluated by agarose gel electrophoresis to confirm the target bands.

For the identification of gene edited lines genomic DNA was extracted from the leaves of regenerated plantlets. PCR screening was initially performed using the primers Hygjc2 F and Hygjc2 R to detect the hygromycin phosphotransferase marker gene (Table S5; Fig. S7). To further characterize the target site mutations in the positive plants genomic DNA was amplified using the specific primers JD F and JD R (Table S3). The target PCR products were purified and cloned into the pEASY T3 vector for sequencing. The recombinant plasmids were transformed into *E. coli* and positive single colonies were verified by colony PCR and subsequently sequenced to confirm the editing efficiency.

2.3.4 Quantitative real-time PCR (qRT-PCR) analysis

Total RNA was extracted from leaves of wild-type and transgenic potato plants using a total RNA rapid extraction kit. First-strand cDNA was synthesized using a reverse transcription kit. qRT-PCR was performed with a qPCR kit on a real-time fluorescence quantitative PCR system. β -actin was used as the internal reference gene, and StCCR6-specific primers were designed with Primer-BLAST (Table S1). Relative expression levels were calculated using the $2^{-\Delta\Delta\text{Ct}}$ method, with three independent biological replicates.

2.4 Analysis of Physiological Indices in Overexpressed and Gene-Edited Plants

2.4.1 Determination of Lignin, Cellulose, and Hemicellulose Contents in Transgenic Plants

The contents of lignin cellulose and hemicellulose in the leaves of wild type and transgenic potato plants overexpressing *StCCR6* or edited via CRISPR/Cas9 were determined using specific assay kits. All biochemical assay kits were purchased from Suzhou Keming Biotechnology Co. Ltd. Suzhou China. The extraction and quantification procedures for lignin cellulose and hemicellulose were performed strictly in accordance with the manufacturers instructions.

2.4.2 Microscopic Observation of Transverse Stem Sections in Transgenic Plants

To observe cell wall ultrastructure stems from 30-day-old wild type and *StCCR6* overexpressing potato plants exhibiting uniform growth were selected as experimental materials. Segment samples with a length of 3 mm and a width of 1 mm were excised from identical positions of the aboveground stems. The collected samples were initially fixed with 2.5% (v/v) glutaraldehyde in 0.1 mol·L⁻¹ phosphate buffer pH 6.8 for 2 h. After being rinsed three times with the same phosphate buffer for 15 min each the segments were post fixed with 1% osmium tetroxide. The fixed samples were washed again three times with 0.1·mol L⁻¹ phosphate buffer for 15 min each.

Subsequently the samples were subjected to a graded ethanol series including 50%, 75%, and 90% ethanol for 15 min each followed by 100% ethanol for 20 min. The dehydrated tissues were then transferred into a mixture of 100% ethanol and 100% acetone at a 1 to 1 ratio for 15 min and then into 50% acetone for 5 min. For resin infiltration the specimens were treated sequentially with mixtures of pure acetone and embedding medium at ratios of 1 to 1, 1 to 2, and 1 to 3 with each step lasting for 1 day. The tissues were then embedded in pure resin and polymerized for 3 days.

The polymerized blocks were trimmed and sectioned using an ultramicrotome. Cellular morphology and cell wall structures were examined and observed under a HT7800 transmission electron microscope manufactured by Hitachi High Tech Corporation Beijing China operated at an accelerating voltage of 80 kV. Images were captured at varying ×2,000, ×5,000, and ×10,000. Finally the thickness of the potato stem cell walls was measured and quantified using ImageJ software.

2.4.3 Verification of Disease Resistance in Transgenic Potato Plants

A detached leaf inoculation assay was performed to evaluate the resistance of transgenic potato plants against *Ralstonia solanacearum*. Healthy disease free detached leaves from wild type, *StCCR6* overexpressing, and CRISPR/Cas9-edited potato plants were selected. The petioles of the leaves were wrapped with absorbent cotton and placed abaxial side up on filter paper moistened with sterile distilled water in Petri dishes. A 20 microliter aliquot of *R. solanacearum* bacterial suspension adjusted to an optical density at 600 nm OD₆₀₀ of 0.8 was inoculated into the leaves using a needleless syringe

allowing the bacterial suspension to infiltrate along the leaf veins. The inoculated leaves were maintained in a growth chamber under a photoperiod of 16 h light and 8 h dark. Leaf lesions were observed and recorded daily at 1–5 dpi. At 5 dpi the total lesion area of each leaf was measured and quantified using ImageJ software.

To analyze the physiological and biochemical changes during pathogen infection leaf samples were collected from the wild type, overexpression, and knockout lines at 0 and 5 dpi. The contents of hydrogen peroxide H_2O_2 , superoxide anion radical, and malondialdehyde MDA, as well as the activities of antioxidant enzymes including superoxide dismutase SOD using the WST-8 method, peroxidase POD, and catalase CAT were determined using corresponding specific biochemical assay kits. All extractions and quantifications were executed following the manufacturer's instructions. Each treatment was conducted with three independent biological replicates. Statistical analyses and data visualization were performed using GraphPad Prism 9 software.

2.5 Data Statistics

All experiments in this study were conducted with at least three independent biological replicates and technical replicates. Experimental data are presented as the means plus or minus standard deviation SD. Statistical analysis, significance testing, and data visualization were performed using Microsoft Office Excel 2019 and GraphPad Prism 9 software. Statistically significant differences were determined using appropriate statistical tests. In all figures and tables, asterisks indicate significant differences, whereas ns indicates no significant difference. Specifically, a single asterisk, double asterisks, triple asterisks, and quadruple asterisks represent P value < 0.05, P value < 0.01, P value < 0.001, and P value < 0.0001, respectively.

3. Results

3.1 The Phenylpropanoid Biosynthetic Pathway Is Remodeled at Both the Transcriptional and Metabolic Levels in Response to DON Treatment

To characterize the temporal response of phenylpropanoid metabolism in potato tubers under low-concentration DON exposure, transcriptomic and metabolomic analyses were performed on tubers treated with $5\text{ ng}\cdot\text{mL}^{-1}$ DON for 0, 4, 12, and 48 h. KEGG enrichment analysis showed that the phenylpropanoid biosynthesis pathway (ko00940) was significantly enriched only at 12 h (16 DEGs, gene ratio 0.0265, adjusted P value = 0.002; Fig. 1A) [4]. Although 92 DEGs were mapped to this pathway at 4 h, the enrichment did not reach significance after Benjamini-Hochberg correction (adjusted P = 0.133); at 48 h the pathway contained only 1 DEG and was likewise not significantly enriched (adjusted P = 1.0). Among the associated branches, isoflavonoid biosynthesis (ko00943) was also significantly enriched at 12 h (P_{adj} = 0.004), whereas flavonoid biosynthesis (ko00941) and phenylalanine metabolism (ko00360) did not reach significance at any time point, indicating that DON treatment activated a secondary metabolic network centered on the phenylpropanoid pathway, within which the lignin branch represented the most persistently responsive downstream direction.

Metabolomic analysis revealed a pronounced temporal divergence among phenylpropanoid-related metabolites (Fig. 1B). L-phenylalanine reached its peak of relative accumulation at 12 h. Coniferyl alcohol increased significantly at 12 h, with a $\log_2\text{FC}$ of 6.67 relative to 0 h, and remained at a high level at 48 h ($\log_2\text{FC} = 4.69$). The accumulation of sinapyl alcohol was relatively delayed: it remained low at 4 h, began to rise at 12 h ($\log_2\text{FC} = 2.21$), and peaked at 48 h ($\log_2\text{FC} = 2.32$). In addition, caffeic acid, vanillin, syringaldehyde, sinapic acid, sinapaldehyde, and vanillic acid increased significantly mainly at 48 h, with $\log_2\text{FC}$ values of 3.58 and 3.78 for caffeic acid and vanillin at 48 h, respectively, whereas caffeyl aldehyde and syringic acid showed single-point accumulation peaks at 4 h. The glycosylated coniferin increased at 12 h and declined at 48 h, while syringin decreased at 4 h and recovered at 12 h. These results indicate that DON treatment not only elevated the overall accumulation of lignin monomers and their upstream phenolic intermediates but also imposed a distinct temporal division of labor among different metabolites.

