BRS1 mediates plant redox regulation and cold responses

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Abstract

Background: Brassinosteroid-insensitive 1 suppressor 1 (BRS1) is a serine carboxypeptidase that mediates brassinosteroid signaling and participates in multiple developmental processes in Arabidopsis. However, little is known about the precise role of BRS1 in this context.

Results: In this study, we analyzed transcriptional and proteomic profiles of Arabidopsis seedlings overexpressing BRS1 and found that this gene was involved in both cold stress responses and redox regulation. Further proteomic evidence showed that BRS1 regulated cell redox by indirectly interacting with cytosolic NADP+ dependent isocitrate dehydrogenase (cICDH). One novel alternative splice form of BRS1 was identified in over-expression mutants brs1-1D, which may confer a new role in plant development and stress responses.

Conclusions: This study highlights the role of BRS1 in plant redox regulation and stress responses, which extends our understanding of extracellular serine carboxypeptidases.

Keywords: Serine carboxypeptidase, Cold stress response, Redox, Arabidopsis

Background

Serine carboxypeptidases (SCP) are a class of eukaryotic proteolytic enzymes, belonging to the α/β hydrolase family. These enzymes contain a highly conserved catalytic amino acid triad, Ser-Asp-His [1]. In Arabidopsis, 54 SCP-like genes have been identified and categorized into three classes [2, 3]. However, the functions of majority of these genes have not been described.

BRS1 belongs to class II of the Arabidopsis SCP family [1]. Its over-expression can suppress the phenotype of brassinosteroid (BR) receptor mutant, bri1-5, indicating BRS1 play an important role in BR signaling [4, 5]. In Arabidopsis, BRS1 has five close homologs, of which three can suppress bri1-5 developmental defects [6]. In contrast, no significant phenotypes have been identified in single or double mutants of either BRS1 or its homologs [5–7], indicating BRS1 and its homologs are functionally redundant in Arabidopsis.

Increasing evidence has revealed that SCP and SCP-like (SCPL) proteins play crucial roles in the regulation of stress responses, including regulating wound healing [8], programmed cell death and response to pathogen infections [9, 10]. BRS1 contains a signal peptide and localizes to the extracellular space [4, 5]. Since the extracellular space is central in regulating plant stress responses [11–13], it is likely BRS1 might mediate some of these signaling pathways. Then this hypothesis keeps in largely unknown in current understanding.

In this study, transcriptomic and proteomic analyses demonstrate a role of BRS1 in cold stress responses. Our results indicate BRS1 participates in redox regulation via interaction with cICDH, shedding new light on the function of SCPS in plant stress responses.

Results

Seedling developmental phenotypes of BRS1

The mutant brs1-1D has an enhancer in BRS1 promoter [4, 5], resulting in about 15 times increase in BRS1 transcription compared to that in wild type WS2 (Fig. 1a),...
and showing larger rosette leaves (Fig. 1b) and a longer hypocotyl (Supplementary figure S1). In contrast, BRS1 knockout mutant, brs1-1, showing a significant transcriptional decrease in BRS1 (Fig. 1a), but no significant phenotype was observed [4–7].

Analysis of the transcriptomes of BRS1 seedlings
The RNA sequencing was performed on WS2, brs1-1 and brs1-1D seedlings, and showing the BRS1 in brs1-1 has an insertion of one thymidine at position 533 in the first intron (Fig. 2a) and a significant decrease

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**Fig. 1** The seedling phenotypes in wild type and BRS1 mutants. a The relative expression level of BRS1 in wild type WS2, brs1-1 and brs1-1D. All results are shown as mean ± standard deviation (SD) from three biological replicates with qRT-PCR. The asterisks indicate a statistically significant difference (Student’s t-test, *p < 0.05). b The seedling phenotypes of WS2, brs1-1 and brs1-1D at 14 days after germination (DAG). Scale bar = 1 cm.

