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Modulation of Scion Immunity by Resistant Rootstocks in the Pepper–*Ralstonia solanacearum* Pathosystem

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ABSTRACT: Grafting is a common strategy to control bacterial wilt (*Ralstonia solanacearum*) in susceptible crops, including pepper. However, the contribution of resistant rootstocks to scion immunity, beyond physical pathogen exclusion, requires further elucidation. In this study, susceptible ‘HF8’ scions were grafted onto six rootstock genotypes, and their resistance was evaluated via leaf-clip inoculation, which bypasses root-mediated physical barriers. Scions grafted onto resistant rootstocks exhibited restricted pathogen spread and higher survival rates; specifically, the NK4-HF8 combination demonstrated a 100% survival rate, compared to 11% in susceptible-rootstock combinations and 46% in self-grafted controls. Comparative transcriptomic profiling revealed distinct transcriptional reprogramming in scions on the resistant NK4 compared to the susceptible JD2 following infection. These changes involved the modulation of genes associated with pathogen perception (*NDR1-like*, *Xa21*), defense-associated transcription factors (*CaNAC90*, *CaMYB13*), and specific antioxidant isoforms (*CaAPX3*, *CaAPX6*). Physiological analyses indicated that resistant rootstocks are associated with localized hydrogen peroxide accumulation in the scion, alongside the coordinated activation of an antioxidant gene network including *CaSOD*, *CaCAT*, *CaPOD*, *CaAPX*, and *CaGST*. These findings suggest that resistant rootstocks modulate scion immunity by regulating transcriptional networks linked to oxidative defense and redox homeostasis, offering potential physiological markers for rootstock selection in pepper.

KEYWORDS: *Capsicum annuum*; *Ralstonia solanacearum*; grafting; scion resistance; antioxidant defense

1 Introduction

Bacterial wilt, caused by the soil-borne *Ralstonia solanacearum* species complex, represents a major constraint on agricultural production worldwide, affecting a wide range of plant species [1,2]. Upon root infection, the bacteria rapidly colonize and multiply within the xylem vessels, leading to severe vascular dysfunction and systemic wilting [2,3]. Among susceptible hosts, Solanaceous crops, including pepper (*Capsicum annuum* L.), are particularly vulnerable, with outbreaks leading to yield losses ranging from 20% to 100% [1]. Due to the pathogen’s broad host range, long soil persistence, and high genetic diversity, conventional chemical controls are often ineffective and environmentally unsustainable [3,4]. Consequently, breeding and deploying resistant cultivars remain the most effective strategy for disease management [5]. However, integrating resistance traits into commercial cultivars has proven difficult due to issues like linkage drag and the instability of resistance under environmental stress, such as extreme temperatures and humidity [5,6]. Furthermore, because such resistance is frequently governed by quantitative trait loci

(QTLs), introgressing these defense networks without compromising desirable agronomic traits remains a persistent challenge in traditional breeding [5,7,8].

Grafting susceptible commercial scions onto resistant rootstocks has emerged as an effective strategy for managing soil-borne diseases, including bacterial wilt caused by *R. solanacearum* [9–11]. Graft-mediated resistance is generally attributed to two primary mechanisms: the rootstock functioning as a physical and biochemical barrier, and the modulation of scion defense responses via long-distance root-to-shoot signaling [11,12]. Following the healing of the graft union, the re-establishment of vascular connections allows for the transport of mobile signaling molecules—such as phytohormones, defense-related proteins, and small RNAs—which can systemically prime the scion's immune networks [13–15]. Across diverse host–*R. solanacearum* pathosystems, including tomato and eggplant, previous studies have predominantly focused on the rootstock's role in spatially restricting pathogen entry and early root colonization [9,11]. Similarly, in the pepper pathosystem, while root-level physical defenses are well-documented [16–18], the specific contribution of graft-transmissible systemic immunity remains less characterized. However, under natural field conditions, root physical barriers are often compromised by mechanical wounding, soil fauna, or extreme environmental stress. Because *R. solanacearum* is a highly adapted vascular pathogen, once it breaches the root endodermis and enters the xylem transpiration stream, localized root-level defenses are effectively bypassed [3]. Consequently, graft-transmissible systemic immunity within the scion becomes an essential component of the plant's overall defense, functioning to restrict further bacterial proliferation and delay subsequent vascular occlusion.

Reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), function in plant immunity as both direct antimicrobial agents and secondary messengers that initiate downstream defense cascades [19,20]. The rapid accumulation of ROS, often mediated by respiratory burst oxidase homologs (RBOHs), is a characteristic early physiological response to pathogen perception [21,22]. However, because excessive ROS accumulation can lead to severe cytotoxicity and cellular damage, plants depend on a highly regulated antioxidant defense system to maintain redox homeostasis [23]. This enzymatic network requires the synergistic action of superoxide dismutase (SOD) to dismutate superoxide radicals into H_2O_2 , which is subsequently detoxified by scavenging enzymes including ascorbate peroxidase (APX), catalase (CAT), and peroxidase (POD) [23]. Although elevated antioxidant activity has been correlated with *R. solanacearum* resistance in non-grafted Solanaceous plants [24], the spatial dynamics of this response in grafted systems remain poorly understood. Specifically, it has yet to be determined whether a resistant rootstock can systemically prime a susceptible scion to coordinate this specific ROS-producing and scavenging network following pathogen challenge.