At the transcriptional level, genes of the phenylpropanoid biosynthetic pathway were likewise activated in a relay-like manner from the upstream to the downstream steps (Fig. 1C). At 4 h, C4H1 was rapidly up-regulated with a $\log_2\text{FC}$ of 6.76, and HCT2, PAL2, several 4CL family members, and multiple PER genes were also significantly induced at this time point. At 12 h, midstream and downstream genes were extensively up-regulated, including 4CL1-4CL3, CCR2 (StCCR6, $\log_2\text{FC} = 3.27$), CCR4 ($\log_2\text{FC} = 2.26$), CAD1-CAD4, CCoAOMT1-CCoAOMT4, PER1-PER4, and F5H1-F5H3, among which the $\log_2\text{FC}$ values of CAD3, CAD4, and PER2-PER4 all exceeded 7. At 48 h, CCR1 ($\log_2\text{FC} = 3.65$), CCR4 ($\log_2\text{FC} = 3.97$), CCoAOMT3 ($\log_2\text{FC} = 4.50$), CCoAOMT4 ($\log_2\text{FC} = 7.73$), F5H1 ($\log_2\text{FC} = 7.08$), F5H2 ($\log_2\text{FC} = 6.85$), and most CAD and PER members maintained high expression or increased further. Notably, the PAL family exhibited functional divergence: PAL1 and PAL3 were down-regulated at 12 h, whereas PAL2 and PAL4 were continuously up-regulated from 12 to 48 h. Overall, the CCR/CAD/PER genes of the G-lignin branch were expressed predominantly at 12–48 h, whereas the F5H/COMT genes of the S-lignin branch and their downstream CAD/PER genes peaked at 48 h, suggesting that DON treatment channeled the carbon flux of the phenylpropanoid pathway simultaneously toward the biosynthesis of both G- and S-type lignin monomers.

The temporal dynamics of gene expression and metabolite accumulation were highly concordant: the earlier activation of upstream C4H and HCT genes at 4 h preceded lignin monomer accumulation and supplied precursors for the subsequent bulk synthesis of downstream products; the extensive induction of 4CL, CCR, CAD, and PER at 12 h corresponded to the significant increase of coniferyl alcohol at 12 h; and the sustained high expression of CCoAOMT, F5H, and downstream oxidases at 48 h was consistent with the peaks of sinapyl alcohol, caffeic acid, and vanillin at 48 h. Together, these results demonstrate that DON treatment systemically remodeled the phenylpropanoid biosynthetic pathway of potato tubers at both the transcriptional and metabolic levels, with lignin monomer biosynthesis representing the core responsive direction.

3.2 Multi-Omics Integrated Analysis and Disease-Resistance Candidate Gene Screening in Potato Induced by DON

Multiple CCR family members in the lignin biosynthetic pathway were induced after DON treatment. To identify the members directly associated with lignin monomer accumulation, Pearson correlation analysis was performed between 20 CCR family members and the two major lignin monomers, coniferyl alcohol and sinapyl alcohol (Fig. 2A). CCR2 (StCCR6) was significantly and positively correlated with both coniferyl alcohol ($r = 0.89$, $P = 0.003$) and sinapyl alcohol ($r = 0.71$, $P = 0.047$), representing the only CCR member significantly correlated with both lignin monomers. In contrast, CCR3 (StCCR2a) was significantly correlated only with coniferyl alcohol ($r = 0.90$, $P = 0.002$), but not with sinapyl alcohol ($r = 0.58$, $P = 0.135$), whereas CCR4 (StCCR1b), CCR1, and Soltu.Atl.04_4G021710 were significantly correlated only with sinapyl alcohol ($r = 0.88$, 0.85 , and 0.89 , respectively; $P < 0.01$), with their correlations to coniferyl alcohol not reaching statistical significance. These results preliminarily suggest that StCCR6 may participate in the biosynthetic regulation of both G- and S-type lignin monomers.

To further verify the coordinated changes between genes and metabolites over the time course, a nine-quadrant co-variation analysis was performed between phenylpropanoid pathway genes and metabolites (Fig. 2B), in which the ninth quadrant (Q9) represents simultaneous significant up-regulation of both gene and metabolite. At 4 h, only 20 of the 504 gene-metabolite pairs (4.0%) fell into Q9, indicating that lignin monomer accumulation had not yet been initiated. At 12 h and 48 h, the number of Q9 pairs increased to 180 (35.7%) and 198 (39.3%), respectively, indicating that gene expression and metabolite accumulation became progressively coordinated toward concurrent up-regulation with prolonged treatment. The pairings of StCCR6 with coniferyl alcohol and sinapyl alcohol consistently fell into Q9 at both 12 h and 48 h, in agreement with the correlation analysis.

To decipher the molecular defense mechanism of the phenylpropanoid biosynthetic pathway in the potato response to DON stress at the co-expression module level, weighted gene co-expression network analysis (WGCNA) was conducted. A total of 25,126 DEGs identified from the three transcriptomic comparison groups, together with the accumulation profiles of 22 metabolites involved in the phenylpropanoid biosynthesis pathway (Table S6), were used as phenotypic traits. By selecting a soft-thresholding power of 16, a gene co-expression network was constructed, partitioning all genes into 12 distinct co-expression modules (Fig. 2C). Modules displaying a Pearson correlation coefficient (r) ≥ 0.80 and a P -value ≤ 0.01 were defined as trait-specific positively correlated modules, among which MEskyblue3, MEivory, MEsienna3, MEblue, MEsaddlebrown, MEcoral3, and MEfirebrick3 exhibited positive correlation trends with 11 of the analyzed metabolites.

Based on literature reports, syringyl (S) and guaiacyl (G) lignin monomers participate in plant disease resistance. In this study, analysis was focused on the modules correlated with coniferyl alcohol (mws0093) and sinapyl alcohol (mws0853), which serve as the primary precursors for G- and S-lignin

monomer biosynthesis and were enriched in the metabolomic analysis. Module correlation analysis showed that the mws0093-associated module contained 1,948 genes, whereas the mws0853-associated module contained 1,071 genes; these two modules shared 1,119 overlapping genes, indicating that this core gene set participates in the DON-induced immune response in potato.

Among the 1,119 overlapping genes, 57 transcription factors and 857 functionally annotated genes were identified, of which six genes were mapped to the phenylpropanoid biosynthesis pathway (ko00940). Functional annotation indicated that Soltu.Atl.05_4G018230.1, Soltu.Atl.03_3G006600.1, and Soltu.Atl.01_3G001810.1 encode peroxidases (PODs), which catalyze the polymerization of lignin monomers. Soltu.Atl.12_3G023490.1 encodes a hydroxycinnamoyltransferase (HCT), which catalyzes the transfer of hydroxycinnamoyl moieties to shikimate or quinate, providing substrates for subsequent C3H hydroxylation. Additionally, Soltu.Atl.03_4G021420.1 was identified as encoding a cinnamoyl-CoA reductase (CCR). As a rate-limiting enzyme in the lignin biosynthetic branch, CCR participates in the regulation of metabolic carbon flux, catalyzing the reduction of hydroxycinnamoyl-CoA esters, including p-coumaroyl-CoA and feruloyl-CoA, into their corresponding cinnamaldehydes, representing a key regulatory step in carbon allocation toward lignin monomer biosynthesis.

To further dissect the molecular regulatory network underlying this defense mechanism, a correlation regulatory network was constructed integrating the identified transcription factors, phenylpropanoid pathway genes, and the accumulation levels of coniferyl alcohol (mws0093) and sinapyl alcohol (mws0853) (Fig. 2D). The topological structure of the network was used to analyze the role of the lignin biosynthetic pathway in the potato defense response against stress. Collectively, these multi-omics findings indicate that the CCR gene within the phenylpropanoid pathway participates in the plant immune response, identifying it as a candidate gene, designated StCCR6, for subsequent functional characterization.