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**Fig. 2** Differences in gene sequences and BRS1 transcription in wild type and BRS1 mutants. a The sequence difference of BRS1 in WS2, brs1-1 and brs1-1D. The number above the exon represent the exon 1–9 from 5’ to 3’. A single thymidine base (red) is inserted in the first intron of brs1-1, and the 4 × 35S enhancer (four ellipses in black box) is inserted in the promoter of BRS1 in brs1-1D. b Transcription level (FPKM value) of BRS1 in WS2, brs1-1 and brs1-1D. All data were from RNA-seq and shown as mean ± SD. Three biological replicates were quantified. The asterisks indicate a statistically significant difference (Student’s t-test, *p < 0.05). c Alternative splicing event identification in brs1-1D. “isoform1” is the form in the TAIR 10 annotation. “isoform2” is newly identified in our result. d The relative percentages of isoform1 and isoform 2 in WS2 and brs1-1D. The asterisks indicate a statistically significant difference (**FDR-adjusted pvalue < 0.01**).
in transcription (Fig. 2b). Additionally, no increased transcription was observed on the homologs of BRS1 in brs1-1 (Supplementary figure S2), which indicates there is no transcriptional feedback loop among BRS1 and its homologs. In contrast, the transcriptional level of BRS1 increased by around 20 times in the brs1-1D mutant (Fig. 2b), which is consistent with the presence of four copies of CaMV 35S enhancers inserted in the promoter region of BRS1 [5].

Notably, one novel splice variant isoform was detected in brs1-1D, which had an alternative 5′ splice site (Fig. 2c). Compared with the isoform annotated in the TAIR 10 database, novel isoform had 11 bp less on the 5′ site of exon 8. This new splice product made up 5.02% of the total BRS1 transcripts in brs1-1D (Fig. 2d), but was not detected in wild type and brs1-1 mutant, indicating new transcriptional product exist in brs1-1D besides its transcriptional level change in brs1-1D.

**BRS1 mediates multiple plant stress responses**

With RNA sequencing, a total of 21,873 transcripts were identified, of which 180 were assigned as differentially expressed genes (DEGs, fold change ≥ 2.0, FDR-adjusted p-value < 0.05, FPKM ≥ 1) in brs1-1D compared to wild type, including 114 increased genes and 66 decreased genes (Table S1). In contrast, there were no DEGs identified in brs1-1 seedlings, except BRS1 itself (Table S1).

Gene Ontology (GO) enrichment analysis of DEGs from brs1-1D revealed significant enrichment of terms associated with responding to salicylic acid and jasmonic acid (Fig. 3a). We also found marked enrichment for genes involved in both biotic (innate immune responses, bacterium, fungus and chitin) and abiotic (water deprivation, cold and hypersosmotic salinity) stresses (Fig. 3a). In agreement with these findings, genes associated with redox regulation and cell death were also enriched. These results strongly suggest that BRS1 participates in environmental stress responses.

To support the role of BRS1 in stress response, qRT-PCR was performed on the genes involved in redox regulation, ethylene synthesis and cold response (Fig. 3b). This analysis verifies the transcriptional changes in different genotypes (Table S1), supporting that BRS1 has a role in stress response.

**Cold tolerance is significantly enhanced in brs1-1D**

Notably, three core genes involved in cold signaling, the C-repeat binding factors CBF1, CBF2 and CBF3 [14], all displayed significantly increased transcription in brs1-1D (Fig. 3b). We therefore checked the response of these genes to cold stress. The result showed all these cold transcriptional factors increased two times transcriptional level in brs1-1D (Fig. 4a) after a freeze treatment (4 °C for 3 h), compared to those in wild type plant. Consistently,
cold signaling downstream genes RD29A [15], COR413 [16], KIN2 [17] showed similar increase trend, while redox and ethylene signaling genes kept the same transcriptional level with wild type after freeze treatment (Fig. 4b), indicating the BRS1 has a role in enhancing the cold sensitivity.

Further, the phenotypes of seedling after severe cold shock (-6 °C for 2 h, 3 h and 4 h) were investigated (Fig. 5a), we found the root elongation of brs1-1D was significantly longer than the wild type at 36 h after cold shock (Fig. 5b), suggesting that the over-expression of BRS1 contributes to a higher cold tolerance.

![Fig. 4](image_url) The brs1-1D shows a higher cold sensitivity. The qRT-PCR was performed in each genotype with and without freeze treatment (4 °C for 3 h). The columns represent the fold change of gene expression after freeze treatment compared to untreated control. All results are shown as mean±SD from three biological replicates at least. The asterisks indicate a statistically significant difference (Student’s t-test, * p<0.05).

- **a** Cold signaling genes responses with freeze treatment.
- **b** Stress related genes response with freeze treatment. Including ethylene synthesis related genes (ACS4 and ACO3) and redox related gene PER10

![Fig. 5](image_url) The phenotypes of seedling in cold treatment. 7 DAG seedlings were shifted to -6 °C for 2, 3 and 4 h and then returned to normal conditions (22 °C) for 36 h.

