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A rice DELLA protein OsSLR1 positively regulates rice resistance to southern rice black-streaked dwarf virus infection

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Abstract

Background In the course of long-term confrontation with pathogens, plants have developed complex defense mechanisms to protect themselves from various pathogens. Previous studies have reported that the gibberellin (GA) signaling pathway negative regulator SLENDER RICE 1 (SLR1) in rice activates jasmonic acid (JA)-mediated broad-spectrum antiviral immunity, but the exploration regarding whether OsSLR1 exerts effects on alternative antiviral immune pathways remains limited.

Results Here, we identified that *OsSLR1* was significantly induced after virus infection and overexpression of *OsSLR1* in rice enhance the resistance of rice to southern rice black-streaked dwarf virus (SRBSDV) in rice. Transcriptome analysis revealed that a total of 2,336 differentially expressed genes (DEGs) were detected upon overexpression of *OsSLR1* in rice, including 1,607 upregulated genes and 729 downregulated genes. Gene ontology (GO) enrichment analysis and RT-qPCR analysis revealed that genes related to JA and reactive oxygen species (ROS) were significantly upregulated, while genes associated with abscisic acid (ABA) were significantly downregulated.

Conclusions These results suggest that OsSLR1 positively regulates the antiviral immunity of rice by modulating multiple pathways.

Keywords Rice, OsSLR1, SRBSDV, Transcriptome, JA, ROS, ABA

Background

As a key negative regulator of the gibberellin (GA) signaling pathway, DELLA not only participates in plant growth and development but also plays a crucial role in plant immunity [1–3]. For example, the infection of *Pst* DC300 promotes the protein accumulation level of DELLA, negatively regulating the salicylic acid (SA)-mediated immune response in *Arabidopsis* [4]. Ageratum leaf curl Sichuan virus (ALCScV)-encoded C4 protein negatively regulates the GA signaling pathway by protecting DELLA proteins from degradation by the 26S proteasome, thereby promoting viral infection in *N. benthamiana* [5]. As the only DELLA protein in rice, SLENDER RICE 1 (SLR1) facilitates the disassembly of the OsMYC2/3 and OsJAZ complex, consequently triggering the jasmonic acid (JA)-mediated broad-spectrum antiviral immune response in rice [6]. However, it is unclear whether OsSLR1 regulates antiviral immunity in rice through alternative pathways.

Plant hormones are a class of organic compounds that are widely distributed in nature and deeply involved in plant growth, development, and innate immunity [7, 8].

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Interactions among diverse plant hormones are generally accomplished via the targeting of core components of one hormone by crucial regulators of another [9, 10]. For instance, JA signaling promotes abscisic acid (ABA) biosynthesis to maintain the expression of *RAP2.6*, which is a key factor in lignin biosynthesis and later wound healing processes in *Arabidopsis* [11]. The brassinosteroid (BR) signaling inhibitors glycogen synthase kinase3 (GSK3)–like kinase (OsGSK2) activates JA signaling and enhances plant antiviral defense by promoting the degradation of JA signaling negative regulator OsJAZ4 [12]. Viral proteins encoded by different types of viruses regulate the crosstalk between GA and JA by manipulating the protein stability of OsSLR1, which in turn effectively promotes viral infection [6]. However, it is unclear whether OsSLR1 is involved in the innate immunity of rice mediated by other hormones in rice.

ABA plays a multifaceted role in plant immunity, and the role of ABA in plant resistance against to the invasion of various pathogens depends on the type of plant and pathogen [13–15]. For example, ABA decreases resistance to *Pseudomonas syringae* in *Arabidopsis* but increases resistance to the *A. brassicicola* and *P. cucumerina* [16, 17]. It is reported that ABA is also involved in host-virus interactions. ABA restricts the movement of virus between cells by inducing callus to deposit on plasmodesmata, thus promoting the resistance of *N. benthamiana* to tobacco mosaic virus (TMV) [18]. Besides, ABA facilitates plant resistance to plum pox virus (PPV) via the regulation of RNA diversity, abundance, turnover and translation initiation in *N. benthamiana* [19]. Moreover, ABA also promotes resistance to *Bamboo mosaic virus* (BaMV) mainly by regulating the expression levels of *AGO1* and *AGO2* in *N. benthamiana* and *Arabidopsis* [20]. However, no reports have indicated that whether OsSLR1 participates in the ABA-mediated antiviral immunity in rice.

Reactive oxygen species (ROS) mainly caused by respiratory burst oxidase homologs (RBOHs), play an important role in plant resistance against to the invasion of pathogens [21–28]. Numerous studies have shown that a variety of proteins with gradually differentiated functions can indirectly participate in the process of plant innate immunity by regulating the production of ROS. For instance, the E3 ligases OsRIP1 and OsAPIP6 enhance the resistance to rice blast and sheath blight by promoting the production of ROS in rice [22]. Resistance Gene Analogs3 (TaRGA3) promotes wheat resistance to stripe rust by facilitating the accumulation of ROS [29]. Alfin-like 7 (NbAL7) enhances resistance to TMV by inhibiting the transcriptional activity of ROS-scavenging genes and promoting ROS accumulation [30]. However, it is currently not known whether OsSLR1 participates in the ROS-mediated antiviral immunity in rice.

Southern rice black-streaked dwarf virus (SRBSDV) was first reported in China in 2001, has caused serious damage to rice production in East and Southeast Asia [31–34]. SRBSDV belongs to the genus *Fijivirus* (family *Reoviridae*), is transmitted horizontally by *Sogatella furcifera* (the white-backed planthopper, WBPH) in a persistent circulative propagative manner [33]. The insect vectors infected with SRBSDV carry the virus throughout their lives and insects cannot transmit the virus to its offspring through eggs [35, 36]. Typical symptoms of SRBSDV infected-rice include severely stunted, leaf dark green, stiff and have excessive tillering [33, 37–39]. The SRBSDV genome consists of 10 segments double-stranded RNA, encoding 13 proteins [40]. Recent studies have shown that the P8 protein encoded by SRBSDV promotes viral infection by suppressing the transcriptional activities of auxin response transcription factor OsARF17 and JA signaling transcription factor OsMYC3 [41, 42]. SRBSDV P6 protein attracts insect vectors and promotes viral transmission by blocking the key transcription factor of ethylene signaling OsEIL2 dimerization suppresses ethylene signaling [31]. The SRBSDV nonstructural protein P7-1 promotes viral transmission by inducing the mitophagy receptor BNIP3 (BCL2 interacting protein 3)-mediated mitophagy [43].