3.3 Construction, Screening and Editing Validation of StCCR6 Transgenic Lines

Briefly, for the overexpression lines, microtuber slices were infected with *Agrobacterium* harboring the pBI121-StCCR6 overexpression vector; for the knockout lines, slices were infected with *A. tumefaciens* harboring the pP1C.4-StCCR6 CRISPR/Cas9 vector. The infected slices were then transferred to tuber slice differentiation medium containing 50 mg·L⁻¹ kanamycin to induce callus and adventitious shoots; when the adventitious shoots regenerated from the callus reached 1–2 cm in length, they were subjected to a 2-week shoot elongation culture; subsequently, the elongated shoots were transferred to MS solid medium supplemented with 50 mg·L⁻¹ chlormequat chloride (CCC) and 75 mg·L⁻¹ kanamycin for rooting-based selection. Both the OE and KO lines were generated using the same transformation and selection procedure.

PCR identification of the regenerated plants was performed using GUS gene-specific primers derived from the pBI121 vector, and individuals yielding the expected amplicon were identified as positive

transformants (Fig. S6). The transcriptional level of *StCCR6* was further analyzed by qRT-PCR using leaves from WT Atlantic potato plants and the OE lines (Fig. 3A). The *StCCR6* expression level was elevated 5- to 20-fold in six independent transgenic lines (OE-1 to OE-6) relative to WT, among which OE-3 (~ 19.5-fold) and OE-4 (~ 17-fold) showed the highest expression and were selected for subsequent functional assays.

Phenotypic observation showed that OE-3 and OE-4 exhibited no significant differences from WT in plant height, stem diameter, or leaf number under culture conditions (Fig. 3B), indicating that *StCCR6* overexpression did not exert a negative effect on the basic vegetative growth of potato. In contrast, the KO lines (KO-1 and KO-2, labeled as 1# and 2#) displayed pronounced growth retardation: their plant height was significantly lower than that of WT, with thinner stems, a reduced leaf number, and an overall weaker growth vigor than WT (Fig. 3B). This phenotype is consistent with the growth retardation reported for *Arabidopsis irx4* and *AtCCR1* knockout mutants, suggesting that *StCCR6* plays an indispensable role in maintaining normal growth and development of potato.

Sequence alignment confirmed that both KO-1 and KO-2 carried a 3-bp deletion ($\Delta 3$ bp, with no other indels within the editing window) in the PAM region of target site 2, representing an in-frame deletion that resulted in the loss of one amino acid in the encoded protein (Fig. 3C).

Taken together, *StCCR6* overexpression (OE-3, OE-4) and CRISPR/Cas9 knockout (KO-1, KO-2) potato transgenic lines were successfully obtained, providing the material basis for subsequent functional validation of cell wall structure, lignin content, and disease resistance.

3.4 Overexpression of *StCCR6* in Potato Enhances Resistance to Bacterial Wilt

To investigate the biological role of *StCCR6* in potato disease resistance, functional characterization of the transgenic lines was performed. Microscopic observation of stem cross-sections via transmission electron microscopy (TEM) revealed changes in cell wall ultrastructure (Fig. 4A). Measurements showed that the cell wall thickness of the OE-3 and OE-4 overexpression lines ranged from 0.509 to 0.669 μm and 0.392 to 0.570 μm , with average thicknesses of 0.617 μm and 0.473 μm , respectively. These values were higher than the average cell wall thickness of the wild-type (WT) plants, which was 0.273 μm ranging from 0.186 to 0.337 μm (Fig. 4A). Under 2000x and 5000x magnification fields, the cell wall thickening in the OE lines was not uniform across all observed regions. Conversely, the cell wall thickness in areas of the KO knockout lines was narrower than that of the WT, with average thicknesses of 0.141 μm for KO-1 and 0.185 μm for KO-2. These ultrastructural findings indicated that overexpressing *StCCR6* reinforces the physical cell wall barrier, whereas knocking it out compromises cell wall integrity.

Quantification of the total lignin content in the transgenic potato lines supported the TEM observations (Fig. 4B). Compared with the WT plants, the total lignin content in the OE-3 and OE-4 lines increased by 48.83% and 64.22%, respectively. In contrast, the lignin content in the KO lines decreased to 0.48-fold

and 0.53-fold of that observed in the WT. Concurrently, the contents of cellulose and hemicellulose were determined across the WT, OE, and KO lines. No differences in cellulose and hemicellulose accumulation were observed between the WT and OE lines; however, the contents of both components were lower in the KO lines than in the WT plants.

To evaluate disease resistance, detached leaves from the transgenic and WT plants were inoculated with *Ralstonia solanacearum* (Fig. 4C). At 1 day post-inoculation (dpi), no disease symptoms were observed across genotypes except for KO-1. By 2 dpi, disease symptoms emerged in lines except for the overexpression lines. From 2 to 4 dpi, disease lesions expanded over time across all genotypes. The lesion areas of the OE lines remained smaller than those of the WT, whereas the KO-1 line exhibited larger lesion areas than the WT, and the KO-2 line showed no apparent difference. At 5 dpi, the average lesion areas of the OE-3 and OE-4 leaves were 0.22-fold and 0.56-fold of the WT average lesion area, which was 1.304 cm². Conversely, the average lesion areas of the KO-1 and KO-2 leaves were 3.29-fold and 2.57-fold of that of the WT. These results indicated that *StCCR6* overexpression reduces plant susceptibility to the pathogen, whereas its deficiency increases susceptibility, leading to severe leaf damage.

Physiological and biochemical responses associated with reactive oxygen species (ROS) homeostasis and antioxidant capacity were analyzed in the leaves at 5 days under *R. solanacearum* stress (Fig. 4D). Prior to inoculation, the accumulation of H₂O₂ and superoxide anion radicals showed no differences between the transgenic lines and the WT, with the exception of an anomalous POD activity pattern in the KO-2 line. The baseline content of malondialdehyde (MDA), an indicator of lipid peroxidation and cellular oxidative damage, was lower in the OE lines than in the other genotypes. Additionally, the KO lines displayed lower baseline SOD activity than the WT, the OE-3 line exhibited higher CAT activity than the WT, and the remaining antioxidant enzyme activities did not differ from the WT.

Following pathogen inoculation, the accumulation of H₂O₂ and superoxide anion radicals increased in the KO lines but remained relatively stable with minimal changes in the OE lines. Concurrently, MDA accumulation did not increase in the OE lines; specifically, MDA contents in the OE-3 and OE-4 lines were 41.17% and 35.11% lower than that in the WT, respectively. In contrast, MDA levels in the WT, KO-1, and KO-2 lines rose after infection, with KO-1 and KO-2 reaching 2.41-fold and 2.02-fold of their baseline levels. Although the activities of SOD, POD, and CAT increased across genotypes upon infection, the induction was higher in the OE lines than in the WT and KO lines. The WT plants maintained higher enzyme activities than the KO lines, which exhibited the lowest antioxidant response. For instance, CAT activity in the KO lines increased by 48.05% and 101.42%, which was lower than the 2.3-fold increase in the WT and the 3.02-fold increase in the OE lines. Similar induction trends were observed for SOD and POD activities in the KO lines. Taken together, these findings indicate that under biotic stress, *StCCR6* overexpression suppresses the accumulation of peroxides such as MDA and enhances antioxidant enzyme performance to facilitate defense against pathogen invasion, whereas knocking out *StCCR6* attenuates enzyme activation and compromises defense adaptability.

4. Discussion

By integrating the temporal transcriptome, metabolome, and transgenic validation described above, we propose a working model for low-dose DON-induced resistance of potato against *Ralstonia solanacearum* (Fig. 5). In this model, low-dose DON up-regulates the phenylpropanoid biosynthetic pathway, with *StCCR6* (CCR2) serving as a central regulatory node that drives the accumulation of the lignin monomers coniferyl alcohol and sinapyl alcohol. The monolignols are deposited into the cell wall, where polymerization into lignin reinforces the physical barrier and restricts pathogen invasion. Meanwhile, the up-regulated *StCCR6* concurrently enhances the activities of the ROS-scavenging enzymes SOD, POD, and CAT, which reduces the accumulation of reactive oxygen species and MDA and alleviates oxidative damage.