- **a** The phenotypes of WS2, brs1-1 and brs1-1D grown on 1/2 MS medium under normal conditions and with cold treatment for 2 h, 3 h and 4 h. Scale bar = 2 cm. The black dots on the plate represent the growth position of the seedlings before the cold treatment.
- **b** The root elongation in WS2 and brs1-1D after a severe cold treatment. The root elongation was measured at 36 h after cold shock. Data is from three biological replicates. The same results were repeated at least two times. The asterisks indicate a statistically significant difference (Student’s t-test, ** p<0.01)
BRS1 regulates redox-related proteins

To further understand the function of BRS1, proteomic analysis was performed on WS2, brs1-1 and brs1-1D seedlings using two-dimensional fluorescence difference gel electrophoresis (2D-DIGE) (Fig. 6a). Nineteen proteins were assigned as differentially expressed proteins (t-test, \( p \) value < 0.01), and 15 proteins were identified, of which 5 proteins were associated with redox regulation (Table 1), supporting that BRS1 is involved in redox regulation.

Notably, two protein spots (6 and 7) with significantly changed intensities compared with wild type (Fig. 6b), showing a decrease and increase in expression in brs1-1D, respectively (Fig. 6c), but corresponded to the same protein, the cytosolic NADP+ dependent isocitrate dehydrogenase (cICDH, at1g65930) (Table 1), a critical redox regulator [18]. Together, these data suggests that BRS1 plays important role in redox regulation.

BRS1 participates in redox regulation by interacting with cICDH

To confirm that BRS1 regulates cICDH, the enzyme activity of cICDH was measured in BRS1 mutants. We found that cICDH activity was significantly increased in brs1-1D compared to wild type plants (Fig. 7a), whilst no change was observed in brs1-1 plants. These findings were consistent with our earlier results that only overexpression of BRS1 can alter transcription and phenotypes. Altogether, this suggests that BRS1 regulate the activity of cICDH.

To investigate how the secretory protein BRS1 regulate cICDH, which is localized to the cytosol [19, 20], the cellular localization of BRS1 was evaluated. We observed the expression of BRS1 at the membrane and cell wall (Fig. 7b), which was consistent with the localization to the extracellular space [4, 5], indicating there is not a
physical contact between BRS1 and cICDH. Consistently, yeast two-hybrid and pull-down assays found no direct interaction between cICDH and BRS1 (Table S2), indicating that BRS1 regulates cICDH indirectly.

**Discussion**

BRS1, as a member of SCP, its role is relative clear in SCP family, and its function in plant growth and development has been demonstrated previously [5–7]. In this study, the transcriptomic and proteomic analyses demonstrate BRS1 also has a broad role in biotic and abiotic stress response.

Our results suggest that SA and JA signaling, which are key pathways required for the induction of plant defenses in response to pathogens and insects [21, 22], also contribute to the BRS1-related stress response (Fig. 3a). Similarly, an apoplastic SCPL in rice, OsBISCPL1, also induced stress responses via SA and JA signaling [9], indicating SCPLs may regulate stress responses in a same mechanism.

| No | Chromosome locus | Matched protein | Biological process | Expression Fold |
|----|------------------|-----------------|--------------------|-----------------|
| 1  | AT3G08590        | 2,3-Biphosphoglycerate-independent phosphoglycerate mutase 2 | Carbohydrate metabolism | 1.18 |
| 2  | AT3G57610        | Adenylosuccinate synthetase | AMP biosynthesis | 0.87 |
| 3  | AT3G54050        | Chloroplastic fructose 1,6-bisphosphate phosphatase | Fructose metabolism | 0.88 |
| 4  | AT2G39730        | Rubisco activase | Light activation of rubisco | 1.21 |
| 5  | AT5G15650        | UDP-arabinose mutase | Arabinose metabolism | 0.68 |
| 6* | AT1G65930        | Cytosolic NADP + dependent isocitrate dehydrogenase | Redox | 0.22 |
| 7* | AT1G65930        | Cytosolic NADP + dependent isocitrate dehydrogenase | Redox | 3.12 |
| 8  | AT1G03475        | Coproporphyrinogen III oxidase | | 1.15 |
| 9  | AT5G09530        | Proline-rich protein 10 | Seed germination | 1.24 |
| 10 | AT1G66200        | Cytosolic glutamate synthetase | Glutamine biosynthesis | 1.79 |
| 11 | ATCG00490        | Ribulose-bisphosphate carboxylase | Carbon fixation of photosynthesis | 0.79 |
| 12 | AT3G44310        | Nitrlase 1 | Nitrogen compound metabolism | 1.37 |
| 13 | AT1G75280        | Isoflavone reductase | Redox | 1.26 |
| 14 | AT1G78380        | Glutathione s-transferase tau 19 | Redox | 1.16 |
| 15 | AT1G20340        | DNA-damage resistance protein 112 | Response to UV | 0.67 |

