nature portfolio

Peer Review File

Natural variation in SBRR1 shows high potential for sheath blight resistance breeding in rice

Corresponding Author: Professor Shimin Zuo

Version 0:

Decision Letter:

3rd Jul 2024

Dear Professor Zuo,

Your Article, "A natural variation of rice sheath blight-resistance receptor-like kinase 1 (SBRR1) shows high potential for sheath blight resistance breeding" has now been seen by 3 referees. You will see from their comments below that while they find your work of interest, some important points are raised. We are interested in the possibility of publishing your study in Nature Genetics, but would like to consider your response to these concerns in the form of a revised manuscript before we make a final decision on publication.

To guide the scope of the revisions, the editors discuss the referee reports in detail within the team, including with the chief editor, with a view to identifying key priorities that should be addressed in revision and sometimes overruling referee requests that are deemed beyond the scope of the current study. In this case we encourage you to address reviewers´ comments in full. We hope that you will find the prioritized set of referee points to be useful when revising your study. Please do not hesitate to get in touch if you would like to discuss these issues further.

We therefore invite you to revise your manuscript taking into account all reviewer and editor comments. Please highlight all changes in the manuscript text file. At this stage we will need you to upload a copy of the manuscript in MS Word .docx or similar editable format.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

When revising your manuscript:

*1) Include a “Response to referees” document detailing, point-by-point, how you addressed each referee comment. If no action was taken to address a point, you must provide a compelling argument. This response will be sent back to the referees along with the revised manuscript.

*2) If you have not done so already please begin to revise your manuscript so that it conforms to our Article format instructions, available
here.
Refer also to any guidelines provided in this letter.

*3) Include a revised version of any required Reporting Summary: https://www.nature.com/documents/nr-reporting-summary.pdf
It will be available to referees (and, potentially, statisticians) to aid in their evaluation if the manuscript goes back for peer review.
A revised checklist is essential for re-review of the paper.

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We hope to receive your revised manuscript within four to eight weeks. If you cannot send it within this time, please let us know.

Please do not hesitate to contact me if you have any questions or would like to discuss these revisions further.

Nature Genetics is committed to improving transparency in authorship. As part of our efforts in this direction, we are now requesting that all authors identified as 'corresponding author' on published papers create and link their Open Researcher and Contributor Identifier (ORCID) with their account on the Manuscript Tracking System (MTS), prior to acceptance. ORCID helps the scientific community achieve unambiguous attribution of all scholarly contributions. You can create and link your ORCID from the home page of the MTS by clicking on 'Modify my Springer Nature account'. For more information please visit please visit www.springernature.com/orcid.

We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

Sincerely,
Chiara

Chiara Anania, PhD
Associate Editor
Nature Genetics
https://orcid.org/0000-0003-1549-4157

Referee expertise:

Referee #1: genomics and genetics; rice breeding; rice disease resistance

Referee #2: plant immunity

Referee #3: genetics and genomics; disease resistance

Reviewers' Comments:

Reviewer #1:
Remarks to the Author:
In the manuscript entitled "A natural variation of rice sheath blight-resistance receptor-like kinase 1 (SBRR1) shows high potential for sheath blight resistance breeding", Feng et al reported the isolation of a receptor-like kinase gene (SBRR1) which is associated with sheath blight resistance via genome-wide association study. They further discovered that the elite SBRR1-R allele, carrying a 256-bp insertion in its promoter, expresses elevated SBRR1 transcript levels resulting in higher ShB resistance and an ankyrin (ANK)-repeat domain protein, named SBRR1-interacting-protein 1, facilitates efficient accumulation of SBRR1 protein on plasma membrane, to rapidly upregulate chitinase genes for ShB resistance. They also uncovered the natural elite allele has hidden in natural rice varieties. This manuscript indeed provides new insights into the study of sheath blight resistance. However, this manuscript lacks of mechanism in understanding how SBRR1 regulates sheath blight resistance.

Majors
How the kinase SBRR1 regulates sheath blight resistance? As the authors presented that the kinase dead mutant SBRR1K533E is not able to rescue the susceptibility of sbrr1ko plants to resistance as does SBRR1, how this kinase SBRR1 functions when pathogen Rhizoctonia solani attacks rice plants? What is the specific phosphorylation event mastered by SBRR1 and is this phosphorylation event required for sheath blight resistance? It needs to identify the substrate protein of SBRR1 and test how SBRR1 phosphorylates the substrate protein and whether this phosphorylation is required for SBRR1-mediated sheath blight resistance?

As the authors claimed that the elite SBRR1-R allele, carrying a 256-bp insertion in its promoter, expresses elevated SBRR1 transcript levels resulting in higher ShB resistance, it is required to identify the upstream transcription factor which regulates Sbrr1 expression through acting with the Sbrr1 promoter. And test how this 256-bp insertion elevate the expression of Sbrr1. Does this expression elevation of Sbrr1 is dependent or independent of pathogen Rhizoctonia solani infection?

It is interesting that SBRR1 regulates downstream chitinase genes-associated events for sheath blight resistance. However, there is gap on how plasma membrane located SBRR1 protein regulates the transcription of the chitinase genes in nucleus?

Minors
As Sbrr1 affects many chitinase genes expression (Chit3, Chit4,Chit7, Chit12 and Chit17), why only chitinase genes Chit3 and Chit4 are most important for sheath blight resistance? Only because the expression of these two genes induced more higher by pathogen Rhizoctonia solani infection?

Since chitinase activity is also involved in rice blast resistance, does SBRR1 regulates blast resistance? Is this molecular regulation of SBRR1 in blast resistance same as in sheath blight resistance?

Reviewer #2:
Remarks to the Author:
This study identifies the first gene underlying sheath blight resistance in rice, along with some mechanistic insights. Given the importance of this pathogen, I think the manuscript would appeal to a broad audience.

The genomic and evolutionary analyses of the MS appear to be done well. This is not my primary area of expertise, so I focus my comments more on the molecular and biochemical experiments.