In this study, we investigated whether resistant rootstocks enhance scion defense capabilities independent of physical exclusion mechanisms. To achieve this, a leaf-clip inoculation assay was employed to bypass root-mediated physical barriers [25,26], allowing for the direct evaluation of systemic immunity conferred by six rootstock genotypes to a susceptible pepper scion (HF8). Following the initial phenotypic screening, comparative transcriptomic and physiological analyses were performed on contrasting graft combinations: the resistant NK4 and the susceptible JD2 rootstocks. This approach aimed to characterize the transcriptional networks and ROS-scavenging mechanisms associated with rootstock-induced systemic resistance, thereby providing insights into the molecular dynamics of the pepper–*R. solanacearum* pathosystem [27–29].

2 Materials and Methods

2.1 Plant Materials, Bacterial Strain, and Grafting

Six pepper varieties—JD2 (Jianda 2), NK2 (Nongke 2), JD (Jianda), JZ21 (Jinzuan 21), NK4 (Nongke 4), and NK8 (Nongke 8)—were selected as candidate rootstock genotypes, among which NK2, NK4, and NK8 were provided by RuYiQing Group Co., Ltd., JZ21 by Shandong Weier Seed Co., Ltd., and JD2 and JD by Guangzhou Jinzuo Agricultural Technology Co., Ltd. The susceptible scion cultivar HF8 (Hongfu 8), an F1 hybrid pepper bred by Sakata Seed Corporation (Japan) and sourced from Guangzhou Jinzuo Agricultural Technology Co., Ltd., served as the universal scion for all grafting experiments. These specific genotypes were chosen because they represent elite local breeding lines and commercial cultivars that exhibit a wide spectrum of field resistance to bacterial wilt. By utilizing a panel with pre-established phenotypic diversity, we aimed to construct a gradient of resistance—ranging from highly susceptible to highly resistant—to systematically evaluate root-independent systemic immunity. The susceptible cultivar HF8 (Hongfu 8) served as the universal scion for all grafting experiments. All plants were grown in a controlled greenhouse environment with a temperature of 26°C day/22°C night, a light intensity of 40~100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, a 16 h light/8 h dark photoperiod, and a relative humidity of 70%. Seeds were sown and maintained in plastic pots filled with peat soil. The plants were irrigated regularly with water as needed to maintain optimal soil moisture, without the application of additional fertilizers prior to grafting and inoculation. Grafting was performed using the cleft method when seedlings reached the 4–5 true leaf stage. In addition to heterografts, self-grafted HF8-HF8 plants and non-grafted HF8 plants were included as controls to establish a baseline for defense responses. The highly virulent *R. solanacearum* strain FJC100301 was cultured in liquid medium at 28°C for 48 h and adjusted to a final concentration of 10^8 CFU/mL ($\text{OD}_{600} \approx 0.2$).

2.2 Leaf-Clip Inoculation and Disease Assessment

To evaluate systemic scion resistance independently of root-mediated physical exclusion, a standardized leaf-clip inoculation assay was employed. The top two fully expanded leaves were clipped using scissors dipped in the *R. solanacearum* suspension. Macroscopic disease progression and necrotic lesion areas were specifically recorded and photographed at 5 days post-inoculation (dpi), as this time point provided the optimal phenotypic contrast between resistant and susceptible genotypes prior to the onset of severe systemic wilting in susceptible plants. Disease progression was monitored daily and evaluated using a 0-to-4 disease scoring scale, defined as follows: 0 = no visible symptoms; 1 = minor necrosis or chlorosis strictly localized at the clipped edge; 2 = symptoms spreading into the inoculated leaf blade but not reaching the midrib or petiole; 3 = symptoms extending to the petiole or the main stem immediately adjacent to the inoculated leaf; and 4 = severe systemic wilting or necrosis spreading to uninoculated leaves, leading to eventual plant death. Survival rates were calculated as the percentage of plants with a disease score <4 at 15 dpi. For both the initial screening of non-grafted genotypes and the evaluation of grafted combinations, each treatment consisted of at least 360 plants. All inoculation experiments were independently repeated 3 times to ensure the reproducibility of the disease progression and survival rate data.