In this study, we found that the expression of *OsSLR1* was significantly induced after SRBSDV infected. Next, we identified a total of 2336 DEGs in rice overexpression of *OsSLR1* by RNA-seq, and these DEGs were effectively clustered in 'response to jasmone acid', 'response to abscisic acid', 'peroxidase activity' and 'response to oxidative stress' by gene ontology (GO) analysis. RT-qPCR analysis revealed that overexpression of *OsSLR1* in rice led to a significant upregulation of ROS and JA pathway-related genes and a significant downregulation of ABA pathway-related genes. These results suggest that OsSLR1 positively regulates the antiviral immunity of rice by suppressing the ABA pathway and activating the ROS production and JA pathway.

Materials and methods

Plant materials and growth conditions

The rice (*Oryza sativa* sap *japonica*) varieties used in this study were *Lansheng* (*LS*) and *Nipponbare* (*NIP*). The overexpression of *OsSLR1* plants were created in *LS* background. All these rice plants were grown in the greenhouse at 28–30 °C under 14 h light/10 h dark cycle. The *N. benthamiana* plants used in follow-up subcellular localization were grown in black plastic bowls at 25 °C under 14 h light/10 h dark cycle.

Plants were sourced as follows: 1) *Oryza sativa* (*NIP*) and *N. benthamiana* were stored in our laboratory for a long time. 2) *Oryza sativa* (*LS*) was obtained from Prof Jiayang Li, institute of Genetics and Developmental Biology, The innovative Academy of Seed Design, Chinese Academy of Sciences.

Subcellular localization methods

For the subcellular localization analysis of OsSLR1-GFP, we cloned the coding sequence (CDS) of *OsSLR1* into the pCAMBIA1300 vector, which was driven by the CaMV 35S promoter and carried the GFP tag. The subcellular localization of OsSLR1-GFP was determined in *N. benthamiana* cells using *agrobacterium tumefaciens* strain GV3101. HA-GFP was used as a negative control. In brief, the *agrobacterium* was centrifuged at 5000 rpm for 5 min to collect the organisms, then treated twice with infiltration buffer (10 mM $MgCl_2$, 10 mM MES (pH = 5.6) and 0.2 mM acetosyringone) and incubated at 28 °C for about 2 h. Finally, the final concentration was adjusted to OD600 = 1.0. Then, 4-week-old *N. benthamiana* leaves were soaked in a suspension of *agrobacterium tumefaciens* strains carrying OsSLR1-GFP and HA-GFP for 48 h. After 48 h, the fluorescent signals were captured using a laser confocal microscope (Leica TCS SP10).

Isolation and identification of the overexpression plants

The full length CDS of *OsSLR1* was cloned and the expression vector pCAMBIA1300 was constructed by homologous arm recombination to generate the overexpression of *OsSLR1* transgenic plants. The recombinant plasmid was electroporated into *agrobacterium tumefaciens* strain GV3101 and transformed into *LS* background. Total RNA from T1 generation *OsSLR1-ox* plants was extracted and RT-qPCR analysis was performed. The two lines with high expression were harvested, and the T2 generation of *OsSLR1-ox* plants continued to be selected for further purification of high expression plants. T3 homozygous transgenic plants were used for subsequent experiments.

Insect vectors and virus inoculation assays

SRBSDV is transmitted by *Sogatella furcifera* (the white-backed planthopper, WBPH) and the insect vectors cannot transmit the virus to its offspring through eggs. The offspring must repeatedly feed on the infected plant to continue the transmission. The virus-free nymphs were fed on SRBSDV infected rice seedlings for 3–5 dpi, and then transferred to healthy rice plants for 10–12 dpi. The SRBSDV-infected/virus-free WBPH was inoculated on the rice seedling (rice seedling/insect ratio = 1:3) at the 3 to 4 leaf stage (about 12 dpi of seedlings) for 3 dpi. After 30 dpi, the disease symptoms of the plants were observed and the incidence rate in wild-type and overexpression plants. The rice plants were grown in the field after inoculated for observation and RT-qPCR and western blotting were tested at 30 dpi. All samples of mock and SRBSDV-infected leaves were collected, and then frozen in liquid nitrogen and stored at −80 °C.

Total RNA extraction and RT-qPCR

Follow the manufacturer's instructions to extract total RNA from rice leaves using Trizol reagent. 1 μg of total RNA was treated with gDNA Clean Reaction Mix Ver.2 (Accurate Biotechnology, China) to remove residual genomic DNA and then reverse transcribed into cDNA using 5X Evo M-MLV RT Reaction Mix Ver.2 (Accurate Biotechnology, China). Then, the RT-qPCR was performed on the Light-Cycler480 II Real-Time PCR System (Roche) using the SYBR Green Pro Taq HS Premix (Accurate Biotechnology, China) as the reaction mixture. The actin gene *OsUBQ5* in rice was used as a normalization statistic for the relative mRNA expression level of rice, and the data were finally analyzed using the $2^{-\Delta\Delta Ct}$ method. All experiments were conducted in triplicate biological replicates. The RT-qPCR primer sequences used in this study are listed in Table S1.

Protein extraction and western blotting analysis

The leaves of rice infected with SRBSDV were quick-frozen with liquid nitrogen and rapidly grinded, then the total protein was extracted by lysis buffer (100 mM Tris–HCl (PH = 6.8), 6% SDS). The lysed samples were centrifuged to obtain the supernatant, which was then mixed with 5 × SDS loading buffer (100 mM Tris–HCl (PH = 6.8), 4% SDS, 0.2% BPB, 20% Glycerol and 2% β-mercaptoethanol) and boiled at 100 °C for 5–10 min [44]. The extracted protein was separated by 10% SDS-PAGE gel and then transferred to the PVDF membrane. The infection of SRBSDV was detected using a polyclonal anti-p10 antibody (1:3000 dilution). The total protein was stained with Ponceau staining method to confirm consistent loading.