The differentially expressed proteins were assigned according to the *p* < 0.01 (Student's *t*-test, two tails). Proteins in bold refer proteins are involved in redox. "Expression Fold" is calculated as the ratio of the expression level in brs1-1D to the control WS2. The asterisks indicate two differentially expressed proteins of cICDH. Triplicate biological repeats were performed with independent protein preparations.
The trigger effector production by apoplastic proteases used be the key mechanism to induce plant stress response [13]. Our results shows that both cold sensitivity and cold tolerance are significantly enhanced with BRS1 overexpression, then the underlying mechanism need be further investigated to clarify the precise role of BRS1 in cold perception and/or cold signaling.

Consistent with this notion, we found BRS1 participates in regulating redox homeostasis by indirectly interacting cICDH. The ICDH catalyze the production of NADPH, which is important for redox regulated cell metabolism and promoting redox signaling in response to oxidative stress [23, 24]. cICDH is responsible for more than 90% of total ICDH activity [23, 25]. Therefore, this enzyme plays a crucial role in maintaining redox homeostasis in the cell, and consequently, defense responses [18]. Exactly how BRS1 participates in this pathway still needs further investigation. Moreover, the two isoforms of ICDH showed similar molecular weights but different isoelectric points, implying they may be the different forms produced by protein modification, as described in previous report [26].

The knockout mutant of BRS1 does not have any significant phenotypes [5–7], consistently, no DEGs were identified in the mutant brs1-1 (Table S1). The existence of multiple redundant homologs in Arabidopsis may explain this result. In contrast, we observed many phenotypes [5–7] and identified significant alterations in gene expression upon overexpression of BRS1 in this study. The novel splice variant identified in this study provides a new explanation for significant changes in phenotypes and transcriptional changes in brs1-1D. However, it still needs further investigations to clarify.

**Conclusions**

In this study, transcriptomic and proteomic analyses revealed that BRS1 plays a role in regulating plant responses to cold stress and redox response. We found that BRS1 likely participates in redox regulation in cells through indirect interaction with cICDH. Altogether, our work sheds new light on the roles of SCPs in biotic and abiotic stress responses.

**Methods**

**Plant Materials and Growth Condition**

Wild-type Wassilewskija (WS2) and mutants of BRS1 (brs1-1 and brs1-1D). The brs1-1D is generated by crossing WS2 with an activation-tagging line bri1-5 brs1-1D (CS6127) from the Dr. Jia Li (Lanzhou University, Lanzhou, China) [4, 5, 7]. The brs1-1 obtained from the Wisconsin Arabidopsis knockout pool and identified insertion a thymidine at 533 bp in first intron in this study. T-DNA insertion mutant icdh-2 (SALK_056247) was ordered from the Salk Institute collection of Arabidopsis Biological Resource Center (ABRC) and verified by genotyping as previously described [18]. Theses seeds were grown in growth chamber at 22 °C under 16 h light/8 h dark conditions (light intensity 100 μmol·m⁻²·s⁻¹, humidity 60%) for two weeks after germination to perform phenotypic observation. Seedlings of the three materials were cultured on half-strength Murashige and Skoog (1/2 MS) agar medium (supplemented with 1% (w/v) sucrose and 0.8% (w/v) agar, PH 5.6–5.8) in the same chamber and culture conditions, the total protein and RNA were extracted seven days after germination.

**RNA Extraction and Gene Expression Profiling**

Seven-day-old WS2, brs1-1, and brs1-1D seedlings grown vertically on a 1/2 MS plate were frozen in liquid nitrogen, and triplicate of each material were collected. Total RNA was extracted from whole seedlings using a Tiangen RNAprep pure Plant Kit, and its quality was evaluated with Thermo Scientific NanoDrop2000. The RNA sequencing was completed by Biomarker company (Beijing, China).