Integrated multi-omics analysis connects transcriptome-wide changes with metabolic phenotypes and helps explain plant defense. Joint transcriptomic and metabolomic profiling shows which biosynthetic and catabolic pathways are active under stress. Co-expression networks between differentially expressed genes and metabolites point to specific gene–metabolite pairs that may govern these pathways^[36]. This approach has since been applied to several plant-defense systems. In tea plants, DEGs enriched in phenylpropanoid and flavonoid biosynthesis are linked to resistance against fungal blight^[37]. Under cadmium stress, combined transcriptome–metabolome co-expression analysis tied phenylpropanoid genes (4CL, CHI) to flavonoid accumulation^[38]. In that system, 4CL converts p-coumaric acid into its CoA ester while CHI carries out the isomerization step, together directing flavonoid flux. Pathway enrichment further indicates that the MYB-bHLH-WD40 complex activates downstream structural genes such as CHS, raising total flavonoid content. The same network logic has also singled out flavonoids as defensive metabolites in *Populus tremula* against insect attack^[39].

Dynamic modifications of cell wall architecture constitute a fundamental cornerstone for plants to withstand biotic infestations and establish passive physical barriers^[40]. As a structural component of secondary cell walls, lignin biosynthesis and deposition exert multifaceted physiological roles; this aromatic polymer cross-links cellulose microfibrils to form a three-dimensional network that reinforces cell wall mechanical strength and stabilizes host membrane systems by suppressing lipid peroxidation and reducing MDA accumulation under stress conditions^[41–42]. Cinnamoyl-CoA reductase (CCR) is the first enzyme committed to the lignin monomer-specific pathway, thereby controlling the carbon flux directed into lignin biosynthesis^[17]. Previous genetic studies indicated that CCR deficient Arabidopsis mutants *irx4*, *AtCCR1* knockout lines, and transgenic tobacco lines with compromised CCR functions displayed severe blockages in lignin synthesis, compromised secondary cell wall structural integrity, disrupted vascular development, and distinct growth retardation^[43–44]. Conversely, the expression of rice *OsCCR* accelerates the synthesis of monolignols to mitigate pathogen-mediated damage^[21]. In our study, transmission electron microscopy observations revealed that the cell walls in localized regions of *StCCR6*-overexpressing potato plants were thickened and displayed a compact structural matrix. Physiological quantification further substantiated that the overexpression lines exhibited an elevation in

both lignin and cellulose contents, whereas hemicellulose levels remained largely unchanged. These results indicate that *StCCR6* modulates a structural remodeling of the potato cell wall by driving the biosynthetic flux toward lignin and cellulose, thereby reinforcing the physical barrier to impede subsequent pathogen penetration.

To validate the efficacy of *StCCR6*-mediated cell wall reinforcement against pathogen infection, a detached leaf assay was performed to evaluate disease resistance in potato lines inoculated with the bacterial wilt pathogen *Ralstonia solanacearum*. The *StCCR6*-overexpressing plants exhibited enhanced disease resistance, characterized by a prolonged incubation period before symptom onset and restricted lesion areas compared with the wild-type control. This phenotype confirms that the localized cell wall thickening and optimized tissue compactness observed via electron microscopy diminished the colonization and expansion capabilities of *R. solanacearum* within host foliar tissues. More importantly, physiological profiling post-inoculation demonstrated that the activities of antioxidant enzymes, including SOD, POD, and CAT, were elevated in the overexpression lines, which was tightly coupled with the scavenging of hydrogen peroxide and reactive oxygen species, thereby mitigating the oxidative stress damage triggered by the pathogen. This dual cascade of physical isolation and physiological antioxidant defense cooperatively constitutes the integrated defense network (Fig. 5).

5. Conclusions

In this study, an integrated transcriptomic and metabolomic approach was employed to identify *StCCR6*, and its biological functions were characterized through overexpression and CRISPR/Cas9-mediated knockout strategies. The multi-omics results showed that phenolic acids, flavonoids, and phenylpropanoids constitute the primary differentially abundant metabolites in potato tubers in response to DON induction. The differentially expressed genes were enriched in the phenylpropanoid biosynthetic pathway, with the lignin biosynthetic branch playing a role, within which *StCCR6* encodes an enzyme in the pathway. Transgenic functional validation revealed that compared with the wild-type (WT) plants, *StCCR6* overexpression lines exhibited increased cell wall thickness, elevated total lignin content, reduced leaf lesion areas upon pathogen infection, enhanced antioxidant enzyme activities, and suppressed reactive oxygen species (ROS) accumulation, leading to enhanced disease resistance. Conversely, knocking out *StCCR6* resulted in opposite phenotypes, compromising both structural and physiological defenses. Taken together, these findings indicate that overexpressing *StCCR6* reinforces the physical cell wall barrier and coordinates physiological homeostasis to enhance disease resistance in potato, whereas its deficiency impairs plant immunity. This study provides information on the molecular network governing DON-induced immunity and provides a theoretical foundation and candidate gene resources for future potato disease control and resistance breeding.

Declarations

Author contributions Yujie Han: Writing-original draft, Writing-review & editing, Conceptualization, Data curation, Formal analysis. Yaling Liu: Data curation, Formal analysis, Visualization. Pingping Sun: Investigation, Validation. Chong Du: Investigation, Resources. Fumeng He: Validation, Visualization. Jiaqi Wang: Investigation. Linlin Wang: Resources. Xinqi Zhang: Data curation. Yingnan Wang*: Conceptualization, Writing-review & editing, Supervision. Fenglan Li*: Conceptualization, Writing-review & editing, Funding acquisition, Supervision.

Data Availability Statement: The RNA-seq datasets in this study are available from the NCBI Sequence Read Archive under project PRJNA943451.

Declaration of competing interest

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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References

1. Anders S, Cowling W, Pareek A, Gupta KJ, Singla-Pareek SL, Foyer CH. Gaining acceptance of novel plant breeding technologies. *Trends Plant Sci.* 2021;26:575–87.
2. Solvik BS, Strand TA. Biotin: a scoping review for Nordic Nutrition Recommendations 2023. *Food Nutr Res* 68 (2024).
3. Liu T, Song C, Wang D. Study on the production of alum-free potato noodles. *Sci Technol Food Ind.* 2013;34:262–5.
4. Qi T, He F, Zhang X, Wang J, Zhang Z, Jiang H, Zhao B, Du C, Che Y, Feng X, Wang Y, Li F. Genome-wide identification and expression profiling of potato (*Solanum tuberosum* L.) universal stress proteins reveal essential roles in mechanical damage and deoxynivalenol stress. *Int J Mol Sci.* 2024;25:1341.
5. Yuan R. Common diseases of potato and their control methods. *Agricultural Science-Technology Inform* (2024) 118–21.
6. Jose J, Éva C, Bozsó Z, Hamow KÁ, Fekete Z, Fábián A, Bánfalvi Z, Sági L. Global transcriptome and targeted metabolite analyses of roots reveal different defence mechanisms against *Ralstonia solanacearum* infection in two resistant potato cultivars. *Front Plant Sci.* 2023;13:1065419.