The transgenic line phenotypes are convincing. I am missing the generation tested. Are they all homozygous or earlier generations? This information should be provided.

claims of increased yield, etc should be toned down. The data rely on one greenhouse and one smaller field trial. See: https://www.nature.com/articles/d41586-023-02895-w

phosphorylation: blot in fig 2e is heavily cropped, positive and negatives should be run on the same gel. Samples should be treated with a phosphatase to show removal of the phosphorylation signal.
Is SBRR1 phosphorylated in planta? Can it phosphorylate SIP1 (in vitro or in vivo)? The size shift in fig 5e for SIP1 indicates it could be phosphorylated. One can check phosphorylation after IP using a gel shift assay + phosphatase if anti pS/pT is not strong enough.

Fig 2 b-h could be labeled better for easier interpretation.

Fig 4 – several of the graphs indicate significant differences but error bars are overlapping. Statistics should be double-checked or raw data provided (Fig 4f, 4k, 4i).

Fig 5- 5b the signal for GST input looks stronger than GST IP. Were samples diluted after IP? There should be a stronger signal after IP for GST. 5c – there is no negative control for IP, the authors need to use an antibody that localizes to the ER/PM in rice to demonstrate a specific interaction. The PIP1;1 aquaporin antibody could be used (Fig 6).

Localization: western blots should be provided to demonstrate no GFP cleavage in rice protoplasts (Fig 6). Western blots should also be provided to demonstrate expression in Y2H in the main and supplemental figures.

Fig S13 – western blots are closely cropped. Is SBRR1 cleaved in planta or is only full length detectable?

The domain architecture of both SBBR1 and SIP1 should be provided in a main figure.

Fig S15 – plasmolysis should be used to demonstrate PM localization after transient expression in Nicotiana. Or the PM localization should be strengthened for the rice protoplasts as described above.

Reviewer #3:
Remarks to the Author:
The fungal pathogen Rhizoctonia solani can infect various staple crops such as rice, wheat, maize, soybean, resulting in severe crop diseases, which not only poses a threat to global food security, but also leads to significant economic losses. To the best of my knowledge, so far, there has been no successful cloning of an effective gene against sheath blight from natural rice varieties. In this study, the authors employed comprehensive genetic analysis combined with biochemical evidence to successfully clone the first major quantitative disease resistance gene, SBRR1-R, against sheath blight in rice. They elucidated the mechanism behind its disease resistance and demonstrated the significant practical value of their research findings. A few comments listed below I think may further improve the manuscript.
1. Fig. 2e, the kinase activity of His-SBRR1 looks prominent. However, it would be more convincing to use the isotope labeled the ATP to analysis the kinase activity of His-SBRR1. Also, In Fig. 2, the paper presents the disease resistance phenotype of sheath blight through the measurement of disease lesion length. It is better to utilize fungal biomass and cell staining data as additional criterions to validate the observed disease resistance phenotype for some critically transgenic plants.
2. Fig. 4b, as these are in vitro interaction data of purified recombinant proteins, I would like to suggest the authors to detect and then quantify by immunoblot (that easily saturates and is making the interaction assay less quantitative than simply quantifying on stained gels). Affinity assays would provide more convincing support for the claims made.
3. Fig. 5, the authors showed that the SIP1 physically interacts with SBRR1, and the data from Fig. 6 suggested that the

SIP1 is required for the transportation of SBRR1 to the plasma membrane, could the authors comment on mechanism underlying here, and have the authors checked whether the SIP1 altered SBRR1 kinase activity in vivo? In addition, considering that SBRR1 is a protein kinase, can SBRR1 phosphorylate SIP1 and is it possible that SIP1 is one of the phosphorylation substrates of SBRR1?
4, Fig. S14, the Y2H data suggested that, the SBRR1-ECD (366-353) is required for its interaction with SIP1, and SIP1-M2 is required for its interaction with SBRR1, have the authors used other assays to conform these interactions?
5. The SBRR1-R showed elevated expression after R. solani infection due to its 256 bp insertion in the promoter region. So, I would like to suggest the authors predict the possible upstream regulators that may activate higher expression of SBRR1-R, but not SBRR1-S.
6. Although the data supported the chitinase genes contribute a lot to the resistance of SBRR1-R mediated resistance, the remaining resistance is unclear. The authors showed that the kinase activity of SBRR1 is required for its full resistance, so, it is better to add some explanations on how the SBRR1 mediated full resistance to the pathogen by comparing the transcriptomic data of SBRR1-OE and WT or sbrr1-ko plants.
7. There are still some minor errors in the text. Such as, which supplementary material does "Supplementary xxx" refer to in Line 511? The text in the horizontal axis of Figure 5e overlaps. The “promotor” in Line 213 should be “promoter”. The “timepoints” in Line 388 should be “time points”. The “Light panel” in the legend of Fig. 1 should be “Left panel”.

Version 1:

Decision Letter:

7th Jan 2025

Dear Professor Zuo,

Your Article, "A natural variation of rice sheath blight-resistance receptor-like kinase 1 (SBRR1) shows high potential for sheath blight resistance breeding" has now been seen by 3 referees. You will see from their comments below that while they find your work of interest, some important points are raised. We are interested in the possibility of publishing your study in Nature Genetics, but would like to consider your response to these concerns in the form of a revised manuscript before we make a final decision on publication.

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here.
Refer also to any guidelines provided in this letter.

*3) Include a revised version of any required Reporting Summary: https://www.nature.com/documents/nr-reporting-summary.pdf
It will be available to referees (and, potentially, statisticians) to aid in their evaluation if the manuscript goes back for peer review.
A revised checklist is essential for re-review of the paper.

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EXTENDED DATA FIGURES

When re-submitting your manuscript, please ensure that any supplementary figures and tables that are crucial to the manuscript’s conclusions are converted into Extended Data figures and tables to increase visibility of these data. Extended

Data figures and tables are online-only (present in the online PDF and full-text HTML versions of the paper), peer-reviewed display items that provide essential background to the article but are not included in the main article due to space constraints. A maximum of ten Extended Data display items (figures and tables) is permitted.