2.3 ROS Detection and Histochemical Staining

To capture early physiological defense responses prior to extensive tissue collapse, H_2O_2 accumulation was visualized at 3 dpi using 3,3'-diaminobenzidine (DAB) staining. For this histochemical analysis, at least 30 representative scion leaves from different individual plants were collected per treatment combination. Leaves were immersed in 1 mg/mL DAB solution (pH 3.8) in the dark for 5 h, followed by boiling in 95%

ethanol to remove chlorophyll. For quantitative assessment, the H₂O₂ content was determined using a Hydrogen Peroxide Assay Kit (Biyuntian, Shanghai, China) according to the manufacturer's instructions. The absorbance was measured at 560 nm using a spectrophotometer, and the H₂O₂ concentration was calculated based on a standard curve. The quantitative H₂O₂ assay was performed with three independent biological replicates, with each replicate comprising pooled leaf tissue collected from 30 individual plants per treatment.

2.4 RNA Extraction, Transcriptome Sequencing, and RT-qPCR

Total RNA was extracted from scion leaves of the NK4-HF8, JD2-HF8, and HF8-HF8 combinations at 3 dpi to specifically investigate primary transcriptional reprogramming rather than secondary cell death responses. For sampling, the top two clipped and inoculated leaves were collected from each plant. Each treatment consisted of three independent biological replicates, and each replicate was a pooled sample of leaves from 3 individual plants. RNA quality and integrity were assessed using an Agilent 2100 Bioanalyzer. For transcriptomic profiling, mRNA was purified using poly-T oligo-attached magnetic beads and fragmented. cDNA libraries were constructed using the NEBNext[®] Ultra[™] RNA Library Prep Kit for Illumina[®] following the manufacturer's recommendations. The libraries were then sequenced on an Illumina NovaSeq 6000 platform to generate 150 bp paired-end reads.

For data processing, raw reads were processed using fastp to remove adapter sequences, reads containing poly-N, and low-quality reads. The resulting high-quality clean reads were mapped to the *Capsicum annuum* reference genome GCF_002878395.1. Gene expression levels were quantified and normalized using the TPM metric. Differentially expressed genes (DEGs) were identified using a threshold of $|\log_2 \text{Fold Change}| \geq 1$ and a false discovery rate (FDR) < 0.05. Following DEG identification, Gene Ontology (GO) terms and KEGG pathway enrichment analyses were performed using Goseq R package. Enrichment significance was defined by an adjusted *p*-value (FDR) < 0.05. For RT-qPCR validation, first-strand cDNA was synthesized using the HiScript IV RT SuperMix for qPCR (+gDNA wiper) (Vazyme R423-01). RT-qPCR was performed using 2× RealStar Fast SYBR qPCR Mix (High ROX; Genstar, A303) on the QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific) to determine the relative mRNA expression levels of *CaRBOHD*, *CaSOD*, *CaCAT*, *CaPOD*, *CaAPX* and *CaGST*. The *CaActin* gene was used as the internal reference to normalize expression data using the $2^{-\Delta\Delta Ct}$ method. Primers used in this study are listed in Supplementary Table S1.

2.5 Statistical Analysis

Data were analyzed using GraphPad Prism 8.0. Significant differences between treatments were determined using Duncan's multiple range test at a significance level of *p* < 0.05. To determine statistical significance among three or more groups, a One-Way Analysis of Variance (ANOVA) was performed, followed by Tukey's Honestly Significant Difference (HSD) post hoc test for multiple comparisons. For comparisons between two specific groups (e.g., RT-qPCR and H₂O₂ quantification), an unpaired Student's *t*-test was utilized.

3 Results

3.1 Screening of Candidate Rootstock Genotypes for Foliar Resistance Using a Root-Independent Assay

To assess the inherent capacity for systemic immunity independent of root structures, the foliar resistance of six candidate rootstock genotypes was evaluated using a leaf-clip inoculation assay using on

non-grafted seedlings. This method allows for the direct observation of *R. solanacearum* progression from the inoculation site at the leaf apex towards the vascular tissues of the petiole and stem.

The candidate genotypes displayed distinct disease progression profiles. In the susceptible genotypes (JD2, NK2, and JD), pathogen spread was rapid; by 5 dpi, disease symptoms were evident along the leaf veins, accompanied by widespread chlorosis and tissue necrosis (Fig. 1A). Conversely, the JZ21 and NK8 genotypes restricted the infection predominantly to the leaf apex, resulting in smaller lesion areas. The NK4 genotype demonstrated the most restricted disease progression, with macroscopic symptoms limited to minor yellowing at the immediate clipping site and no visible vein discoloration (Fig. 1A,B).

These foliar symptom phenotypes generally corresponded with the overall plant survival rates following vascular invasion. NK4 plants exhibited a 99% survival rate, followed by NK8 at 93%. JZ21 and JD showed moderate survival rates of 45% and 40%, respectively, whereas JD2 and NK2 recorded the lowest survival rates at 11% (Fig. 1C). Notably, the NK2 genotype presents an interesting exception: while it showed moderate foliar lesion restriction compared to JD2, its final survival rate was equally low (11%). This highlights that localized foliar defense does not always translate to robust systemic vascular survival. These data indicate that the selected genotypes possess varying, inherent capacities to restrict *R. solanacearum* proliferation. This characterized phenotypic divergence provides a baseline for evaluating subsequent rootstock-mediated effects on scion immunity.