7. Yang Y, Hou L, Mo J, Li J, Zhou B, Jiang X, Fan D, Zhang Y. Occurrence and control of potato bacterial wilt. *South Agric*. 2018;12:17–9.
8. Meneely J, Greer B, Kolawole O, Elliott C. T-2 and HT-2 toxins: toxicity, occurrence and analysis: a review. *Toxins (Basel)*. 2023;15:481.
9. Ma C, Zhai C, Shang C, Jia Y. Induction of peroxidase activity in maize leaves by low-concentration toxin filtrate of *Bipolaris maydis*. *Chin J Plant Pathol* (2008) 504–8.
10. Xue H, Bi Y, Wei J, Tang Y, Zhao Y, Wang Y. New method for the simultaneous analysis of types A and B trichothecenes by ultrahigh-performance liquid chromatography coupled with tandem mass spectrometry in potato tubers inoculated with *Fusarium sulphureum*. *J Agric Food Chem*. 2013;61:9333–8.
11. Dixon R. Molecular communication in interactions between plants and microbial pathogens. *Annu Rev Plant Physiol Plant Mol Biol*. 1990;41:339–67.
12. Ma C, Zhai C, Wang L, Chen X, Li Y, Guo X, Cui S, Li G. Induced resistance by the toxin filtrate of *Bipolaris maydis* race T cultivation. *Agricultural Sci China*. 2006;5:678–84.
13. Zhao B, Yu H, Liu D, Wang J, Feng X, He F, Qi T, Du C, Wang L, Wang H, Li F. Combined transcriptome and metabolome analysis reveals adaptive defense responses to DON induction in potato. *Int J Mol Sci*. 2023;24:8054.
14. Dong N-Q, Lin H-X. Contribution of phenylpropanoid metabolism to plant development and plant-environment interactions. *J Integr Plant Biol*. 2021;63:180–209.
15. Vance CP, Kirk TK, Sherwood RT. Lignification as a mechanism of disease resistance. *Annu Rev Phytopathol*. 1980;18:259–88.
16. Doppler M, Kluger B, Bueschl C, Steiner B, Buerstmayr H, Lemmens M, Krska R, Adam G, Schuhmacher R. Stable isotope-assisted plant metabolomics: investigation of phenylalanine-related metabolic response in wheat upon treatment with the deoxynivalenol virulence factor. *Front Plant Sci*. 2019;10:1137.
17. Li X, Bonawitz ND, Weng JK, Chapple C. The growth reduction associated with repressed lignin biosynthesis in *Arabidopsis thaliana* is independent of flavonoids. *Plant Cell*. 2010;22:1620–32.
18. Lacombe E, Hawkins S, Van Doorselaere J, Piquemal J, Goffner D, Poeydomenge O, Boudet A-M, Grima-Pettenati J. Cinnamoyl CoA reductase, the first committed enzyme of the lignin branch biosynthetic pathway: cloning, expression and phylogenetic relationships. *Plant J*. 1997;11:429–41.
19. Zhou R, Jackson L, Shadle G, Nakashima J, Temple S, Chen F, Dixon RA. Distinct cinnamoyl CoA reductases involved in parallel routes to lignin in *Medicago truncatula*. *Proc Natl Acad Sci U S A*. 2010;107:17803–8.
20. Wadenbäck J, von Arnold S, Egertsdotter U, Walter MH, Grima-Pettenati J, Goffner D, Gellerstedt G, Gullion T, Clapham D. Lignin biosynthesis in transgenic Norway spruce plants harboring an antisense construct for cinnamoyl CoA reductase (CCR). *Transgenic Res*. 2008;17:379–92.
21. Kawasaki T, Koita H, Nakatsubo T, Hasegawa K, Wakabayashi K, Takahashi H, Umemura K, Umezawa T, Shimamoto K. Cinnamoyl-CoA reductase, a key enzyme in lignin biosynthesis, is an effector of

- small GTPase Rac in defense signaling in rice. *Proc Natl Acad Sci U S A*. 2006;103:230–5.
22. Chen G. Correlation analysis and functional study of CCR gene with seed coat development and powdery mildew resistance in *Cucurbita pepo*. Gansu Agricultural University; 2013.
23. van der Rest B, Danoun S, Boudet A-M, Rochange SF. Down-regulation of cinnamoyl-CoA reductase in tomato (*Solanum lycopersicum* L.) induces dramatic changes in soluble phenolic pools. *J Exp Bot*. 2006;57:1399–411.
24. Piquemal J, Lapierre C, Myton K, O'Connell A, Schuch W, Grima-Pettenati J, Boudet A-M. Down-regulation of cinnamoyl-CoA reductase induces significant changes of lignin profiles in transgenic tobacco plants. *Plant J*. 1998;13:71–83.
25. Tamasloukht B, Wong Quai Lam MS-J, Martinez Y, Tozo K, Barbier O, Jourda C, Jauneau A, Borderies G, Balzergue S, Renou J-P, Huguet S, Martinant JP, Tatout C, Lapierre C, Barrière Y, Goffner D, Pichon M. Characterization of a cinnamoyl-CoA reductase 1 (CCR1) mutant in maize: effects on lignification, fibre development, and global gene expression. *J Exp Bot*. 2011;62:3837–48.
26. Liu D, Wu J, Lin L, Li P, Li S, Wang Y, Li J, Sun Q, Liang J, Wang Y. Overexpression of cinnamoyl-CoA reductase 2 in *Brassica napus* increases resistance to *Sclerotinia sclerotiorum* by affecting lignin biosynthesis. *Front Plant Sci*. 2021;12:732733.
27. Zhang W, Wei R, Chen S, Jiang J, Li H, Huang H, Yang G, Wang S, Wei H, Liu G. Functional characterization of CCR in birch (*Betula platyphylla* × *Betula pendula*) through overexpression and suppression analysis. *Physiol Plant*. 2015;154:283–96.
28. Sui J, Sun J, Yang J, Liu P, Ning W, Song Y, Qu C. Cloning and expression analysis of TsCCR gene from *Toona sinensis*. *Mol Plant Breed* 16 (2018).
29. So HA, Chung E, Cho CW, Kim KY, Lee JH. Molecular cloning and characterization of soybean cinnamoyl CoA reductase induced by abiotic stresses. *Plant Pathol J*. 2010;26:380–5.
30. Liu C, Yu H, Rao X, Li L, Dixon RA. Absciscic acid regulates secondary cell-wall formation and lignin deposition in *Arabidopsis thaliana* through phosphorylation of NST1. *Proc Natl Acad Sci U S A*. 2021;118:e2010911118.
31. Hu Y, Di P, Chen J, Xiao Y, Zhang L, Chen W. Isolation and characterization of a gene encoding cinnamoyl-CoA reductase from *Isatis indigotica* Fort. *Mol Biol Rep*. 2011;38:2075–83.
32. Escamilla-Treviño LL, Shen H, Uppalapati SR, Ray T, Tang Y, Hernandez T, Yin Y, Xu Y, Dixon RA. Switchgrass (*Panicum virgatum*) possesses a divergent family of cinnamoyl CoA reductases with distinct biochemical properties. *New Phytol*. 2010;185:143–55.
33. Chen C, Wang Z, Cao X, Wang Z. Cloning and bioinformatics analysis of cinnamoyl-CoA reductase gene from *Salvia miltiorrhiza*. *Acta Bot Boreali-Occidentalia Sinica*. 2011;31:1963–8.
34. Chen W, Gong L, Guo Z, Wang W, Zhang H, Liu X, Yu S, Xiong L, Luo J. A novel integrated method for large-scale detection, identification, and quantification of widely targeted metabolites: application in the study of rice metabolomics. *Mol Plant*. 2013;6:1769–80.
35. Nahirňak V, Almasia NI, González MN, Massa GA, Décima CA, Oneto SE, Feingold HE, Hopp C. Vazquez Rovere, State of the art of genetic engineering in potato: from the first report to its future

- potential. *Front Plant Sci.* 2021;12:768233.
36. Wu M, Northen TR, Ding Y. Stressing the importance of plant specialized metabolites: omics-based approaches for discovering specialized metabolism in plant stress responses. *Front Plant Sci.* 2023;14:1272363.
37. Zheng S, Du Z, Wang X, Zheng C, Wang Z, Yu X. Metabolic rewiring in tea plants in response to gray blight disease unveiled by multi-omics analysis. *Metabolites.* 2023;13:1122.
38. Jiao Z, Shi Y, Wang J, Wang Z, Zhang X, Jia X, Du Q, Niu J, Liu B, Du R, Ji G, Cao J, Lv P. Integration of transcriptome and metabolome analyses reveals sorghum roots responding to cadmium stress through regulation of the flavonoid biosynthesis pathway. *Front Plant Sci.* 2023;14:1144265.
39. Pastierovič F, Mogilicherla K, Hradecký J, Kalyniukova A, Dvořák O, Roy A, Tomášková I. Genome-wide transcriptomic and metabolomic analyses unveiling the defence mechanisms of *Populus tremula* against sucking and chewing insect herbivores. *Int J Mol Sci.* 2024;25:6124.
40. Bacete L, Mélida H, Miedes E, Molina A. Plant cell wall-mediated immunity: cell wall changes trigger disease resistance responses. *Plant J.* 2018;93:614–36.
41. Vanholme R, Demedts B, Morreel K, Ralph J, Boerjan W. Lignin biosynthesis and structure. *Plant Physiol.* 2010;153:895–905.
42. Dixon RA, Barros J. Lignin biosynthesis: old roads revisited and new roads explored. *Open Biol.* 2019;9:190215.
43. Laskar DD, Jourdes M, Patten AM, Helms GL, Davin LB, Lewis NG. The *Arabidopsis* cinnamoyl CoA reductase *irx4* mutant has a delayed but coherent (normal) program of lignification. *Plant J.* 2006;48:674–86.
44. Baucher M, Halpin C, Petit-Conil M, Boerjan W. Lignin: genetic engineering and impact on pulping. *Crit Rev Biochem Mol Biol.* 2003;38:305–50.