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Note: This URL links to your confidential home page and associated information about manuscripts you may have submitted, or that you are reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

We hope to receive your revised manuscript within four to eight weeks. If you cannot send it within this time, please let us know.

Please do not hesitate to contact me if you have any questions or would like to discuss these revisions further.

Nature Genetics is committed to improving transparency in authorship. As part of our efforts in this direction, we are now requesting that all authors identified as 'corresponding author' on published papers create and link their Open Researcher and Contributor Identifier (ORCID) with their account on the Manuscript Tracking System (MTS), prior to acceptance. ORCID helps the scientific community achieve unambiguous attribution of all scholarly contributions. You can create and link your ORCID from the home page of the MTS by clicking on 'Modify my Springer Nature account'. For more information please visit please visit www.springernature.com/orcid.

We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

Sincerely,
Chiara

Chiara Anania, PhD
Associate Editor
Nature Genetics
https://orcid.org/0000-0003-1549-4157

Referee expertise:

Referee #1:

Referee #2:

Referee #3:

Reviewers' Comments:

Reviewer #1 (Remarks to the Author):

The authors have provided some data and have improved this manuscript. However, it still lacks of data on how this kinase activates chitinase genes expression. It would be better to provide these data or make some mechanism explanation on how this kinase gene regulates disease resistance against sheath blight.

Reviewer #2 (Remarks to the Author):

The authors have addressed most of my previous comments and the manuscript is greatly improved. I only have a few remaining suggestions, below.

For phos-tag experiments, there are critical controls missing. Thus, it is not clear if the upper band corresponds to phosphorylated SBBR1. Non-transformed rice (or kinase dead variant of SBBR1) should be used as a control. Phos-tag is not always specific for detecting phosphorylation. An experiment should also be performed demonstrating the upper signal disappears upon incubation with a phosphatase (Fig S9a, Fig S2e). This does not need to be done for each gel but can be done for one to demonstrate specificity.

Fig 2g – can the authors provide a blot exposure with higher background? It appears overly edited for decreased contrast. For the claims of yield - although the data are promising, they are not large-scale tests with sufficient numbers to make strong claims. I suggest the authors note this limitation in the discussion and the opportunity for more extensive testing in the future.

Reviewer #3 (Remarks to the Author):

The authors appropriately addressed all of my concerns and those of the other reviewers' comments. They performed additional kinase assay experiments and analysis for comprehensive enhancements to the SBRR1 manuscript. The authors provide sufficient evidence to support their conclusion that natural variation of SBRR1 for sheath blight resistance in rice. I have no further objections to publication of this MS in Nature Genetics.

Version 2:

Decision Letter:

Our ref: NG-A65534R1

24th Feb 2025

Dear Dr. Zuo,

Thank you for submitting your revised manuscript "A natural variation of rice sheath blight-resistance receptor-like kinase 1 (SBRR1) shows high potential for sheath blight resistance breeding" (NG-A65534R1). It has now been seen by the original referees and their comments are below. We find that the paper has improved in revision, and therefore we'll be happy in principle to publish it in Nature Genetics, pending minor revisions to comply with our editorial and formatting guidelines.

If the current version of your manuscript is in a PDF format, please email us a copy of the file in an editable format (Microsoft Word or LaTex)-- we can not proceed with PDFs at this stage.

We are now performing detailed checks on your paper and will send you a checklist detailing our editorial and formatting requirements soon. Please do not upload the final materials and make any revisions until you receive this additional information from us.

Thank you again for your interest in Nature Genetics Please do not hesitate to contact me if you have any questions.

Sincerely,
Chiara

Chiara Anania, PhD
Associate Editor
Nature Genetics
https://orcid.org/0000-0003-1549-4157

Point by point responses to Reviewer comments:

Reviewers' Comments:

Reviewer #1:

Remarks to the Author:

In the manuscript entitled "A natural variation of rice sheath blight-resistance receptor-like kinase 1 (SBRR1) shows high potential for sheath blight resistance breeding", Feng et al reported the isolation of a receptor-like kinase gene (*SBRR1*) which is associated with sheath blight resistance via genome-wide association study. They further discovered that the elite *SBRR1-R* allele, carrying a 256-bp insertion in its promoter, expresses elevated *SBRR1* transcript levels resulting in higher ShB resistance and an ankyrin (ANK)-repeat domain protein, named SBRR1-interacting-protein 1, facilitates efficient accumulation of SBRR1 protein on plasma membrane, to rapidly upregulate chitinase genes for ShB resistance. They also uncovered the natural elite allele has hidden in natural rice varieties. This manuscript indeed provides new insights into the study of sheath blight resistance. However, this manuscript lacks of mechanism in understanding how *SBRR1* regulates sheath blight resistance.

Response: Thanks for the reviewer's recognition on the study and pointing out several critical and valuable suggestions for manuscript improvement. We have carefully revised the manuscript and addressed these comments as thoroughly as possible. After revision, we feel that the manuscript is greatly improved.

Majors

1. How the kinase SBRR1 regulates sheath blight resistance? As the authors presented that the kinase dead mutant SBRR1^{K533E} is not able to rescue the susceptibility of sbrr1-ko plants to resistance as does SBRR1, how this kinase SBRR1 functions when pathogen *Rhizoctonia solani* attacks rice plants? What is the specific phosphorylation event mastered by SBRR1 and is this phosphorylation event required for sheath blight resistance? It needs to identify the substrate protein of SBRR1 and test how SBRR1 phosphorylates the substrate protein and whether this phosphorylation is required for SBRR1-mediated sheath blight resistance?

