Characterization of BRS1 functions in plant stress 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 is involved in both biotic and abiotic stress responses and redox regulation. Further proteomic evidence shows that BRS1 regulates cell redox by indirectly interacting with cytosolic NADP+-dependent isocitrate dehydrogenase (cICDH). We identified two novel splice products of *BRS1,* which might play important roles in development and stress responses in plants.

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, 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 the 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]. The functions of the majority of these genes have not been described.

BRS1 belongs to class II of the Arabidopsis SCP family [1]. Its overexpression can rescue the receptor mutant of brassinosteroid (BR), *bri1-5*, indicating BRS1 might play an important role in BR signaling [4, 5]. 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 [4, 6, 7]. This suggests that BRS1 and its homologs are functionally redundant in Arabidopsis, making difficulties to clarify the precise roles of BRS1.

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.

Environmental stress leads to the accumulation of reactive oxygen species (ROS) in plant cells and results in oxidative stress [14-16]. Therefore, to adapt to environmental stress, it is vital to control redox homeostasis to maintain normal cellular metabolism, and/or trigger programmed cell death [17, 18].

In this study, transcriptomic and proteomic analyses demonstrate a role for BRS1 in

biotic and abiotic stress responses. Our results indicate BRS1 participates in redox regulation via interaction with cICDH, shedding new light onto the function of SCPs in plant stress responses.

Results

Seedling developmental phenotypes of *BRS1*

BRS1-1D was over-expressed BRS1 with an enhancer in BRS1 promoter [4, 5], showing larger rosette leave (Fig. 1a) and a longer hypocotyl (Fig. 1b, c, d and e). However, we saw no visible phenotypes with the BRS1 knockdown mutant, *brs1-1* (Fig. 1), consistent to no significant phenotype was observed in *BRS1* mutant [4-7].

Analysis of the transcriptomes of BRS1 seedlings

We performed RNA sequencing on the BRS1 seedlings (Fig. 1b). A total of 21,873 transcripts were identified, of which 180 differentially expressed genes (DEGs, fold change ≥2.0, FDR-adjusted P-value <0.05, FPKM ≥ 1) were identified in the *brs1-1D* seedlings when compared to wild type. This included increased expression of 114 genes and decreased expression of 66 genes (Supplementary Table S1). In contrast, no gene significantly changed its transcription in mutant *brs1-1* seedlings, except *BRS1* itself (Supplementary Table S1). This is consistent with the finding of no visible morphological difference shown in *brs1-1* plants (Fig. 1).

However, this was an unusual result as we expected some genes in the network would be affected in the *brs1-1* plants. We, therefore, used our RNA sequencing data to investigate the transcription of mutated *BRS1* in our seedlings. The transcription of *BRS1* in the mutant *brs1-1* was decreased significantly due to the insertion of a thymidine at position 786 in the first intron (Fig. 2a and b), which resulted in a mis-splicing event (Fig. 2c). In contrast, the transcriptional level of *BRS1* increased by

around 20 times in the *brs1-1D* mutant (Fig. 2a), which is consistent with the presence of four copies of CaMV 35S enhancers inserted in the promoter region of *BRS1* (Fig. 2b) [4].

Interestingly, the transcriptional level of the first exon in *brs1-1* maintained a similar level to that of the wild type (Fig. 2c), indicating there is a not feedback loop to enhance *BRS1* transcription. We also saw no increased expression of the homologs of *BRS1* when *BRS1* expression was disrupted (Supplementary Figure S1).

Notably, we detected novel splice variants in *brs1-1D*, which retained multiple introns compared to the wild type (Fig. 2c). The two new splice products (4 and 5) made up a lower proportion of the total *BRS1* transcripts in *brs1-1D*, then the level still equal to the total transcripts of *BRS1* in wild type (Fig. 2c), indicating these novel splice products may have important functions.