Figures

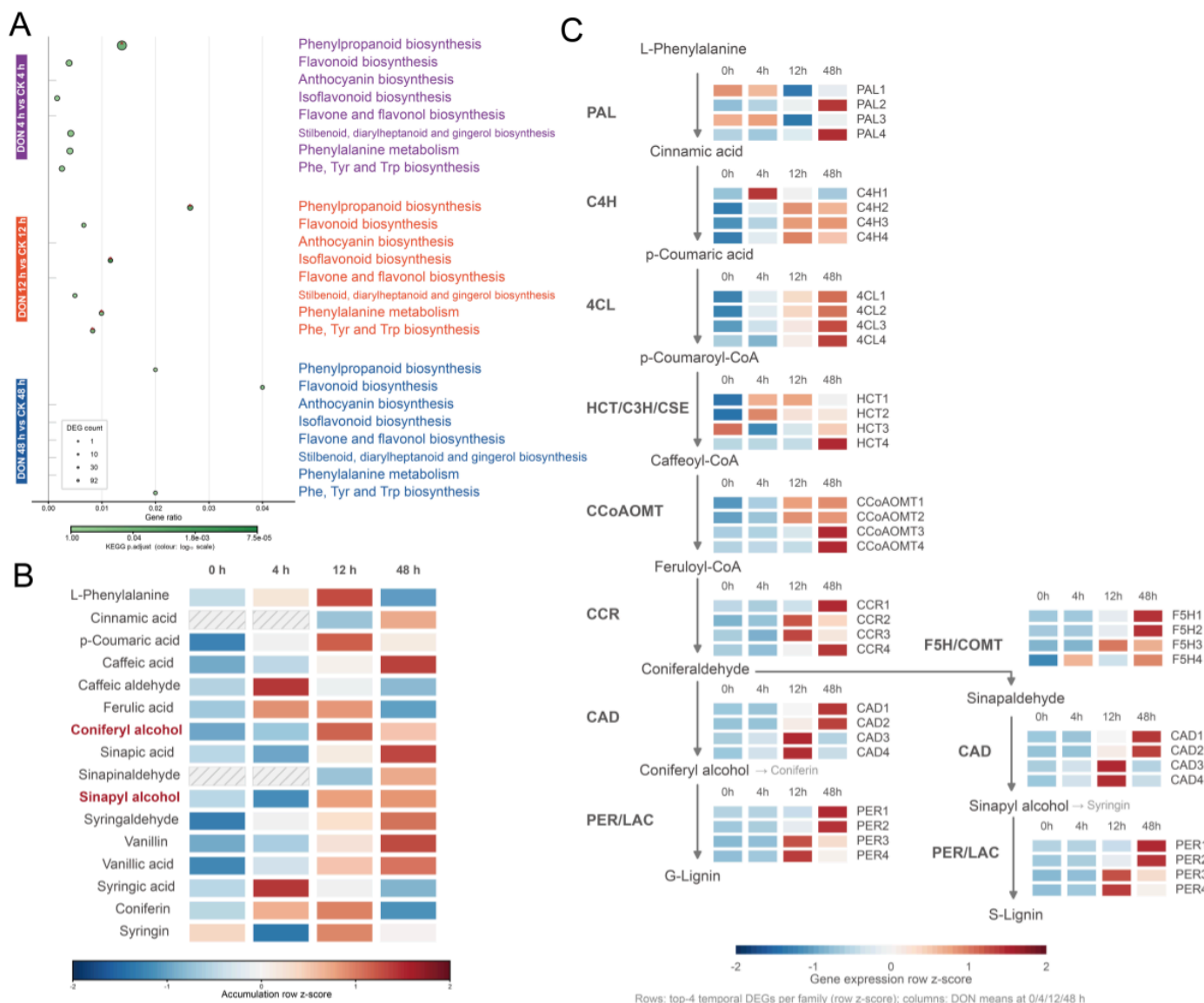


Figure 1

DON treatment reprograms the phenylpropanoid biosynthetic pathway at both the transcriptional and metabolic levels in potato tubers. (A) KEGG pathway enrichment of differentially expressed genes (DEGs) in the DON 4 h, 12 h, and 48 h comparison groups. The phenylpropanoid biosynthetic pathway (ko00940) was significantly enriched only at 12 h ($P_{adj} = 0.002$), with no significant enrichment at 4 h ($P_{adj} = 0.133$) or 48 h ($P_{adj} = 1.0$). Circle size represents DEG count; the x-axis shows the gene ratio. Significance was determined by hypergeometric test with Benjamini–Hochberg correction ($P_{adj} < 0.05$). (B) Accumulation patterns of 16 phenylpropanoid pathway metabolites in DON-treated tubers across 0, 4, 12, and 48 h, shown as z-scores of relative peak areas. Coniferyl alcohol and sinapyl alcohol (highlighted in red) are the primary G- and S-lignin monomer precursors, respectively. (C) Expression patterns of phenylpropanoid pathway genes arranged along the canonical biosynthetic route, shown as z-scores of TPM values.