Response: We agree with the reviewer that these are interesting and attractive points. Actually, when we confirmed that SBRR1 is a kinase protein and the kinase activity is

required for the resistance, we are very curious about how this kinase SBRR1 regulates sheath blight resistance. **We are well aware of the significance of these points and have made great efforts to address them.**

(1) **For the first point "how this SBRR1 kinase functions when pathogen *Rhizoctonia solani* attacks rice plants?":** We have tried many methods with both adult plants and young seedlings and hoped to extract enough SBRR1 protein in response to the pathogen attacks to analyze its phosphorylation events after pathogen infection. However, due to the limitations on either uneven inoculation for young seedlings or difficulties on protein extraction from adult plants, we had not obtained significant progresses on this issue before the original manuscript submission. Fortunately, in the past several months, we have tried a new inoculation method, named mycelia-containing cellulose acetate membrane inoculation method (NP-Fig.1, NP: these figures will not be provided in the manuscript), on young seedlings. In this new method, during pathogen cultivation, we place a cellulose acetate membrane on the PDA plate and then a small PDA block carrying the pathogen on the cellulose acetate membrane (NP-Fig.1a). After incubation for 2-3 days, pathogen mycelia would grow to all sides of the plate to cover the whole membrane; then the cellulose acetate membrane is used to inoculate plants by placing the mycelia side on the plants (NP-Fig.1b). Using this method, we now can quickly inoculate many plants within a short time. Especially, this method allows a similar quantity of mycelia to inoculate on all rice tissues that are close to the cellulose acetate membrane. After inoculation, we observed that most plant tissues showed similar disease progression and symptoms at 24, 48 and 72 hours post inoculation (HPI) (NP-Fig.1c), which greatly facilitates us in collecting large amounts of inoculated rice tissues at similar disease degree.

This breakthrough in inoculation method allowed us to obtain enough rice tissues with similar disease degree at seedling stage after inoculation and enough SBRR1 protein in response to pathogen infection for subsequent kinase activity analysis and identification of phosphorylation events. Therefore, using the Phos-tag SDS-PAGE assay, we successfully observed that the phosphorylation level of SBRR1 remained unchanged at 6 HPI, but slightly increased at 12 HPI, then greatly increased and peaked at 18 HPI (Fig.2e; Supplemental Fig.9a, b). These results show that the phosphorylation level of SBRR1 is induced by pathogen infection and reaches the highest level around 18 HPI.

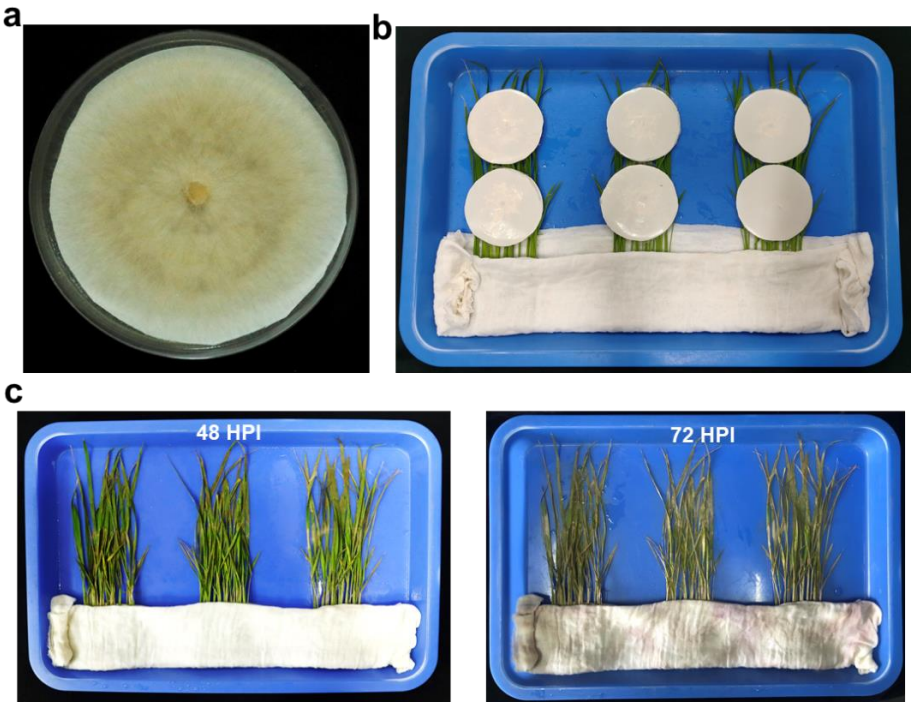
(2) For the second point "What is the specific phosphorylation event mastered by SBRR1 and is this phosphorylation event required for sheath blight resistance?": We adopted a LC-MS/MS to compare the SBRR1 phosphorylation events in rice plants at 18 HPI with *R. solani* and those with mock (inoculation with water) treatment. We found that compared with the control, four amino acid residues (S678, T682, T683 and Y733) of SBRR1 were phosphorylated after *R. solani* infection; these amino acids were not phosphorylated without *R. solani* infection (Supplemental Fig.10). We further calculated the phosphorylation levels of the four residues based on the data of three biological replications and found that T683 and T682 were significantly changed showing a phosphorylated/unphosphorylated peptide ratio of 1.8 and 2.8, respectively, while S678 and Y733 showed slight changes with a ratio of 0.35 and 0.17, respectively (Fig.2f). Except for Y733, the other three amino acids are all located within the kinase activation loop of SBRR1, and in general, the phosphorylation of one to three residues in this loop is required for kinase activation (Taylor et al., 1992, Annu. Rev. Cell Biol., 8, 429-462; Lemmon and Schlessinger, 2010, Cell, 141, 1117-1134). *In vitro*, we found that mutations of TT682/683 to AA682/683, but not a S186A mutation, absolutely abolished SBRR1 autophosphorylation (Fig.2g; Supplemental Fig.9c), suggesting the phosphorylation of TT682/683 is not only induced by *R. solani*, but also required for SBRR1 activity.