DAB and NBT staining

H_2O_2 accumulation was detected by DAB staining as described previously [45, 46]. 10 dpi seedling leaves of *LS* and *OsSLR1-ox* plants were cut into small pieces (approximately 1 cm length) and vacuum infiltrated in DAB solution (1 mg/mL DAB, 100 mM Tris–HCl (pH = 3.8)). After overnight incubation at 25 °C, the samples were destained by destaining solution (50% ethanol, 50% acetic acid) for 10 h.

NBT staining was applied to analyze ROS production as described previously [46, 47]. In short, 10-dpi seedling leaves of *LS* and *OsSLR1-ox* plants were cut into small

pieces in NBT solution (6 mM NBT, 10 mM sodium citrate (pH = 6.0)). Incubated overnight at 37 °C, and then destained with 75% ethanol twice for 4 h. Finally, rinsed with absolute ethanol to remove chlorophyll.

RNA library construction and sequencing

LS and *OsSLR1-ox* rice samples were collected after 30 dpi of natural growth in the field, and total RNA was extracted by the above method after rapid and sufficient grinding of liquid nitrogen. Three rice leaves were selected as one biological replicate, and three biological replicates were treated each. RNA purity and quantification were evaluated by Nanodrop 2000 spectrophotometer (Thermo Scientific, USA). RNA integrity was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Then the libraries were constructed using VAHTS Universal V6 RNA-seq Library Prep Kit according to the manufacturer's instructions. The transcriptome sequencing was conducted by OE Biotech (Shanghai, China). The libraries were sequenced on llumina Novaseq 6000 platform and 150 bp paired-end reads were generated. Raw reads of fastq format were firstly processed using fastp and the lower quality reads were removed to obtain the clean reads. The clean reads were mapped to the reference genome using HISAT2. FPKM of each gene was calculated and the read counts of each gene were obtained by HTSeq-count.

Statistical analysis

Differences of RT-qPCR were analyzed using Student's *t*-test. Each experiment was repeated at least three times, all values are presented with mean ± standard deviation. Asterisks indicate significant differences ($^{*}P < 0.05$, $^{**}P < 0.01$). All images of RT-qPCR were performed using Graphpad Prism 9.5.0.

Results

Overexpression of *OsSLR1* increased the resistance of rice to SRBSDV

Previous studies have shown that the transcription levels of many defense or stress related genes were significantly changed after viral infection [7, 37]. In this work, we identified that the expression level of *OsSLR1*, a DELLA protein in rice, was significantly upregulated after SRBSDV infection for 30 dpi (Fig. 1a). In order to further explore the structure and function of OsSLR1, we selected the DELLA homologous genes AtRGA, AtGAI, AtRGL1, AtRGL2, AtRGL3 in *Arabidopsis*, and the only DELLA protein OsSLR1 in rice for domain analysis. Each of the five DELLA proteins in *Arabidopsis* shows a different role in a specific process, and in some cases there is functional redundancy, so its conserved domains are slightly different. Analysis of the protein sequence showed that the N-terminal region of the DELLA protein in *Arabidopsis* and rice contained the DELLA domain and TVHYNP motif, and the C-terminal region contained a GRAS domain. (Fig. 1b). We next constructed a fusion protein of OsSLR1 with C-terminal green fluorescent protein (GFP) to determine the subcellular localization of OsSLR1 and used HA-GFP as a control. *Agrobacterium* mediated transient expression system was used to introduce OsSLR1-GFP and HA-GFP constructs into *N. benthamiana* cells. The result of subcellular localization showed that HA-GFP located in the cytoplasm and nucleus, and OsSLR1-GFP was located in the nucleus (Fig. S1).

Based on the above research results, we have further clarified the role that OsSLR1 plays in the process of SRBSDV infecting rice. We constructed transgenic rice lines overexpression of *OsSLR1*, and the transgenic expressing the *OsSLR1* gene was created in *LS* background, and the expression level of *OsSLR1* was analyzed by RT-qPCR (Fig. S2). We next used homozygous T3 generation transgenic rice lines *OsSLR1-ox* and wildtype *LS* to challenge with SRBSDV-infected or virus-free insect vector. After about 30 dpi of growth in the field, our investigation revealed that SRBSDV infected-*LS* plants exhibited the typical stunted, leaf dark green, stiff and have excessive tillering symptoms in wildtype plants, but symptoms were milder in the *OsSLR1-ox* plants (Fig. 1c). RT-qPCR assay showed that the accumulation of SRBSDV RNA (*S2* and *S10*) in the *OsSLR1-ox* plants was significantly lower than that in *LS* plants (Fig. 1d) and western blotting assay showed that the accumulation of SRBSDV protein (P10) in the *OsSLR1-ox* plants was significantly lower than that in *LS* plants (Fig. 1e). Together, these results indicated that OsSLR1 plays positive roles in rice's defensive response against SRBSDV infection.

Overexpression of *OsSLR1* reprograms transcriptome and activates multiple defense responses in rice

To delve deeper into the functional roles of OsSLR1 in the molecular biological processes, transcriptome analysis was performed in wild-type *LS* and Os*SLR1-ox* rice plants grown naturally in the field for 30 dpi. We established the threshold by setting the significance fold change (FC > 2) or FC < 0.5 ($P < 0.05$), to screen for differentially expressed genes (DEGs). We identified 2336 differentially expressed genes (DEGs) in total: 1607 upregulated and 729 downregulated in *OsSLR1-ox* compared with *LS* plants (Fig. 2a). Hierarchical clustering analysis showed that the expression of DEGs. Interestingly, based on hierarchical clustering analysis, we found that 68.79% (1607/2336) genes were activated and 31.21% (729/2336) genes were suppressed in *OsSLR1-ox* compared with *LS* plants (Fig. 2b). These analyses