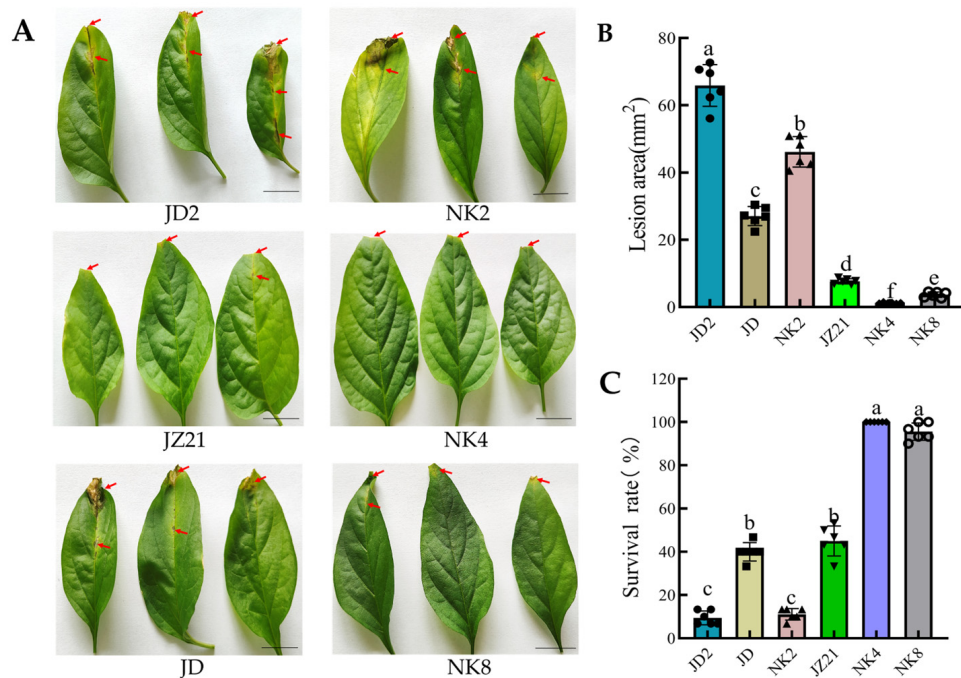


Figure 1: Evaluation of inherent resistance to *Ralstonia solanacearum* in six pepper genotypes using a standardized leaf-clip inoculation assay. (A) Disease phenotypes and symptom development at 5 days post-inoculation (dpi). Red arrows indicate the inoculation site or highlight lesions along the veins. Scale bars = 2 cm (B) Quantitative assessment of necrotic lesion area across the six genotypes. Data are represented as individual data points for each independent biological replicate ($n = 6$ plants), with the bars indicating the mean \pm SD. (C) Survival rates of the six genotypes calculated at the end of the observation period. Different lowercase letters indicate significant differences according to Tukey's HSD test ($p < 0.05$).

3.2 Rootstock Genotypes Modulate the Systemic Resistance of Susceptible Scions

To investigate whether the resistance levels observed in the candidate genotypes could be systemically transmitted to a susceptible scion, ‘HF8’ scions were grafted onto the six previously characterized rootstocks. Following leaf-clip inoculation, the disease progression in the ‘HF8’ scions corresponded with the inherent resistance patterns of their respective rootstocks.

At 5 dpi, scions grafted onto the resistant rootstocks (NK4-HF8 and NK8-HF8) exhibited restricted symptom development, with no visible vein discoloration, consistent with the phenotypes of the non-grafted NK4 and NK8 plants. In contrast, scions grafted onto the susceptible rootstocks (JD2-HF8 and NK2-HF8) developed pronounced chlorosis and distal vein lesions, exhibiting susceptibility similar to the non-grafted JD2 and NK2 genotypes (Fig. 2A–H).

The final survival rates further supported this rootstock-mediated effect. The NK4-HF8 combination recorded a 100% survival rate, indicating significantly enhanced resistance in the susceptible HF8 scion. Conversely, the survival rate of the JD2-HF8 combination was 11%, mirroring the inherent survival rate of the JD2 rootstock and lower than the 46% survival observed in the self-grafted HF8-HF8 controls (Fig. 2I). These data demonstrate that the immune status of the susceptible scion is modulated by the underlying rootstock, providing evidence that resistant rootstocks confer systemic protection against *R. solanacearum* infection.