Actually, although we obtained these phosphorylation data recently, we have previously generated some T2 transgenic plants in Dongjing (DJ) background with several SBRR1-mutation versions on some amino acids putatively associated with SBRR1 kinase activity according to our previous experiences (Zhao et al., Plant Cell, 2019, 31, 2131-2151). Fortunately, the transgenic rice plants overexpressing *SBRR1*$^{TT682/683AA}$ (TT682/683AA mutations) under control of the 35S promoter were included (Supplementary Fig.9d). Therefore, we further extend the above findings *in vivo*, and found that upon *R. solani* infection, both *SBRR1*$^{TT682/683AA}$-OE lines showed similar susceptible levels as the DJ control, while all displayed significantly lower resistance than SBRR1-OE transgenic plants (Fig.2h), validating the importance of TT682/683 in SBRR1-mediated ShB resistance. Together, these data clearly show that the kinase activity of SBRR1 and especially the phosphorylation of TT682/683 are required for SBRR1-mediated ShB resistance. **In the revised version, we have added these new data and believe they can address your point.**

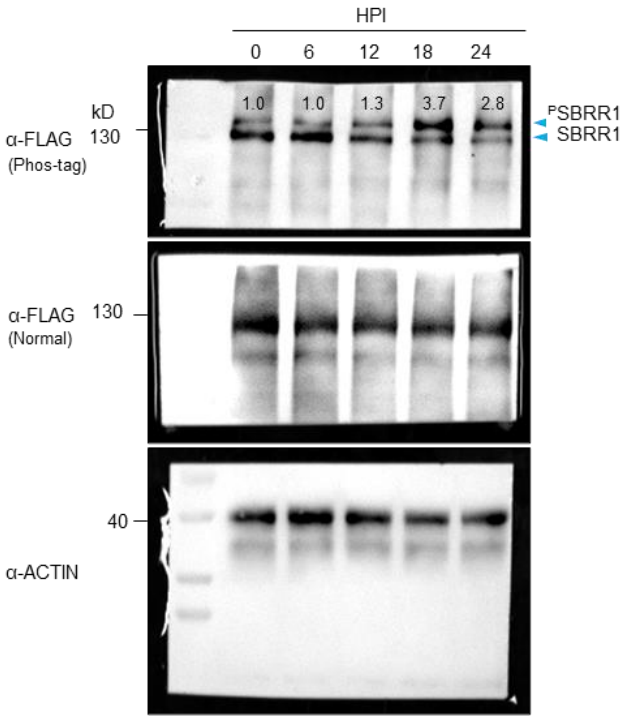
(3) For the third point "It needs to identify the substrate protein of SBRR1 and test how SBRR1 phosphorylates the substrate protein and whether this phosphorylation is required for SBRR1-mediated sheath blight resistance?": We feel regretful that to date we have not been able to identify the substrate protein of SBRR1, nor the kinase proteins that phosphorylate SBRR1 in response to pathogen infection. We totally understand the significance of this point. However, the identification of SBRR1 substrates and elucidation of its downstream signaling is not something trivial and, even with luck, usually takes many years to accomplish. In fact, we have also tried for at least five years to identify the substrates of SBRR1 by using Y2H screening and mass-spectrometry identification after pull down of SBRR1 protein complex, as well as bioinformatic analysis. However, to date, we have only identified SIP1 (acting as a regulator for SBRR1 subcellular localization, but not as a substrate), as described in this manuscript. We have also tested several other known RLK and RLCK proteins, including OsSERK2 (BAK1 rice orthologue), CERK1, RLCK185, RLCK176, and found that SBRR1 does not interact with any of them, suggesting that the SBRR1-triggered immune signaling is not directly associated with these well-known kinases. **Thus, identifying the substrates of SBRR1 is currently too difficult for us and beyond the scope of this manuscript,** we will continue to contribute efforts to tackle these challenges with multi-disciplinary collaborations in future.

In addition, we think the current, revised manuscript is very attractive because it contains several important findings: 1) The identification of *SBRR1* and its elite allele *SBRR1-R*; 2) the molecular evolution and breeding potential of *SBRR1-R*; 3) the identification of bHLH57, a positive regulator that differentiates expression of *SBRR1-R* and *SBRR1-S*; 4) the localization of SBRR1 aided by SIP1 is required for its resistance; 5) the resistance mechanism of *SBRR1*, which is mainly associated with the quick induction of chitinase 3 and 4 genes.

Some new data are listed as below.



NP-Fig. 1, A new mycelia-contained cellulose acetate membrane inoculation method. a, *Rhizcotonia solani* mycelia cultivated for 3 days on the PDA plate on a cellulose acetate membrane; b, Young seedlings were covered with the cellulose acetate membrane containing mycelia for inoculation; after inoculation, the plants were kept in an incubator with humidity ranged from 80% to 95% and temperature from 26℃ to 30℃. c, Disease symptoms at 48 and 72 hours post inoculation (HPI).



Supplementary Fig. 9a, Comparison of phosphorylation levels of SBRR1 protein in

SBRR1-OE1 at different time points post *R. solani* inoculation. Total protein was separated in SDS-PAGE containing Phos-tag acrylamide or normal SDS-PAGE. CIP, calf intestinal alkaline phosphatase. ACTIN was used as a loading reference.