Overexpression of BRS1 alters multiple stress responses

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

BRS1 regulates redox-related proteins

We next performed proteomic analysis on seedlings using two-dimensional difference gel electrophoresis (2D-DIGE) (Fig. 4a). 19 proteins were assigned as differentially

expressed proteins, and 14 proteins were identified, of which 5 proteins were involved in redox regulation (Table 1), supporting that BRS1 is involved in redox regulation.

The 2D-DIGE revealed two spots (6 and 7), which were most changed compared to wild type, showing a decrease and increase in *brs1-1D*, respectively (Fig. 4). These spots corresponded to the same protein, the cytosolic NADP+-dependent isocitrate dehydrogenase (cICDH, at1g65930) (Table 1), a critical redox regulator [19]. Together, these data suggest that BRS1 is involved 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. 5a), whilst no change was seen in *brs1-1* plants. These findings are consistent with our earlier results that showed only overexpression of BRS1 altered transcription. Altogether this suggests that BRS1 regulates the activity of cICDH.

To understand how the secretory protein BRS1 could regulate cICDH, which is localized to the cytosol, the cellular localization of BRS1 was evaluated. We observed expression of BRS1 at the membrane and cell wall of mature epidermal cells (Fig. 5b), consistent with localization to the extracellular space [4, 5]. Unexpectedly, we also found cICDH-GFP to be localized to the membrane of *Nicotiana benthamiana* leaves (Fig. 5c), indicating a potential interaction might occur in this area. However, yeast two-hybrid and pull-down assays found no direct interaction between cICDH and BRS1 (Supplementary 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 [4, 6, 7]. In this study, the transcriptome suggested its important role in stress response, highlighting BRS1 as the apoplastic protease, share the important function in biotic and abiotic stress defense [13, 20].

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

The trigger effector production by apoplastic proteases used be the key mechanism to induce plant stress response [13]. Our results showed that systemic stress response was induced upon *BRS1* over-expression (Fig. 3). Thus this pathway is likely involved in plant defense more generally, rather than being limited to a specific pathogen.

Consistent with this notion, we found BRS1 regulates redox homeostasis, and therefore plays a critical role in controlling apoplastic ROS [13]. ROS regulation is vital to induce widespread stress responses, and essential for SA and JA signaling in response to various stressors [23, 24].

The ICDHs catalyze the production of NADPH, which is important for redox regulated cell metabolism and promoting redox signaling in response to oxidative stress [25, 26]. cICDH is responsible for more than 90% of total ICDH activity [25, 27]. Therefore, this enzyme plays a crucial role in maintaining redox homeostasis in the cell, and consequently, defense responses [19]. Exactly how BRS1 participates in this pathway still needs further investigation.

The knockdown mutant of *BRS1* does not have any significant phenotypes [4, 6, 7];

consistently, we found no transcriptional changes in the mutant *brs1-1* (Supplementary Table S1). However, this finding suggests that there are no pathways dependent on BRS1. In contrast, we observed many phenotypes and identified numerous alterations in gene expression upon overexpression of *BRS1* [7]. The existence of multiple redundant homologs in Arabidopsis may explain this result [4, 6, 7]. However, in this study the concurrence of the novel splice variants, phenotypes and activity of cICDH in *brs1-1D* (Fig. 2c), implying a splicing-based functional variation of BRS1, which may play a special role in those highly redundant gene family to overcome their redundancy nature in changing environments.

Conclusions

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

Methods

Plant Materials and Growth Condition

The plant materials used in this study are as follows: 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 laboratory of Jia Li (Lanzhou University, Lanzhou, China) [4, 5, 7]. The mutant *brs1-1* also came from the laboratory of Jia Li, which was originally obtained from the Wisconsin Arabidopsis knockout pool. This mutant identified insertion a thymidine at 786 bp in first intron through our RNA sequencing and has been used in previous research [7]. 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, PH5.6-5.8) in the same chamber and culture conditions, and total protein and RNA were extracted seven days after germination. 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 [19].