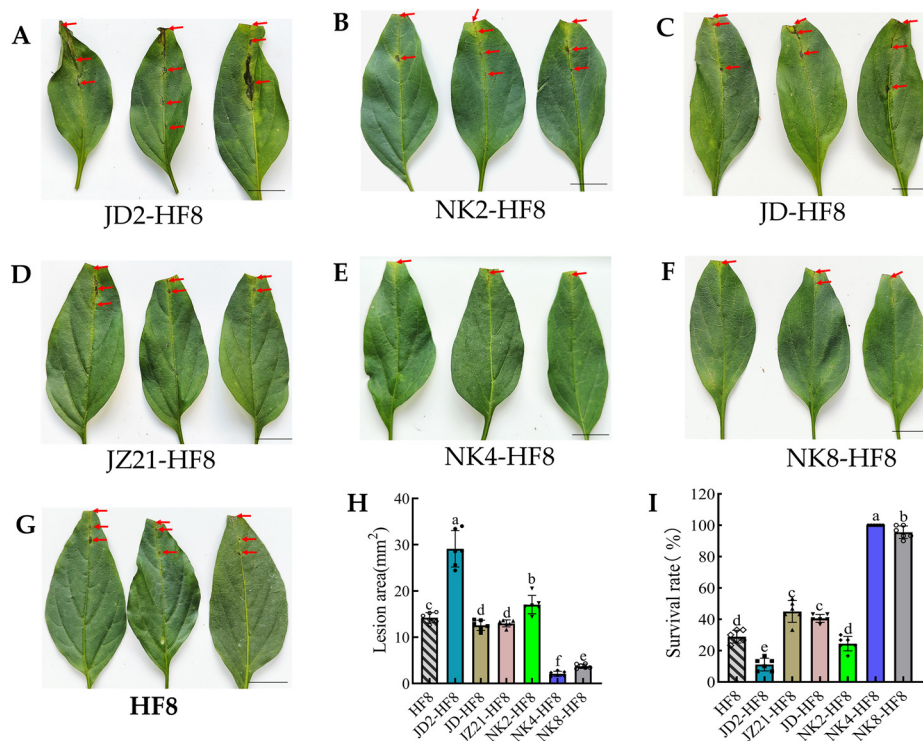


Figure 2: Rootstock genotype determines the resistance level of susceptible HF8 scions. (A–G) Disease phenotypes of HF8 scions grafted onto different rootstocks (NK4, NK8, JZ21, JD, NK2, JD2) following leaf-clip inoculation. The disease phenotype of the scion corresponds to the rootstock genotype. Scale bars = 2 cm. (H) Quantitative analysis of lesion area development in grafted scions. Data are represented as individual data points for each independent biological replicate ($n = 6$ plants), with the bars indicating the mean \pm SD. (I) Survival rates of HF8 scions grafted onto the six different rootstocks. Different lowercase letters indicate significant differences according to Tukey’s HSD test ($p < 0.05$).

3.3 Transcriptomic Analysis Reveals Rootstock-Specific Gene Regulation in Scions

To investigate the molecular basis of graft-induced immunity, a comparative transcriptomic analysis was performed on scions grafted onto the resistant NK4 and the susceptible JD2 rootstocks, utilizing the infected self-grafted HF8–HF8 combination as a baseline reference. This approach facilitated the identification of rootstock-specific transcriptional changes under identical *R. solanacearum* infection pressure.

The analysis revealed a divergence in transcriptional profiles. NK4–HF8 scions exhibited 285 DEGs (71 up-regulated and 208 down-regulated) relative to the HF8–HF8 control (Fig. 3A; Supplementary Table S2). The substantial proportion of down-regulated genes in the NK4–HF8 combination is consistent with an attenuated damage response. As the resistant NK4 rootstock restricts early pathogen proliferation, the scion incurs less structural damage. Consequently, damage-associated genes—which are typically induced in the susceptible HF8–HF8 control—remained at lower expression levels in the NK4–HF8 scions. These down-regulated transcripts include disease-induced proteases (e.g., *aspartyl protease AED3* [LOC107867358], Log₂FC = −2.97), cell wall-degrading enzymes (e.g., *endoglucanase 6* [LOC107851575], Log₂FC = −2.21; *endo-1,4-beta-xylanase 5* [LOC107840064], Log₂FC = −2.39), and late-stage stress and *ethylene-responsive factors* (e.g., ERF038-like [LOC107872529], Log₂FC = −1.82) (Supplementary Table S2). In contrast, JD2–HF8 scions mounted a narrower transcriptional response, yielding 74 DEGs (34 up-regulated and 40 down-regulated) (Fig. 3B and Supplementary Table S3). Only 22 DEGs were shared between the two combinations, indicating a specific transcriptional response associated with the NK4 rootstock (Fig. 3C).