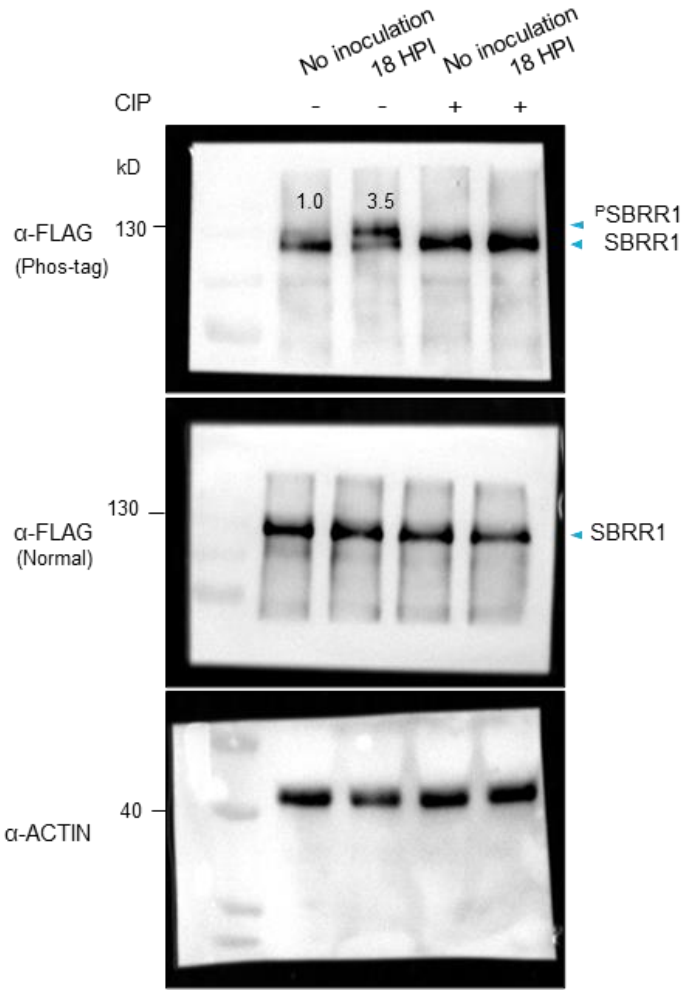
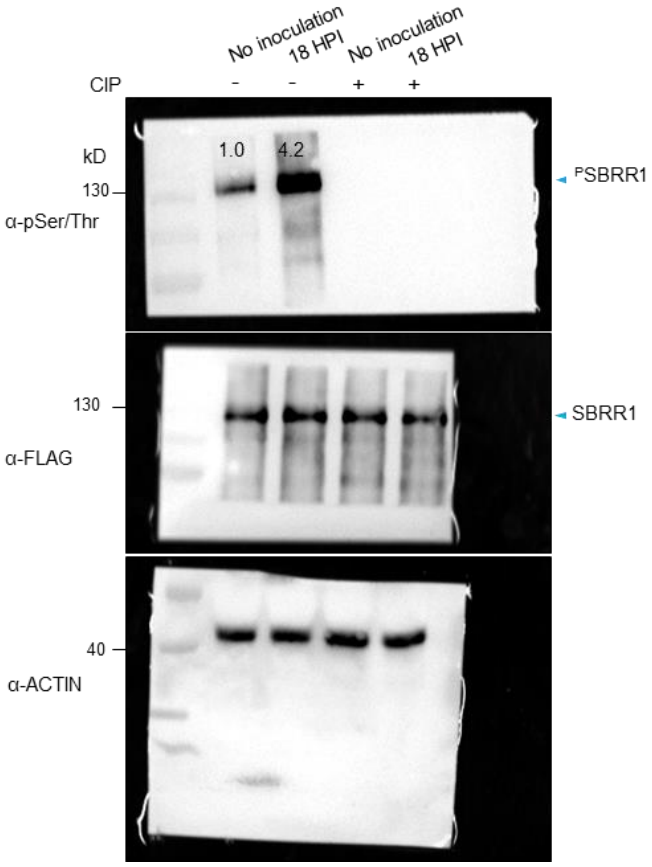


Fig. 2e, Comparison of phosphorylation levels of SBRR1 protein in *SBRR1*-OE1 with or without *R. solani* infection. Total protein was separated in SDS-PAGE containing Phos-tag acrylamide or normal SDS-PAGE. CIP, calf intestinal alkaline phosphatase. ACTIN was used as a loading reference.



Supplementary Fig. 9b, Immunoblotting assay using a pSer/Thr antibody to detect phosphorylation levels of SBRR1 protein in *SBRR1*-OE1 line with or without *R. solani* infection. CIP, calf intestinal alkaline phosphatase. ACTIN was used as a loading reference.

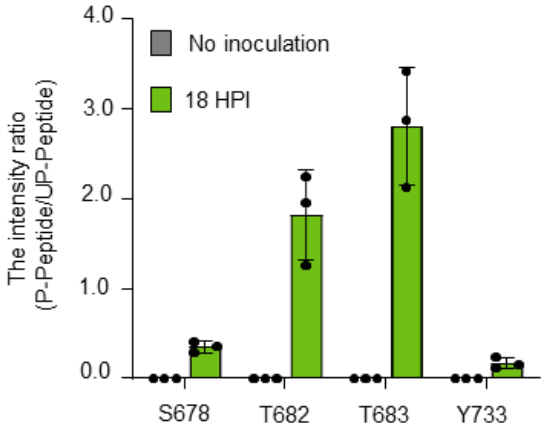
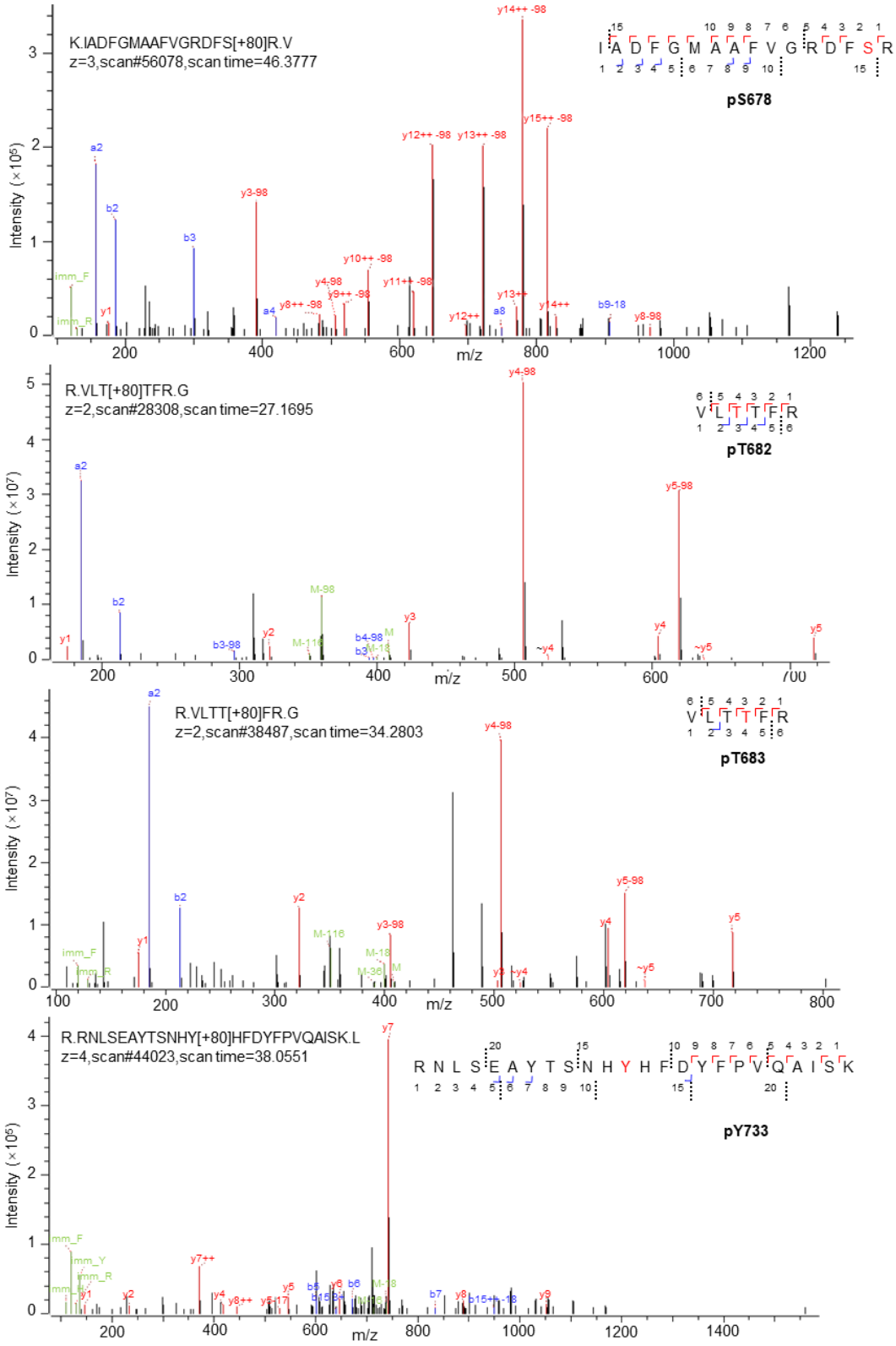