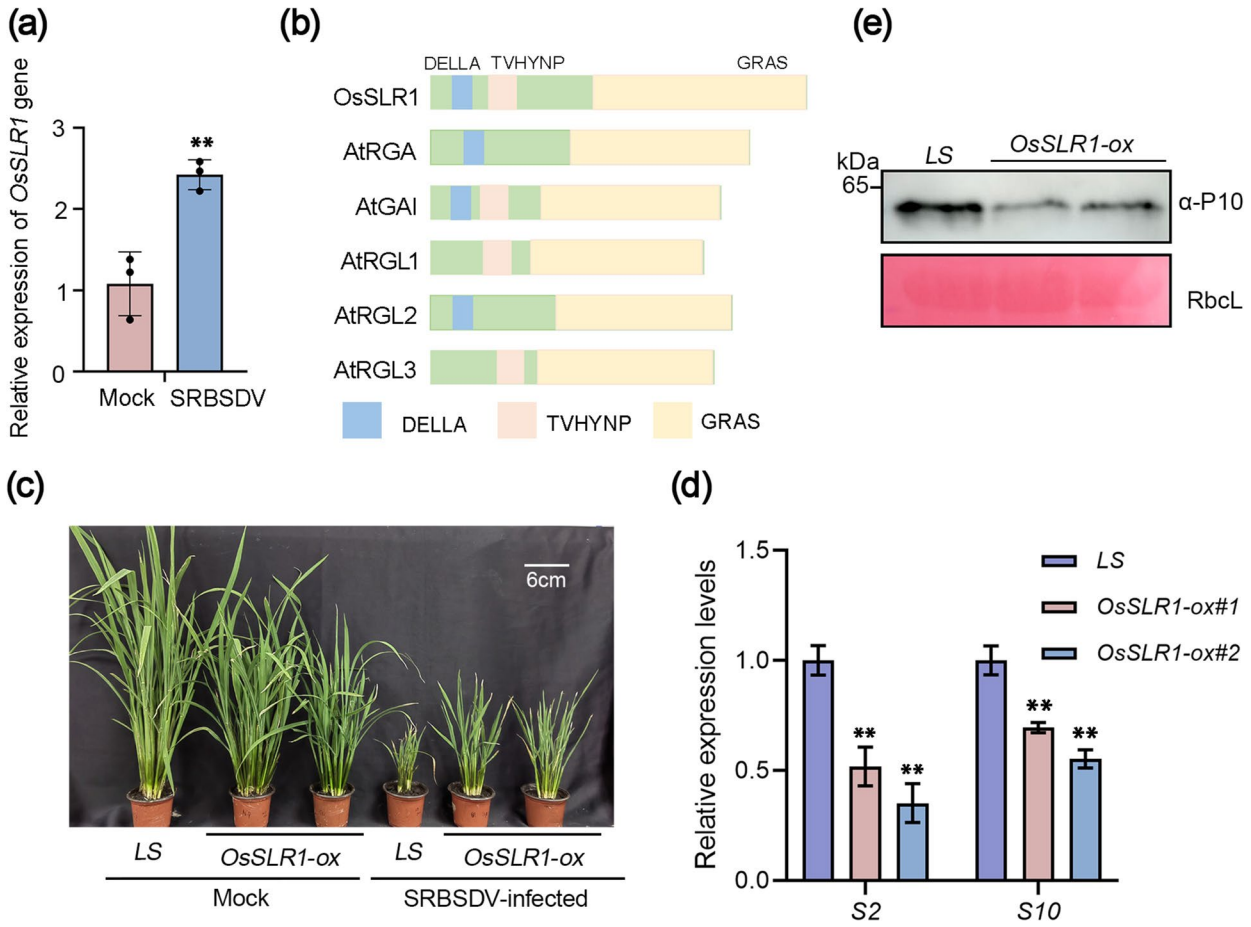

Fig. 1 Overexpression of *OsSLR1* increased the resistance of rice to SRBSDV. **a** RT-qPCR analysis of *OsSLR1* gene expression levels in SRBSDV-infected and MOCK plants. Significant differences were identified using Student's *t*-test, all values are presented with mean ± standard deviation of 3 biological replicates. Asterisks indicate significant differences (**P* < 0.05, ***P* < 0.01). **b** Domain prediction for OsSLR1, AtGAI, AtRGA, AtRGL1, AtRGL2, AtRGL3. **c** Symptoms in *LS* and *OsSLR1-ox* lines following mock-inoculation or SRBSDV infection. Scale bar = 6 cm. **d** RT-qPCR results showing the relative expression of viral RNA (two different SRBSDV genomic RNA segments *S2* and *S10*). *OsUBQ5* was used as the internal reference gene. Significant differences were identified using Student's *t*-test, all values are presented with mean ± standard deviation of 3 biological replicates. Asterisks indicate significant differences (**P* < 0.05, ***P* < 0.01). **e** Western blot to assess the accumulation of SRBSDV P10 in SRBSDV-infected *OsSLR1-ox* lines and *LS* at 30 dpi. The RbcL was used as an internal reference

demonstrated that the majority of DEGs were activated for expression in *OsSLR1-ox* plants.

To further determine the biological significance of OsSLR1, we performed GO analysis for all DEGs. GO analysis showed a significant abundance of defense-related categories, such as response to abscisic acid, response to jasmonic acid, induced systemic resistance, peroxidase activity and response to oxidative stress (Fig. 2c). Overall, these results suggest that overexpression of *OsSLR1* gene affects transcription levels in a large number of defense-related genes.

OsSLR1 activates JA signaling pathway in rice

Our previous studies have reported that OsSLR1 hijacks the JA negative regulator OsJAZs and activates the transcription of downstream OsMYC2/3, thereby activating the JA signaling pathway in rice [6]. Our further hierarchical clustering analysis showed that the JA-related genes were significantly upregulated in *OsSLR1-ox* plants compared with *LS* plants, indicating that OsSLR1 affected the expression of genes related to the JA pathway at the transcriptional level (Fig. 3a). Next, we extracted total RNA from *LS* and *OsSLR1-ox* seedlings that had grown for 10 dpi and detected the expression levels of JA-related genes. RT-qPCR assay showed that *OsAOC*, *OsCOI1a*, *OsCOI1b*, *OsJamyb*, *OsJAZ4* and *OsJAZ11* genes were significantly upregulated in *OsSLR1-ox* plants compared with wild-type *LS* (Fig. 3b-g). Moreover, JA-responsive genes *OsPR1a* and *OsPR1b* were significantly upregulated in *OsLSR1-ox* plants compared with wild-type *LS*