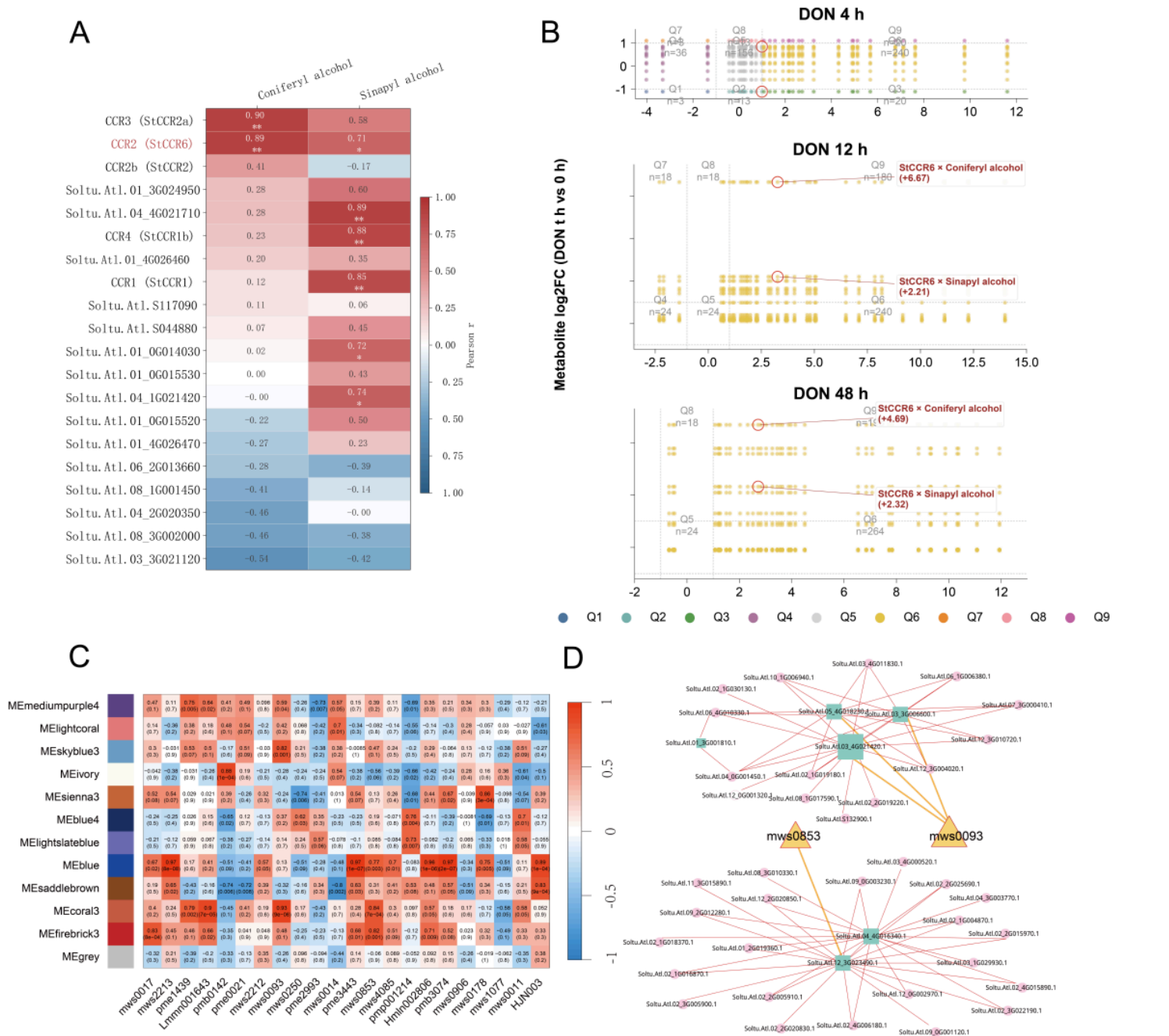


Figure 2

StCCR6 was pinpointed as the pivotal gene connecting DON-triggered transcriptional reprogramming with lignin monomer biosynthesis. (A) A Pearson correlation analysis was performed between the expression of the 20 StCCR family members and the contents of the two lignin monomers (coniferyl alcohol and sinapyl alcohol) at four sampling time points (0, 4, 12, and 48 h) under both CK and DON treatments. StCCR6 (CCR2) is the only CCR member significantly correlated with both G- and S-lignin monomer precursors. Significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (two-tailed t-test). (B) Nine-quadrant co-variation analysis of phenylpropanoid pathway genes and metabolites at DON 4 h, 12 h, and 48 h versus CK 0 h. Quadrant 9 (Q9; both gene and metabolite significantly upregulated) increased from

4.0% at 4 h to 35.7% at 12 h and 39.3% at 48 h. StCCR6 paired with both coniferyl alcohol and sinapyl alcohol was located in Q9 at 12 h and 48 h (log2FC values indicated). (C) WGCNA module–trait relationships. Twelve co-expression modules were identified from 25,126 DEGs and correlated with 22 phenylpropanoid pathway metabolites. Cell values are Pearson correlation coefficients with Padj in parentheses. (D) Transcription factor–CCR–metabolite co-expression network. StCCR6 occupies a central position and is directly connected to coniferyl alcohol (mws0093) and sinapyl alcohol (mws0853); a parallel HCT-centered subnetwork (Soltu.Atl.12_3G023490.1) additionally connects to sinapyl alcohol.

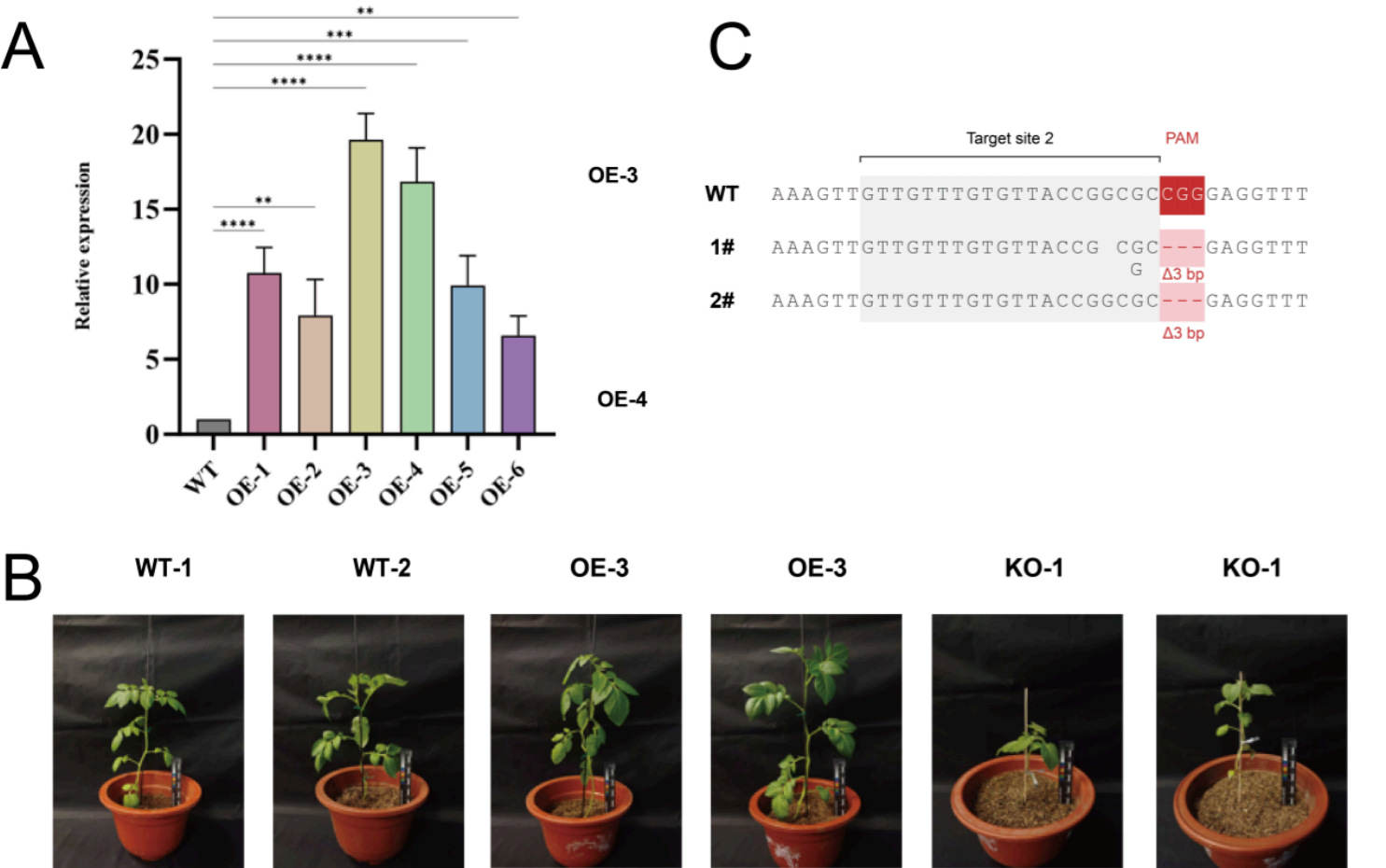


Figure 3

StCCR6 overexpression and CRISPR/Cas9 knockout lines were generated and verified.(A) Relative StCCR6 expression in WT and six independent overexpression lines (OE-1 to OE-6) determined by qRT-PCR. OE-3 and OE-4, showing the highest expression, were selected for downstream functional analyses. (B) Representative phenotypes of wild-type (WT), StCCR6-overexpressing (OE-3, OE-4), and StCCR6-knockout (KO-1, KO-2) plants at four weeks after transplanting. Both KO lines displayed a marked dwarf phenotype, whereas both OE lines were morphologically similar to WT. (C) Sanger sequencing alignment of the StCCR6 CRISPR/Cas9 target site 2 region. Both edited lines (1# and 2#, corresponding to KO-1

and KO-2, respectively) carry a 3-bp deletion spanning the PAM (CGG) of the sgRNA target site, resulting in an in-frame deletion of one codon.