Fig. 2f, f, LC-MS/MS-dectected intensity ratios of phosphorylated (P-peptide) to un-phosphorylated peptide (UP-peptide) at S678/T682/T683/Y733 sites of SBRR1 protein in *SBRR1*-OE1 line with or without *R. solani* infection (n=3).



Supplementary Fig. 10, Identification of four phosphorylation sites (S678, T682, T683 and Y733) in SBRR1 protein after *R. solani* attack by quantitative mass spectrometry analysis. The b and y ions are marked in spectra with respective peptide sequence above the spectrum.

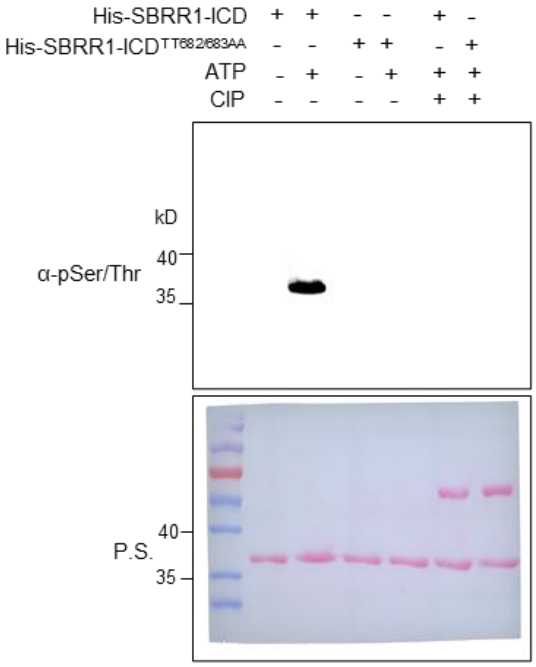
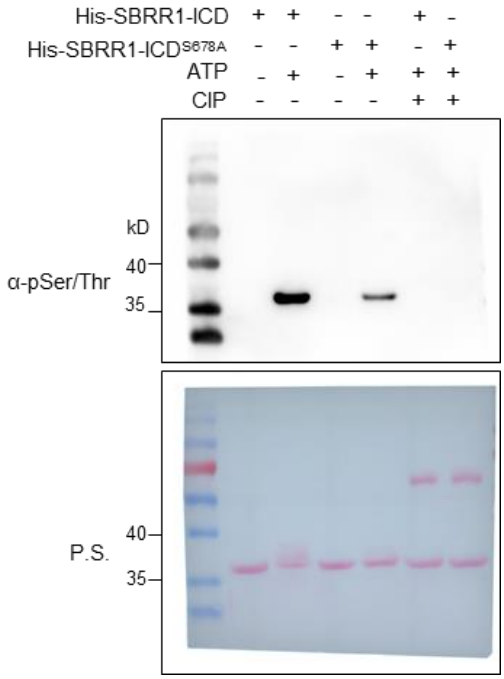


Fig. 2g, Immunoblot assay using a pSer/Thr antibody to detect autophosphorylation in His-SBRR1-ICD$^{TT682/683AA}$. CIP, calf intestinal alkaline phosphatase. Ponceau S stains His-SBRR1-ICD and His-SBRR1-ICD$^{TT682/683AA}$ proteins. P.S., Ponceau S staining.



Supplementary Fig. 9c, Immunoblot assay using a pSer/Thr antibody to detect autophosphorylation in His-SBRR1-ICD^{S678A}. CIP, calf intestinal alkaline phosphatase. Ponceau S stains His-SBRR1-ICD and His-SBRR1-ICD^{S678A} proteins. P.S., Ponceau S staining.