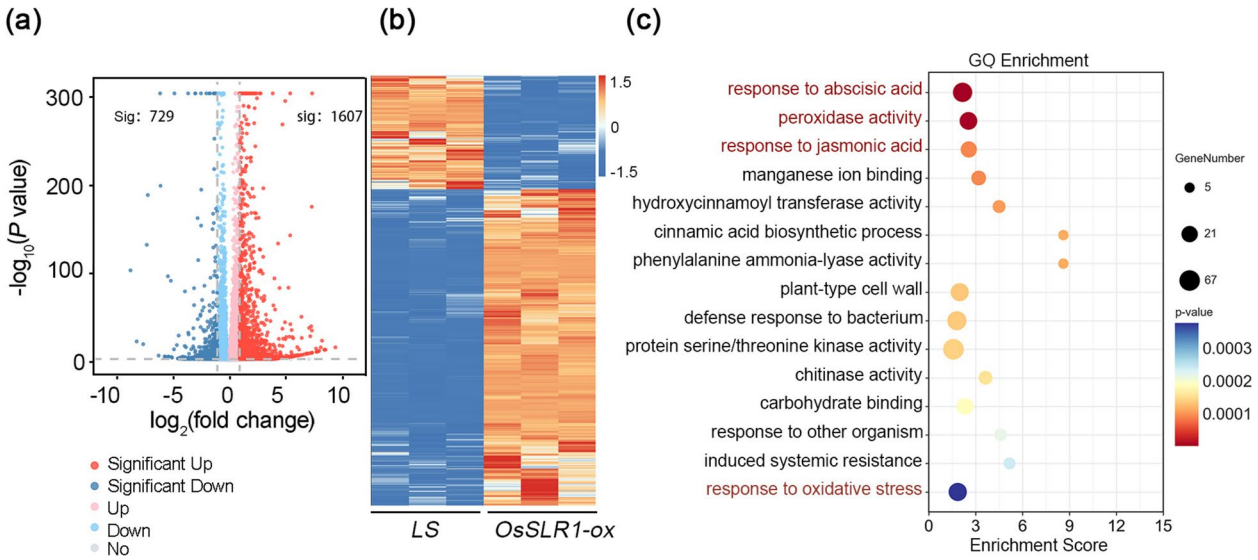

Fig. 2 Transcriptomic analysis of regulatory gene expression profiles in plants overexpressing *OsSLR1* (**a**) Volcano plot showing all DEGs in *OsSLR1-ox* compared with *LS* plants. The threshold standards were set at a fold change (FC) > 2 or FC < 0.5 and $P < 0.05$. **b** Hierarchical clustering showing all the DEGs. **c** GO showing all the DEGs, defense-related genes are highlighted in red

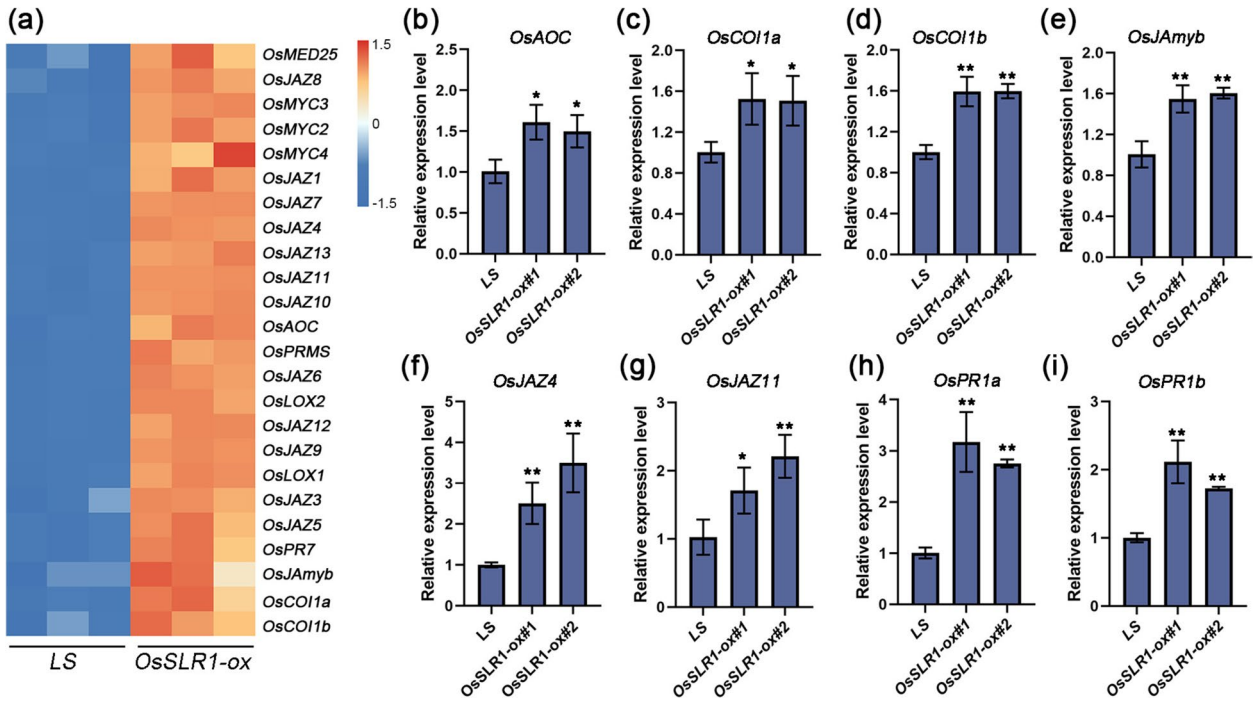

Fig. 3 Overexpression of Os*SLR1* activates the JA pathway. **a** Hierarchical clustering analysis of JA-related genes. **b**-**i** RT-qPCR analysis the expression levels of JA-related genes in *LS* and *OsSLR1-ox* plants. *OsUBQ5* is used as an internal reference gene to normalize relative expression. Significant differences were identified using Student's *t*-test, all values are presented with mean ± standard deviation of 3 biological replicates. Asterisks indicate significant differences (*$P < 0.05$, **$P < 0.01$)

(Fig. 3h-i). These results implied that the JA pathway was activated in *OsSLR1-ox* plants.