**Identification of Differentially Expressed Genes (DEGs) and GO enrichment analysis**

Raw transcriptome sequencing data cleaned using Trimomatic (v 0.36). The clean reads were aligned to the Arabidopsis thaliana reference genome (TAIR 10) using HISAT2 (v 2.1.0) and the expression of genes were profiled using StringTie (v1.3.3) [27, 28].

Identification all of differential genes in brs1-1 and brs1-1D compared to control WS2, respectively. The corrected read count data of genes were imported into the R package DESeq2 (v1.26.0) [29] to identify DEGs with the standard of a fold change ≥ 2.0, a false discovery rate (FDR)-adjusted p-value < 0.05, and expression (FPKM ≥ 1) in at least one sample for each comparison.

The GO descriptions were obtained by Annotation-Hub ("AH75734"), and used the R package clusterProfiler (v3.14.0) [30] with the “enrichGO” function for GO enrichment analysis. The statistical significance of the enrichment of GO was examined using the hypergeometric distribution test, followed by multiple-test correction using the Benjamini–Hochberg method. GO terms with q-value < 0.01 for further analysis.

**Alternative splicing analyses**

Clean reads from all samples were aligned to the Arabidopsis thaliana reference genome (TAIR 10) using STAR (v 2.7.3a) [31]. Regtools (v0.5.2) were used to extract exon-exon junctions from RNA-seq BAM files with the
performed. Sample, at least quadruplicate protein preparations were immediately or were stored in aliquots at -80 °C. For each standard [33]. The final proteins underwent 2D-DIGE proteins via Bio-Rad Bradford method using BSA as a standard [33]. The pH of protein samples was adjusted to 8.5 with HCl and NaOH, and the concentration of proteins was determined by Bio-Rad Bradford method using BSA as a standard [33]. The supernatant was collected by centrifugation at 18,000 g for 20 min at 4 °C, and the pellet was resuspended in 3 mL extract buffer for repeat extraction. The combined supernatant was supplemented with chilled acetone to 80% (V/V) (4 times volume acetone of the supernatant) and incubated at -20 °C overnight to precipitate proteins. Proteins were pelleted by dissolved in 100 μL lysis buffer (7 M urea, 2 M thiourea, 4% w/v CHAPS, 20 mM Tris–HCl, pH 8.5), and the debris was removed by centrifugation at 18,000 g for 20 min. Finally, the pH of protein samples was adjusted to 8.5 with HCl and NaOH, and the concentration of proteins was determined by Bio-Rad Bradford method using BSA as a standard [33]. The final proteins underwent 2D-DIGE immediately or were stored in aliquots at -80 °C. For each sample, at least quadruplicate protein preparations were performed.

Protein Preparation for Two-Dimensional Fluorescence Difference Gel Electrophoresis (2D-DIGE) Analysis

Seven-day-old seedlings (1 g) were harvested and ground into fine powder in liquid nitrogen and further mixed with 4 mL ice-cold extract buffer (20 mM Tris–HCl, PH 8.0, 1 mM EDTA, 20 mM NaCl, 5 mM MgCl₂, 10 mM DTT, 2 mM phenylmethanesulfonyl fluoride, 1 μg/mL leupeptin, 10 μg/mL aprotinin, 1 μg/mL chymostatin and 1% phosphorylase inhibitor mixture). The supernatant was collected by centrifugation at 18,000 g for 20 min at 4 °C, and the pellet was resuspended in 3 mL extract buffer for repeat extraction. The combined supernatant was supplemented with chilled acetone to 80% (V/V) (4 times volume acetone of the supernatant) and incubated at -20 °C overnight to precipitate proteins. Proteins were pelleted by dissolved in 100 μL lysis buffer (7 M urea, 2 M thiourea, 4% w/v CHAPS, 20 mM Tris–HCl, pH 8.5), and the debris was removed by centrifugation at 18,000 g for 20 min. Finally, the pH of protein samples was adjusted to 8.5 with HCl and NaOH, and the concentration of proteins was determined by Bio-Rad Bradford method using BSA as a standard [33]. The final proteins underwent 2D-DIGE immediately or were stored in aliquots at -80 °C. For each sample, at least quadruplicate protein preparations were performed.