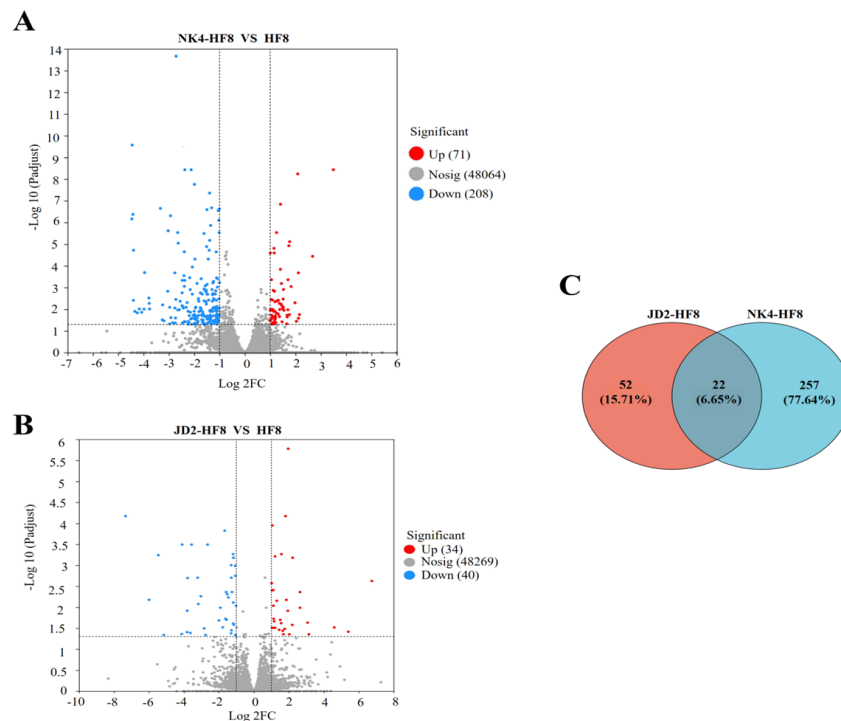


Figure 3: Comparative transcriptomic profiling reveals distinct, rootstock-specific transcriptional reprogramming in susceptible HF8 scions. (A,B) Volcano plots illustrating the distribution of differentially expressed genes (DEGs) in scions grafted onto the highly resistant NK4 rootstock (NK4–HF8 vs. HF8) (A) and the highly susceptible JD2 rootstock (JD2–HF8 vs. HF8) (B) at 3 dpi. (C) Venn diagram displaying the overlap and specificity of DEGs between the JD2–HF8 and NK4–HF8 combinations.

While standard functional enrichment analysis of the NK4–HF8 DEGs did not yield significantly enriched pathways—likely due to the highly specific and restricted nature of the transcriptional response—a manual, literature-guided curation of the DEG list was conducted. This directed search specifically screened for gene families with established roles in plant immunity, successfully identifying a subset of up-regulated genes associated with defense signaling and redox homeostasis. For early pathogen perception, the NK4 rootstock induced genes encoding NDR1-like proteins (LOC107854707, Log2FC = 1.73) and the immune receptor kinase Xa21 (LOC124895600, Log2FC = 1.67). Downstream of this initial signal transduction, NK4 up-regulated *CaWRKY53* (LOC107851739, Log2FC = 1.52) and specific transcription factors, including *CaNAC90* (LOC107846948, Log2FC = 2.67) and *CaMYB13* (LOC107874571, Log2FC = 1.20). Furthermore, the transcriptomic data indicated the up-regulation of ascorbate peroxidase genes, such as *CaAPX6* (LOC107868078, Log2FC = 1.41) and *CaAPX3* (LOC107859857, Log2FC = 1.07), alongside *glutathione S-transferase U9* (LOC107866635, Log2FC = 2.40). These expression patterns suggest the mobilization of redox-scavenging and detoxification networks in the resistant graft combination.

3.4 Enhanced ROS Burst and Antioxidant Gene Expression Validate the Transcriptomic Changes

To functionally validate the transcriptomic data physiologically, ROS dynamics and antioxidant gene expression were compared between the NK4–HF8 and JD2–HF8 scions. At 3 dpi, DAB staining indicated distinct differences in H₂O₂ accumulation. JD2–HF8 scions exhibited faint H₂O₂ staining at the infection site, whereas NK4–HF8 scions displayed dense, yellowish-brown H₂O₂ deposits (Fig. 4A–D). Quantitative assays confirmed that the H₂O₂ content in NK4–HF8 was significantly higher than in JD2–HF8 ($p < 0.0001$), indicating elevated systemic ROS accumulation (Fig. 4E). This physiological response corresponded with the induction of the NADPH oxidase gene *CaRBOHD* (Fig. 4F), an enzyme involved in ROS production during plant-pathogen interactions [21,22].

To evaluate the response to this oxidative accumulation and mitigate potential cytotoxicity, the expression of genes within the scion's antioxidant system was quantified using targeted RT-qPCR. Consistent with the ROS accumulation, RT-qPCR analysis of ROS-scavenging and detoxification genes—specifically *CaSOD*, *CaCAT*, *CaPOD*, *CaAPX*, and *CaGST*—revealed significantly higher expression levels in the NK4–HF8 scions compared to the JD2–HF8 combination (Fig. 4G–K).