OsSLR1 negatively regulate ABA signaling pathway in rice

Our previous study demonstrated that ABA negatively regulate rice resistance to rice black-streaked dwarf virus (RBSDV, together with SRBSDV belongs to the second group of the genus *Fijivirus*, family *Reoviridae*) by inhibiting the JA pathway and ROS production [45]. Through hierarchical clustering analysis of the transcriptome data, we found that ABA-related genes were significantly downregulated in *OsSLR1-ox* plants (Fig. 4a). We further extracted total RNA from *LS* and *OsSLR1-ox* seedlings that had grown for 10 dpi and detected the expression levels of ABA-related genes. RT-qPCR assay showed that the ABA-related genes Os*AAO2*, Os*AAO3*, Os*LEA3*, Os*PYL3*, Os*ABIL4* and Os*AP2-39* were significantly downregulated compared with wild-type *LS* (Fig. 4b-g). These results indicated that the ABA pathway was suppressed in *OsSLR1-ox* plants.

OsSLR1 activates ROS pathway in rice

Our transcriptome analysis findings indicated that, beyond its participation in the JA and ABA pathways, OsSLR1 potentially plays a role in the generation of ROS (Fig. 2c). Upon encountering pathogen assaults, plants generate substantial ROS, triggering an ROS burst and initiating the plant's defensive reaction against pathogens [48, 49]. Therefore, we conducted a hierarchical clustering analysis on ROS-related genes and found that the expression levels of ROS-related genes were significantly upregulated in *OsSLR1-ox* plants (Fig. 5a) Consistent with this finding, RT–qPCR assay indicated that ROS-related genes expression levels including *OsRBOHA*, *OsRBOHB*, *OsRBOHD*, *OsRBOHF*, *OsRBOHH*, and *OsRBOHI* were significantly upregulated in *OsSLR1-ox* plants compared to wild-type *LS* (Fig. 5b-g). To observe ROS levels, rice seedling leaves of *LS* and *OsSLR1-ox* were stained with 3,3′-diaminobenzidine (DAB). Leaves of *OsSLR1-ox* plants showed deeper staining compared to wild-type *LS* (Fig. 5h). Moreover, nitroblue tetrazolium (NBT) staining was conducted to investigate ROS

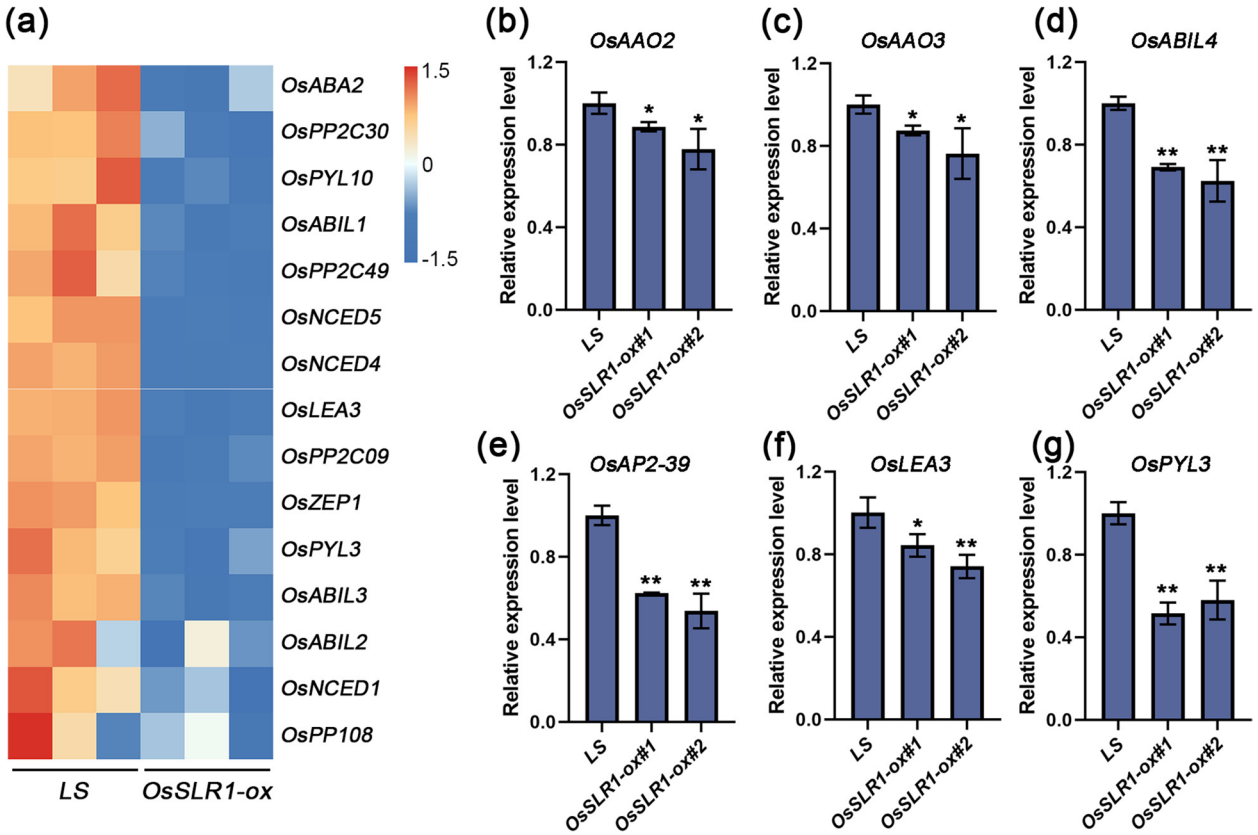

Fig. 4 Overexpression of Os*SLR1* suppresses the ABA pathway. **a** Hierarchical clustering analysis of ABA-related genes. **b**-**g** RT-qPCR analysis the expression levels of six ABA-related genes in *LS* and *OsSLR1-ox* plants. *OsUBQ5* is used as an internal reference gene to normalize relative expression. Significant differences were identified using Student's *t*-test, all values are presented with mean ± standard deviation of 3 biological replicates. Asterisks indicate significant differences (**P* < 0.05, ***P* < 0.01)