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).

Analysis of Transcriptome Data

Raw transcriptome sequencing datas cleaned using Trimmomatic (v 0.36) under default parameters. The clean reads were aligned to the TAIR 10 reference genome using HISAT2 (v 2.1.0) and the expression of genes were profiled using StringTie (v1.3.3) [28, 29]. The reads in the AT4G30610 (*BRS1*) gene region using StringTie for transcript assembly and reads coverage using “genomeCoverageBed” statistics in BEDtools (v 2.29.0).

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

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) 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 [30].

The GO descriptions were obtained by AnnotationHub ("AH75734"), and used the R package clusterProfiler (v3.14.0) 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 enrichment analysis.

Protein Preparation for Fluorescent Two Dimension Difference Gel Electrophoresis (2D-DIGE) Analysis

Seven-day-old seedlings (1 g) were harvested and ground to a 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 18,000g 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 18,000g for 20 min. Finally, the pH of protein

samples was adjusted to 8.5 with HCl and NaOH, and the concentration of proteins were determined as previously described Bio-Rad Bradford method using BSA as a standard [31]. 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 [32]. To minimize systemic and inherent biological differences, it is recommended to combine four independent protein preparations for each sample [33].

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) [32]. 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℃ 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 20071214; 5,742,110 sequences) by use of Mascot software2.2.03(http://www.matrixscience.com; Matrix Science, London,U.K.).

Enzyme activities

Seven-day-old of seedlings (0.1 g) were ground to a 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 minutes to remove insoluble materials, and measure ICDH activity by spectrophotometry [19, 34]. Determination of protein concentration as previously described Bio-Rad Bradford method using BSA as a standard [31].

List of 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

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

Funding

This work was funded by the National Key Research and Development Program of China (2016YFD0101602) and the National Natural Science Foundation of China (31370318). This Funding was not involved in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Author Contributions

SX conceived and designed the research and contributed to writing and revising the manuscript. DZ performed experimental work and data analysis, and wrote and revised the manuscript. PZ performed bioinformatics analysis. All authors have read and approved the manuscript.

Acknowledgements

We thank Jia Li (Lanzhou University, Lanzhou, China) for kindly providing the mutants of *BRS1*.

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Figure legends:

Figure 1. A *BRS1* knockdown mutant does not show phenotypic alterations compared to wild type

a. Comparison of the phenotypes of wild type WS2, *BRS1* knockdown mutant *brs1-1* and BRS1 overexpression allele *brs1-1D* at 14 days after germination (DAG). Plants were grown in a growth chamber at 22°C under long-day conditions. Scale bar = 1 cm.

b. The phenotypes of WS2, *brs1-1* and *brs1-1D* seedlings grown on 1/2 MS medium under long-day conditions. Photos captured 7 DAG. Scale bar = 1 cm.

c. The phenotypes of WS2, *brs1-1* and *brs1-1D* seedlings grown on 1/2 MS medium in the dark. Photos captured 5 DAG. Scale bar = 1 cm.

d. Comparison of hypocotyl lengths of seedlings grown as pictured in **(b)**. Means ± standard error (SE) are shown from three independent experiments, n≥ 20 in each experiment.

e. Comparison of the hypocotyl lengths of seedlings grown as pictured in **(c)**. Means ± SE are shown from three independent experiments, n ≥ 20 in each experiment. Asterisks indicate a statistically significant difference based on the Student's t-test (*P < 0.05).