These results suggest that resistant rootstocks influence scion immunity by promoting localized ROS production while concurrently activating an antioxidant gene network. This coordinated regulation likely restricts pathogen spread and protects the scion against oxidative damage, a physiological mechanism that aligns with the attenuated damage responses observed in the transcriptomic profiling.

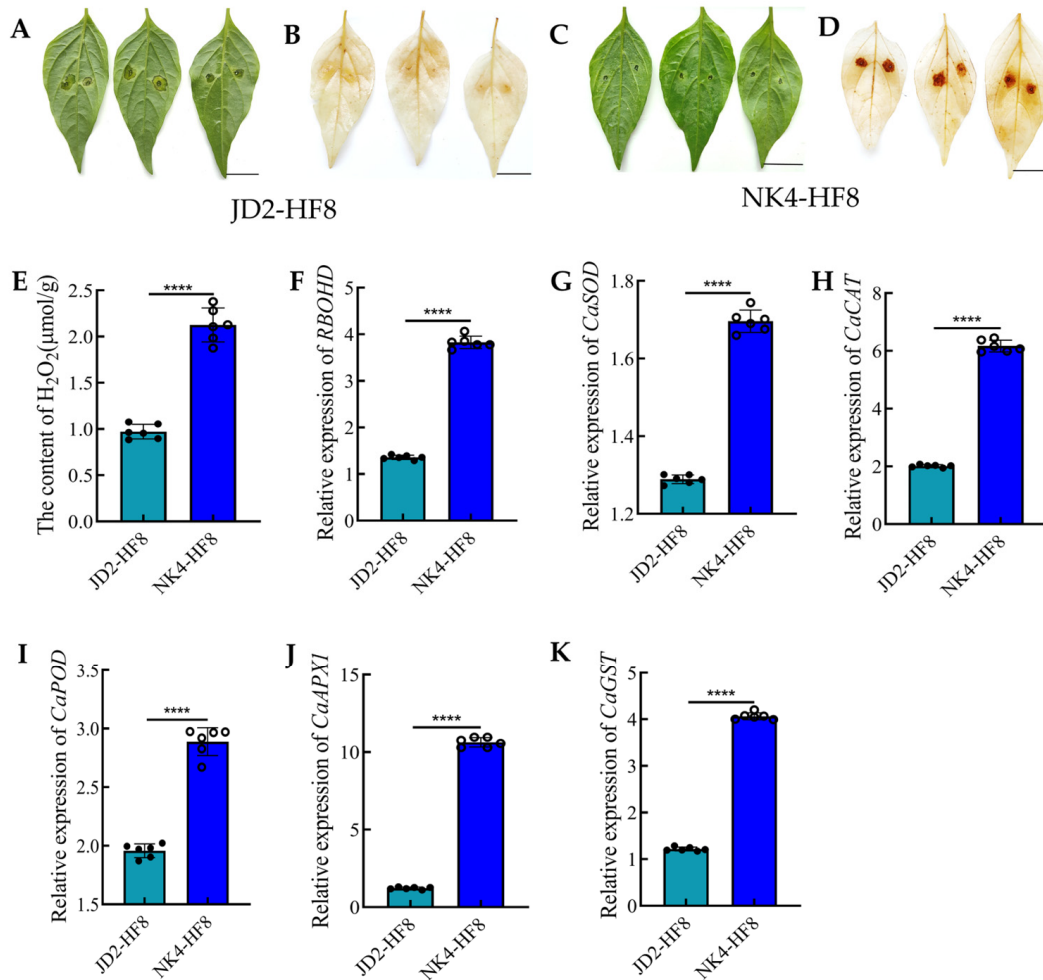


Figure 4: Resistant rootstocks prime scions for rapid ROS accumulation and antioxidant defense activation. (A–D) Histochemical detection of hydrogen peroxide (H₂O₂) accumulation (DAB staining) and oxidative response patterns in scion leaves at 3 dpi. (E) Quantification of (H₂O₂) content in scion leaves. (F) Relative expression levels of the NADPH oxidase gene *CaRBOHD* in scions grafted onto different rootstocks. (G–K) Relative expression levels of key antioxidant genes in scion leaves, showing upregulation in scions grafted onto resistant rootstocks. Asterisks (****) indicate a significant difference between JD2-HF8 and NK4-HF8 at the $P < 0.0001$ level.