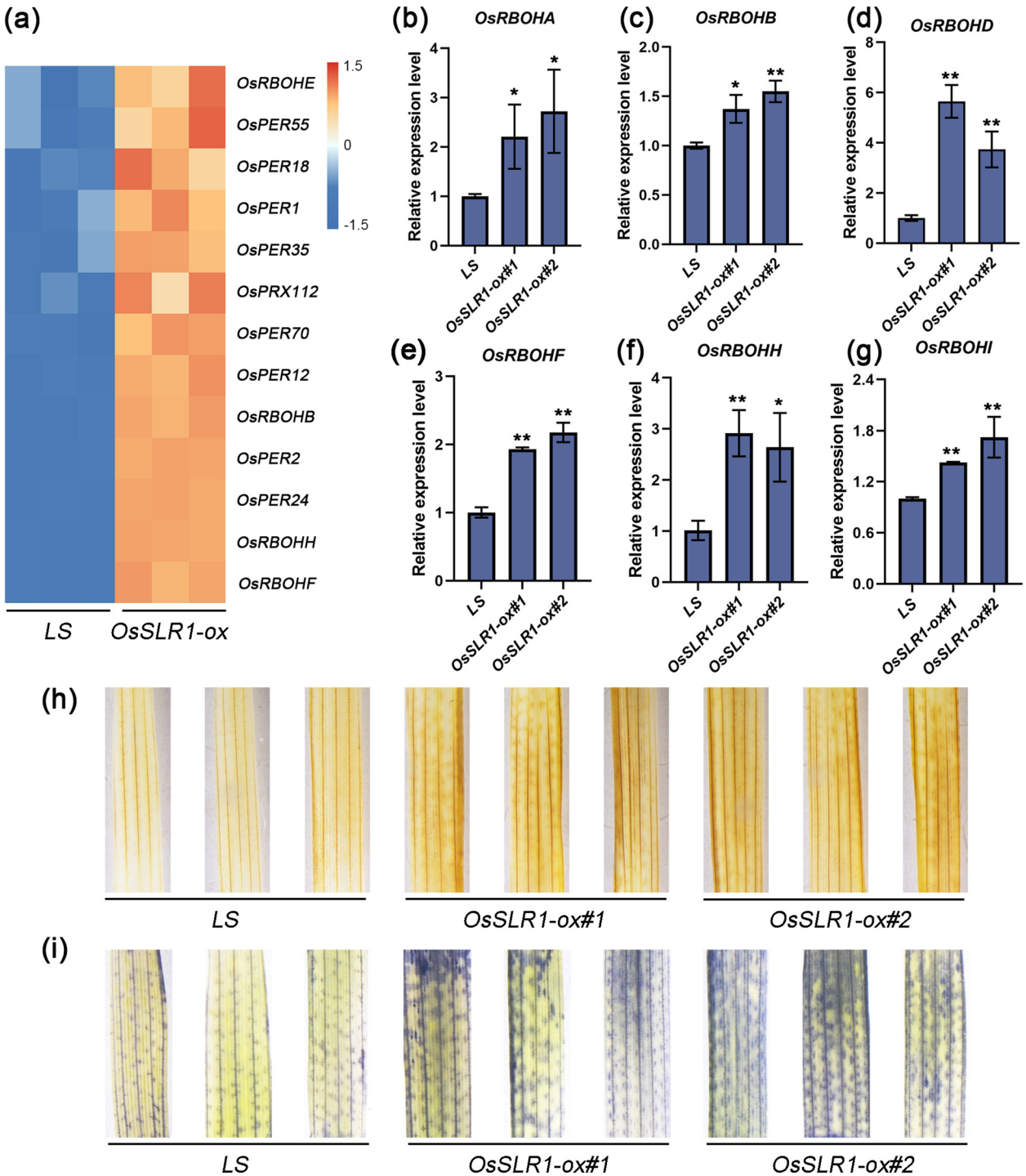

Fig. 5 Overexpression of Os*SLR1* activates the ROS pathway. **a** Hierarchical clustering analysis of ROS-related genes (**b**-**g**) RT-qPCR analysis the expression levels of six ROS-related genes in *LS* and *OsSLR1-ox* plants. *OsUBQ5* is used as an internal reference gene to normalize relative expression. Significant differences were identified using Student's *t*-test, all values are presented with mean ± standard deviation of 3 biological replicates. Asterisks indicate significant differences (**P* < 0.05, ***P* < 0.01). **h** Diaminobenzidine (DAB) staining in 10 dpi rice seedlings of *LS* and *OsSLR1-ox* plants. The representative samples were shown. **i** Nitroblue tetrazolium (NBT) staining in 10 dpi rice seedlings of *LS* and *OsSLR1-ox* plants. The representative samples were shown

production, revealing that rice leaves of *OsSLR1-ox* plants showed more ROS accumulation compared to *LS* (Fig. 5i). These results indicate that ROS pathway was activated in *OsSLR1-ox* plants.

Discussion

Plant hormone signaling networks play an important role in resisting the invasion of pathogens [12]. Crosstalk between hormones is essential for plant growth and adaptation to environmental stresses [50]. The SA signaling key transcription factor OsNPR1 activates JA signaling and enhances the antiviral response by disrupting the OsJAZ-OsMYC complex and facilitating the transcriptional activation activity of OsMYC2 in rice [51]. As a master negative regulator in the GA signaling, DELLA not only promotes the expression of the JA-responsive genes *AtLOX3*, *AtLOX4*, *AtJAZ1* and *AtJAZ3* at the transcriptional level, but also enhances the transcriptional regulation of AtMYC2 thereby activating JA signaling in *Arabidopsis* [52]. In addition, JA promotes the accumulation of RGL3 (DELLA protein in *Arabidopsis*) through direct binding of MYC2 to the RGL3 promoter. Meanwhile, RGL3 promotes plant resistance to *B. cinerea* through positive regulation of JA signaling in *Arabidopsis* [53]. Previous study revealed that antagonistic interactions between JA and GA signaling are regulated through OsJAZ9 and OsSLR1(DELLA proteins in rice) in rice [54]. Our previous study found that OsSLR1, activates the JA antiviral response by competing with OsMYC2/3 for binding to the JA signaling negative regulator OsJAZs protein [6]. In this study, RNA-seq and RT-qPCR analysis found that OsSLR1 also enhance the expression level of JA-related genes at transcript level in rice, which was consistent with previous studies.