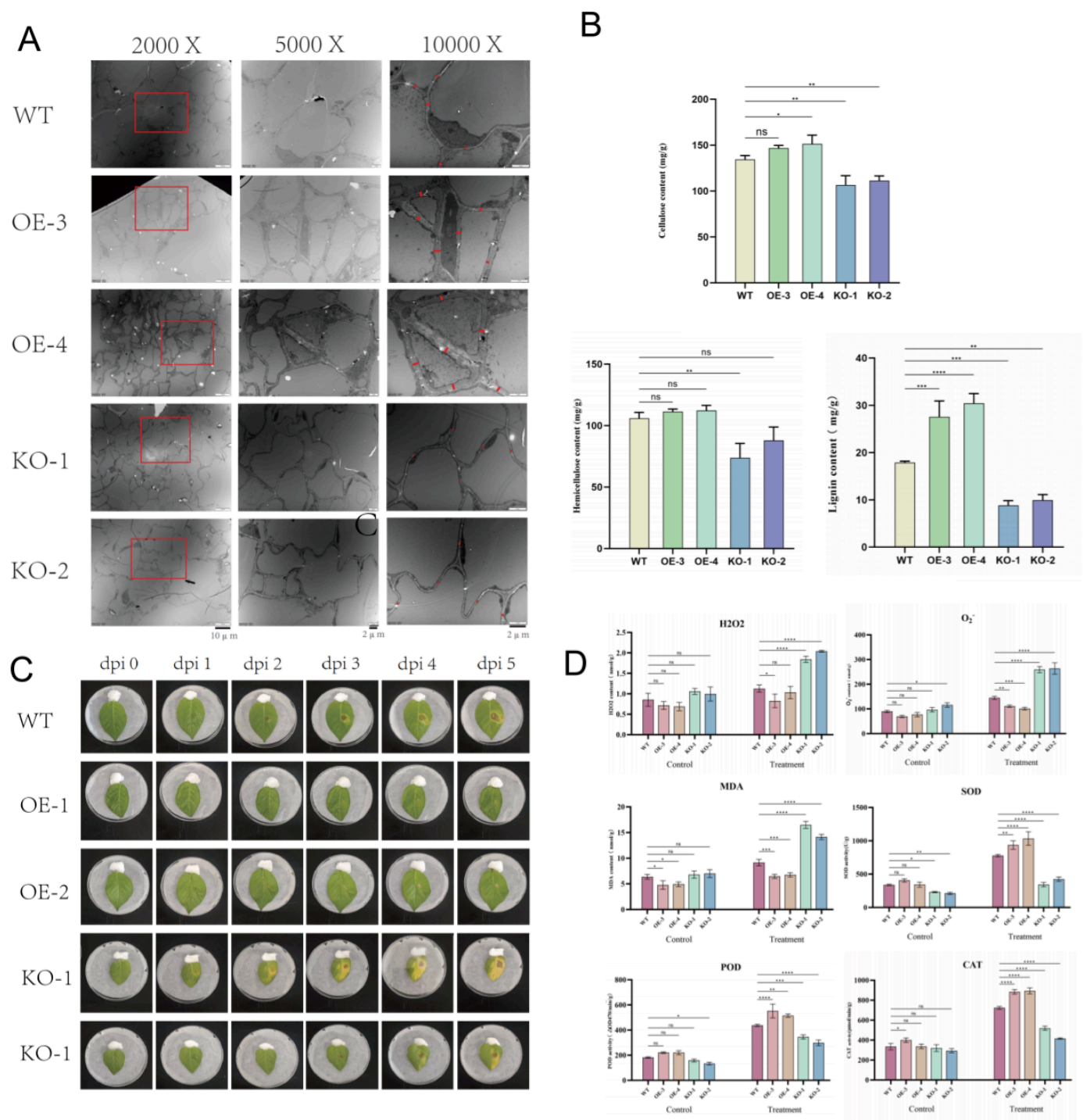


Figure 4

StCCR6 positively regulates lignin-mediated resistance to bacterial wilt in potato. (A) Transmission electron microscopy (TEM) of tuber cell walls in WT, OE (OE-3, OE-4), and KO (KO-1, KO-2) lines at 2,000 $\times$, 5,000 $\times$, and 10,000 $\times$ magnification. The boxed regions in the 2,000 $\times$ panels mark the areas enlarged in the 5,000 $\times$ and 10,000 $\times$ views. Red arrows indicate cell wall layers. Scale bars, 10 μ m (2,000 $\times$) and 2 μ m (5,000 $\times$ and 10,000 $\times$). (B) Cellulose, hemicellulose, and lignin contents in WT, OE, and KO lines. Data are

means $\pm$ SD (n = 3). Significance was determined by one-way ANOVA with Tukey's multiple-comparison test; *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; ns, not significant. (C) Disease progression in detached leaves of WT, OE, and KO lines following inoculation with *Ralstonia solanacearum*. Photographs were taken daily from 0 to 5 days post-inoculation (dpi). (D) Quantification of H_2O_2 , O_2^{2-} , MDA, SOD, POD, and CAT under control and pathogen-inoculated conditions across the five genotypes. Data are means $\pm$ SD (n = 3). Significance was determined by two-way ANOVA with Tukey's test; asterisks as in (B).

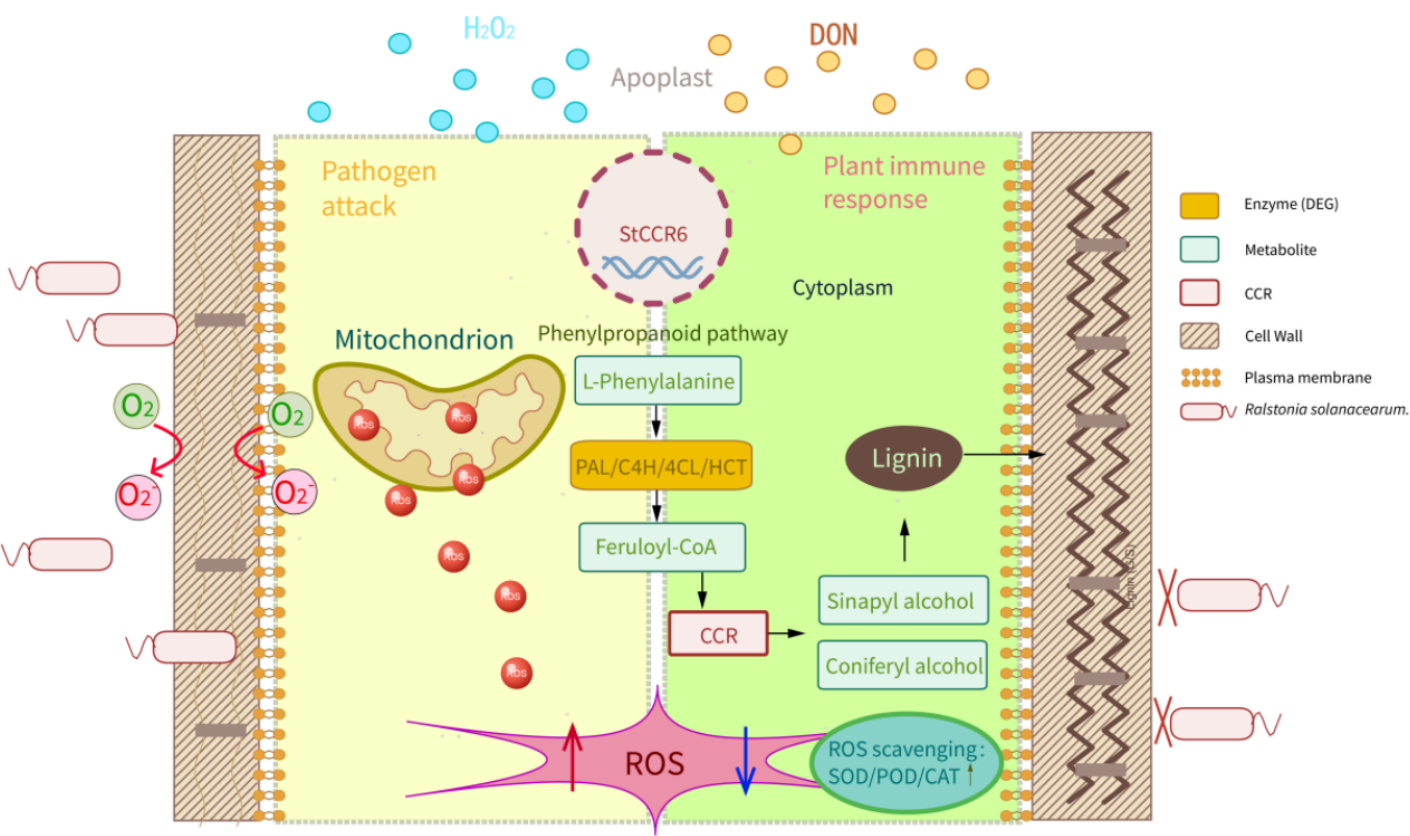


Figure 5

Proposed model for low-dose DON-induced monolignol accumulation and lignified cell wall defense against *Ralstonia solanacearum* in potato.

Supplementary Files

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