2D-DIGE and Image Analysis

According to the manufacturer’s instructions (GE Healthcare), the equivalent amounts of brs1-1 and brs1-1D proteins were labeled with Cy3 and Cy5 minimum fluorescent dyes (400 pmol dye/50 μg protein), respectively. The internal standard WS2 protein was labeled with CY2 and mixed with two different labeled proteins in equal amounts. Adjust the mixed-labeled protein to a total volume of 450 μL with rehydration buffer (8 M urea, 13 mM DTT, 4% w /v CHAPS, 0.5% Pharmalyte pH 3–10), and then load on an IPG test strip holder containing an IPG test strip with 24 cm pH 4–7 linear gradient (GE Healthcare). Experimental methods of isoelectric focusing and SDS-PAGE as previously described [34]. To minimize systemic and inherent biological differences, it is recommended to combine four independent protein preparations for each sample [35].

Fluorescent images of gels were scanned by Typhoon 9400 scanner (GE Healthcare) and the images were analyzed using DeCyder 6.5 software in accordance with the DeCyder User Manual (GE Healthcare) [34]. Approximately 2000 spots were detected in each image, and then spots that showed significant differential expression were determined by ANOVA and Student's t-test (p < 0.05). 19 spots with significant differential expression were selected for mass spectrometric identification.

Protein Identification

Coomassie brilliant blue staining was performed on the scanned 2-D-DIGE gel, and then differential protein spots were found by position comparison, but it was difficult to detect proteins with low background expression. Therefore, a 2-DE gel prepared with 1 mg of internal standard protein was used for staining to show spots that could not be determined from the 2D-DIGE gel.

After 19 differential protein spots were excised from 2-D-DIGE gel, each spot was destained in destaining buffer (25 mM ammonium bicarbonate, 50% v/v acetonitrile). Destained spots were dehydrated by acetonitrile and spun-dry, and digested with sequencing grade modified trypsin (Roche) at 37°C for 16 h. The matrix-assisted laser-desorption ionization (MALDI) mass spectra were produced on an Ultroflex II MALDI time-of-flight/time-of-flight mass spectrometer (MALDI-TOF/TOF MS) (Bruker Daltonics, Germany) with use of FlexAnalysis 2.4 software. After tryptic peptide masses were transferred to a BioTools 3.0 interface (Bruker Daltonics), peptide mass fingerprintings (PMFs) were searched against the NCBInr protein database (http://www.ncbi.nlm.nih.gov/; NCBInr 20,711,214; 7,542,110 sequences) by use of Mascot software2.2.03(http://www.matrixscience.com; Matrix Science, London, U.K.).

Enzyme activities

Seedlings (0.1 g) at 7 days after germination were ground into fine powder in liquid nitrogen and mixed with the extract buffer (1 mL 0.1 M NaH₂PO₄ (pH 8.0), 5 mM MgCl₂, 14 mM 2-mercaptoethanol). Vortex the homogenate, centrifuge at 12,000 g for 5 min to remove insoluble materials, and measure ICDH activity by spectrophotometry [18, 20]. Determination of protein concentration as previously described Bio-Rad Bradford method using BSA as a standard [33].

Confocal imaging

For protein localization of BRS1, the corresponding seedlings root tips from transgenic Col-0 plants expressing 35S-BRS1-GFP were stained in 0.1 mg/
ml propidium iodide for 8 min. Seedlings were photographed using a confocal fluorescence microscope (Leica, TCS SP8). GFP was excited with a 488 nm laser and detected at 495–535 nm. Propidium iodide was excited with a 552 nm laser and detected at 530–680 nm. Images were further analyzed using Adobe Photoshop and ImageJ.

Abbreviations
SCP: Serine carboxypeptidase; BRS1: brassinosteroid-insensitive 1 suppressor 1; ROS: Reactive oxygen species; DEGs: Differentially expressed genes; FPKM: Fragments per kilobase of exon per million fragments mapped; SA: Salicylic acid; JA: Jasmonic acid; cICDH: Cytosolic NADP-dependent isocitrate dehydrogenase.

Supplementary Information
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Authors’ Contributions
SX conceived and designed the research and contributed to writing and revising the manuscript. DZ and YZ performed experimental work and data analysis. DZ and JW wrote and revised the manuscript. PZ performed bioinformatics analysis. All authors have read and approved the manuscript.

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Availability of data and materials
All data generated or analyzed in this study are included in this article and the supplementary files. The raw data of RNA sequencing were submitted to the NCBI database with the bioproject ID: PRJNA657702.

Declarations
Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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