The presence of ABA and GA antagonized the interaction during most stages of plant growth and development. For example, the central factor in GA/ABA homeostasis and antagonism in post-germination stages ABA-INSENSITIVE 4 (ABI4), enhances ABA biosynthesis and GA catabolism in *Arabidopsis* [55]. NAC (NAM, ATAF1/2, CUC2) transcription factor gene OsNAC120 plays a crucial role in maintaining the balance between GA-mediated growth and ABA-induced drought tolerance by transcriptionally activating the GA biosynthetic genes *OsGA20ox1* and *OsGA20ox3* in rice and transcriptionally repressing the ABA biosynthetic genes *OsNCED3* and *OsNCED4* in rice [50]. On the contrary, ABA and GA played a synergistic role during ground tissue (GT) maturation, simultaneous administration of ABA and GA can synergistically suppress perineural asymmetric cell divisions (ACDs) of precocious maturation in the endothelial layer compared to administration of ABA or GA alone [56]. However, it is unclear whether ABA and GA crosstalk in rice regulate rice antiviral immunity. In order to reveal the mechanism of ABA and GA negative regulator OsSLR1 in regulating SRBSDV infection in rice, RNA-seq analysis was performed. It was observed that overexpression of *OsSLR1* significantly downregulated ABA pathway-related genes, and rice was more resistant to SRBSDV infection (Fig. 4a). This implies that GA may synergistically suppress plant immunity in conjunction with ABA.

During the growth and development of plants, they inevitably encounter the invasion of various pathogens, and initiate a series of complex defense mechanisms to fend off these invaders, with the activation of the ROS-related pathway being among them [23, 57]. The burst of ROS not only induce programmed cells death and thus suppress the spread of pathogens, but also act as signaling molecules to transmit signals to activate immunity, thereby giving the host plant the ability to resist pathogens [21, 22, 24]. Plant extracellular ROS signaling is mainly produced by plasma membrane nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [58, 59]. The receptor-like cytosolic kinase RIPK promotes ROS production and facilitates host immune defense response by phosphorylating an NADPH-generating enzyme NADP-ME2 and enhancing vitro activity in *Arabidopsis* [26]. Also, CYSTEINE-RICH RLK2 (CRK2) positively regulates the resistance to *Pseudomonas syringae* pv tomato DC3000 by phosphorylating the NADPH oxidase RESPIRATORY BURST OXIDASE HOMOLOG D (RBOHD) and inducing ROS production in *Arabidopsis* [60]. In addition, PBL13 interacting RING domain E3 ligase (PIRE) regulates ROS production by regulating polyubiquitination of RBOHD and promoting RBOHD accumulation in *Arabidopsis* [61]. Therefore, in this study, we focused on the peroxidase activity. Interestingly, we observed the expression levels of *OsRBOHD* was induced in *OsSLR1-ox* rice plants by RT-qPCR analysis (Fig. 5d). Previous study reported that DELLA protein inhibit plant growth and enhance resistance by regulating ROS content and activating plant defenses [62]. However, it has not been reported whether OsSLR1 regulates ROS in rice. In this study, we found that the expression of ROS-related genes in *OsSLR1-ox* rice plants was upregulated (Fig. 5b-g), and we hypothesize that OsSLR1 may be involved in rice resistance to SRBSDV by activating plant immunity through ROS.

Plants have evolved complex mechanisms to regulate the balance between growth and defense [63]. There is growing evidence that plant hormone interactions play an important role in this trade-off, such as the growth-related hormones GA, auxin, BR and defense-related JA, SA, etc. A type of mutant with an INDEL that yielded a novel frameshift protein named FJ10

(Frameshift mutation of JAZ10) enhance rice growth and increase resistance to brown planthopper by competing with OsPIL16 for binding to OsSLR1 and suppressing F-box E3 ligase OsFBK16-mediated PAL6 degradation in rice [64]. In rice, BR regulates many important agronomic traits, and crosstalk between BR and GA plays an important role in balancing growth and defense [65]. In our study, we also found that BR-related genes were also significantly upregulated in *OsSLR1-ox* plants. We hypothesized that OsSLR1 coordinate the balance between growth and plant immunity through BR signaling pathway and JA signaling pathway crosstalk. However, the specific regulatory mechanism needs to be further explored.

Conclusions

In conclusion, we report a novel mechanism by which OsSLR1 regulates resistance to SRBSDV infection in rice. *OsSLR1* was significantly upregulated after SRBSDV infected. GO enrichment analysis and RT-qPCR analysis revealed that genes related to JA and ROS were significantly upregulated, while genes associated with ABA were significantly downregulated. On the other hand, overexpression of *OsSLR1* in rice enhance the resistance of rice to SRBSDV. These results reveal that OsSLR1 positively modulates the antiviral immunity of rice by suppressing the ABA pathway and activating the ROS production and JA pathway. This will help us to improve the construction of OsSLR1 regulatory host immune signaling network.

Abbreviations

GA	Gibberellin
SLR1	SLENDER RICE 1
JA	Jasmonic acid
SRBSDV	Southern rice black-streaked dwarf virus
DEGs	Differentially expressed genes
GO	Gene ontology
ROS	Reactive oxygen species
ABA	Abscisic acid
SA	Salicylic acid
BR	Brassinosteroid
RBOHs	Respiratory burst oxidase homologs
WBPH	White-backed planthopper
LS	*Lansheng*
NIP	*Nipponbare*
CDS	Coding sequence

Supplementary Information

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Supplementary Material 1.

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Authors' contributions

J.D., J.C. and Z.S. conceived and designed the project.J.D. performed the experiments. J.D., Z.Y., L.L., Y.L., H.Z., J.C. and Z.S. analyzed data. J.D. wrote the manuscript. Z.Y., L.L. and Z.S. supervised and revised the manuscript. All authors read and approved the manuscript.

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Data availability

The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2021) in National Genomics Data Center (Nucleic Acids Res 2022), China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA023483) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa. Please access it from the following link: https://bigd.big.ac.cn/gsa/browse/CRA023483.

Declarations

Ethics approval and consent to participate

Our study did not involve any human or animal subjects, material, or data. We declare that the plant material in the experiment was collected and studied by relevant institutional, national, and international guidelines and legislation.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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