Figure 2. Differences in gene sequences and *BRS1* transcription in wild type and *BRS1* mutants

a. Transcription levels (FPKM value) of *BRS1* in WS2, *brs1-1* and *brs1-1D* as measured by RNA sequencing. All results are expressed as mean ± standard deviation (SD). The asterisks indicate a statistically significant difference (Student's t-test, *P < 0.05).

b. Summary of the differences in gene sequences between WS2, *brs1-1* and *brs1-1D*. *BRS1* has nine exons and eight introns. A thymidine base (red) is inserted in the first intron of *brs1-1*, and the 4 X 35S enhancer is inserted in the promoter of *BRS1* in *brs1-*

1D.

c. The transcription of *BRS1* was measured in WS2, *brs1-1*and *brs1-1D*. The counts of different reads of *BRS1* and their distribution in genes are shown above. Data is from three biological replicates per sample. The types of different *BRS1* transcripts present in the different samples are shown. The percentage value shows the ratio of individual transcripts to total transcripts. The red box highlights the normal transcript of *BRS1* in TAIR10.

Figure 3. Enrichment analysis of DEGs in transcripts of *brs1-1D* compared to wild type

Analysis of GO terms associated with DEGs between *brs1-1D* and WS2 seedlings (q-value < 0.01). The first red column represents DEGs that are up-regulated in *brs1-1D* compared to WS2; the second blue column represents DEGs that are down-regulated. Terms found enriched in the up-regulated DEGs are divided into four categories (red vertical lines). The term enriched in the down-regulated DEGs is indicated with the blue vertical line. Gene ratio indicates the ratio of DEGs clustered into different terms, divided by the total number of DEGs.

Figure 4. Identification of two isoforms of cICDH and comparison of protein expression levels

a. Representative 2D-DIGE image of the two most significant differential protein spots in seedlings from WS2, *brs1-1* and *brs1-1D*. Samples from WS2, *brs1-1* and *brs1-1D* were labeled with Cy2 (blue), Cy3 (green), and Cy5 (red), respectively. White arrows indicate differential protein spots.

b. Comparison of expression levels of two differential protein spots in WS2, *brs1-1* and *brs1-1D*. The height of the pink circled area represents the level of protein expression.

Figure 5. The enzyme activity of cICDH depends on BRS1

a. Total extractable ICDH activity from wildtype (WS2 and Col-0) seedlings and a range of mutant lines: *brs1-1D*, *brs1-1* and *icdh-2*. Means and SE of eight independent extracts are shown. Asterisks indicate a statistically significant difference based on Student's t-test (*P < 0.05).

b. Subcellular localization of BRS1. Root tips from transgenic Col-0 plants expressing 35S-BRS1-GFP were used to visualize the subcellular localization of BRS1-GFP by confocal microscopy.

c. Subcellular localization of ICDH. *Nicotiana benthamiana* leaves transiently expressing 35S-ICDH-GFP were used to observe the subcellular localization of ICDH-GFP by confocal microscopy.

Table 1. List of differentially expressed proteins identified by mass spectrometry (MS)

No.	Chromosome locus	Matched protein	Biological process	Expression
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**	**Redox**	**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	Nitrilase 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

Proteins listed in bold black are differentially expressed and involved in redox. Points 6* and 7* indicate protein expression is significantly different from control. 'Expression' is calculated as the ratio of the expression level in *brs1-1D* to the control WS2. 'Relative volume' values were determined using ImageMaster Platinum Software. NO indicates the spot was missing in the corresponding gel. For each sample, triplicate biological repeats of 2-DE were performed using independent protein preparations.

Additional files

Additional file 1: Supplementary Table S1. Identification of DEGs in *BRS1* mutants.

Supplementary Table S2. Proteins identified by BRS1-GFP pull-down.

Additional file 2: Supplementary Figure S1. The expression analysis of *BRS1* homologs in wildtype and BRS1 mutants. RNA sequencing was used to calculate the expression levels (FPKM value) of five *BRS1* homologs: *SCPL22*, *SCPL23*, *SCPL25*, *SCPL26* and *SCPL27* in WS2, *brs1-1* and *brs1-1D* plants. Mean ± SD is shown. The asterisks indicate a statistically significant difference (Student's t-test, *P < 0.05).