4 Discussion

While the role of the rootstock as a structural barrier against the soil-borne pathogen *R. solanacearum* has been emphasized in previous literature [16–18], this physical defense can be compromised by mechanical damage or environmental stresses under field conditions. Consequently, once the pathogen breaches the root endodermis and colonizes the xylem, graft-transmissible systemic immunity in the scion functions as an essential defense layer to restrict bacterial proliferation and subsequent wilting. To evaluate this systemic component independently of root-mediated physical exclusion and potential rhizosphere interactions, susceptible HF8 scions were grafted onto six rootstock genotypes with varying levels of inherent resistance. As established in our initial non-grafted screening, these varying levels of localized foliar resistance broadly corresponded with ultimate plant survival, with the minor exception of the NK2 genotype. Following the leaf-clip inoculation assay on the scions, the present phenotypic data demonstrate that the immune status of the susceptible HF8 scion is modulated by the systemic influence of the rootstock. Specifically, HF8

scions grafted onto the resistant NK4 rootstock achieved a 100% survival rate, whereas survival was 11% on the susceptible 'JD2' rootstock and 46% in self-grafted controls. These results suggest that resistant rootstocks maintain the scion in a physiologically primed state, potentially through root-to-shoot signaling molecules traversing the graft union [13–15].

The comparative transcriptomic analysis provided a molecular basis for this systemic protection. The NK4 rootstock triggered a broader transcriptional response (285 DEGs) in the scion compared to the susceptible JD2 rootstock (74 DEGs) under identical infection pressure. A notable characteristic of the NK4–HF8 scion's response was the induction of immune receptors such as NDR1-like and Xa21. These receptors are typically associated with pathogen recognition and the initiation of early immune signaling, suggesting an enhanced sensing capacity in the primed scion. Downstream of this recognition, the specific up-regulation of defense-related transcription factors, including *CaNAC90* and *CaMYB13*, indicates the activation of a complex regulatory network. Consequently, this rapid recognition and transcriptional activation likely contribute to the significant downregulation of genes associated with structural damage and tissue collapse, such as the aspartyl protease AED3 and cell wall-degrading enzymes (endoglucanase 6, endo-1,4-beta-xylanase 5). This attenuated damage profile suggests that the systemic influence of the resistant rootstock enables the scion to restrict early pathogen proliferation, thereby preventing the severe physiological deterioration observed in susceptible combinations.

Physiologically, this rootstock-induced systemic resistance is tightly linked to a coordinated redox regulation strategy. Upon pathogen challenge, scions on resistant rootstocks exhibit significant H₂O₂ accumulation associated with the induction of *CaRBOHD*. This rapid ROS accumulation likely serves as a primary mechanism for inhibiting bacterial spread and facilitating cell wall cross-linking [21,22]. However, unrestricted ROS bursts can lead to severe cellular cytotoxicity. Therefore, this oxidative defense is coupled with the simultaneous mobilization of a coordinated antioxidant network, involving significantly higher expression of *CaSOD*, *CaCAT*, *CaPOD*, *CaAPX*, and *CaGST*. This synchronization allows the scion to utilize reactive oxygen species as antimicrobial agents while maintaining cellular homeostasis and preventing oxidative self-damage [23].

5 Conclusion

In conclusion, the findings of this study provide a mechanistic link between rootstock-to-shoot communication and scion systemic immunity in the pepper–*R. solanacearum* pathosystem. Grafting onto resistant rootstocks effectively mitigates disease symptoms in susceptible scions by pre-activating specific transcriptional networks and a coordinated ROS-scavenging system. This systemic protection strategy operates independently of physical root barriers, offering practical physiological and molecular markers for the evaluation and selection of resistant rootstocks in pepper breeding programs. Future research should focus on identifying the precise long-distance mobile signals—such as specific mobile mRNAs, small RNAs, or metabolites—that mediate this root-to-shoot immune priming.

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Author Contributions: Bizhen Lin and Wenzhong Lin conducted most of the experiments and analyzed the data. Dongmei Lian and Susu Yuan performed the physiological parameter measurements and histochemical staining. Wenzhong Lin and Weiyang Wang conducted the qPCR analysis and data visualization. Bizhen Lin and Weiyang Wang performed the statistical analysis and methodology validation. Weiyang Wang conceived the project, designed and supervised the research, and wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

Availability of Data and Materials: All processed data supporting the findings of this study, including comprehensive transcriptomic datasets, are available within the article and its Supplementary Materials. The raw RNA-seq datasets analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest.

Supplementary Materials: The supplementary material is available online at <https://www.techscience.com/doi/10.32604/phyton.2026.082698/s1>.

Abbreviations

The following abbreviations are used in this manuscript:

ROS	Reactive oxygen species
H ₂ O ₂	Hydrogen peroxide
DEG	Differentially expressed gene
FDR	False discovery rate
log ₂ FC	Log ₂ fold change
RNA-seq	RNA sequencing
RT-qPCR	Quantitative real-time PCR
DAB	3,3'-diaminobenzidine
RBOHD	Respiratory burst oxidase homolog D
SOD	Superoxide dismutase
POD	Peroxidase
CAT	Catalase
APX	Ascorbate peroxidase
GST	Glutathione S-transferase
AED3	Apoplastic, Enhanced Disease Susceptibility 1-dependent 3
NDR1	Non-race-specific disease resistance 1
Xa21	Xanthomonas resistance 21
dpi	Days post-inoculation
CFU	Colony-forming units
OD ₆₀₀	Optical density at 600 nm

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