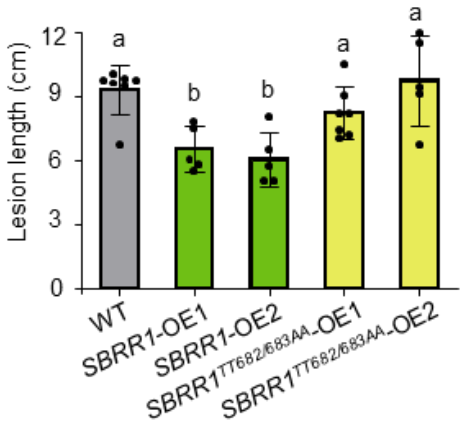


Fig. 2h, ShB resistance of *SBRR1*$^{TT682/683AA}$-OE lines at 7 days after inoculation. The values represent mean ± s.d (n≥5) Different letters indicate significant differences according to *Duncan's* multiple range tests ($P < 0.05$). Scale bar, 5 cm.

2. As the authors claimed that the elite *SBRR1-R* allele, carrying a 256-bp insertion in its promoter, expresses elevated *SBRR1* transcript levels resulting in higher ShB resistance, it is required to identify the upstream transcription factor which regulates Sbrr1 expression through acting with the *SBRR1* promoter. And test how this 256-bp insertion elevate the expression of *SBRR1*. Does this expression elevation of *SBRR1* is dependent or independent of pathogen *Rhizoctonia solani* infection?

Response: Thanks for this valuable suggestion. In the revised version, we have added a section with a subtitle "bHLH57 is a positive regulator that differentiates expression of elite *SBRR1-R and SBRR1-S* " in Results. By using this 256-bp insertion as a bait, we have identified transcription factor bHLH57 via yeast one-hybrid screens. bHLH57 binds to *SBRR1-R* promoter, but not to *SBRR1-S* promoter.

We further divided the 256-bp region into two fragments in yeast one-hybrid assay and narrowed down to a 121 bp region (pSBRR1-M2) that contains a 'CACCGG' motif putatively acting as the binding motif of bHLH57 (Fig.5a). This motif is only present in this 256-bp insertion sequence of *SBRR1-R* genomic region, but not in *SBRR1-S* genomic region. Our further results revealed that: 1) the 'CACCGG' motif is required for bHLH57 binding in yeast one-hybrid assay and EMSA (Fig.5a, b); 2) *in vivo* binding is confirmed by ChIP-qPCR assays (Fig.5c); 3) bHLH57 promotes luciferase expression driven by the *SBRR1-R* promoter, but not by *SBRR1-S* promoter in dual-luciferase assays (Fig.5d).

We also found that *bHLH57* is mainly expressed in leaf sheaths and blades, which is well-correlated with the rice tissues that *R. solani* infects (Supplementary Fig.18). During revision, we have obtained all *bHLH57* transgenic rice lines, including bHLH57 knockout homozygous lines in TG394 (with *SBRR1-S*) and its near-isogenic line TG394-SBRR1^{R} (with *SBRR1-R*) backgrounds (Supplementary Fig.19) and tested their effects. We observed that bHLH57 clearly promotes strong *SBRR1-R* expression upon *R. solani* infection (Fig.5e). For instance, the expression level of *SBRR1* in TG394-SBRR1^{R} (14.2-fold change) plants was significantly higher than that in TG394 (2.2-fold change) at 12 HPI, while in TG394-SBRR1^{R}/*bhlh57-ko* plants, it was comparable with those in TG394 and TG394/*bhlh57-ko* plants, indicating that the **strong induction pattern of *SBRR1* in *SBRR1-R* variety depends on bHLH57.**

Furthermore, we compared the resistance levels of four rice lines (TG394, TG394/*bhlh57-ko*, TG394-SBRR1^{R}, and TG394-SBRR1^{R}/*bhlh57-ko*) and found that TG394-SBRR1^{R} showed stronger resistance than TG394, whereas TG394/*bhlh57-ko* and TG394-SBRR1^{R}/*bhlh57-ko* lines showed similar susceptible levels to ShB (Fig.5f). We also generated bHLH57 overexpression lines in TG394 and TG394-SBRR1^{R} backgrounds (Supplementary Fig.17) and found that TG394-SBRR1^{R}/bHLH57-OE plants showed stronger ShB resistance than both TG394-SBRR1^{R} and TG394/bHLH57-OE plants (Fig.5g). This not only confirms the contribution of *SBRR1-R* on ShB resistance, but also suggests a tight relationship between bHLH57 and *SBRR1-R* for ShB resistance. Additionally, the positive role of bHLH57 in ShB resistance is also reported in a recent study (Liu et al., Plant Cell Reports, 2022, 41:1285-1299).

In the revised version, we have added all these data to a new section in the Results. We believe these data can well address your major concern.

Some new data in the revised version are listed as below.

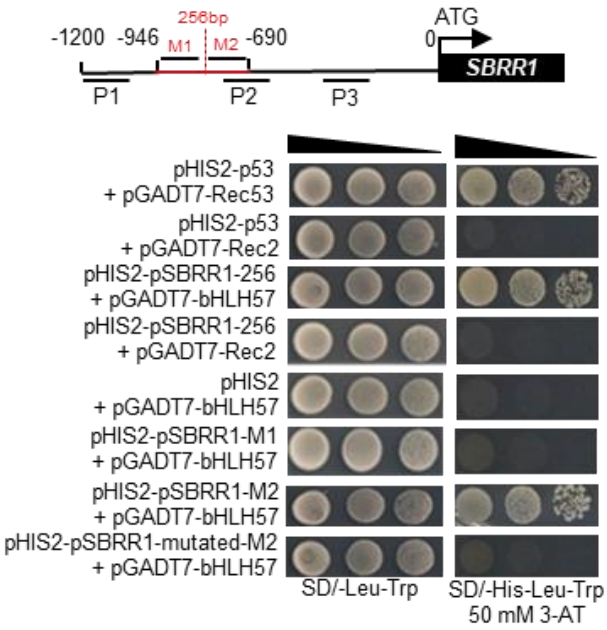


Fig. 5a, Yeast one-hybrid assay to identify the potential bHLH57 binding site. M1 and M2 represent two fragments of the 256bp insertion. The CACCGG motif is found only in the M2 fragment.

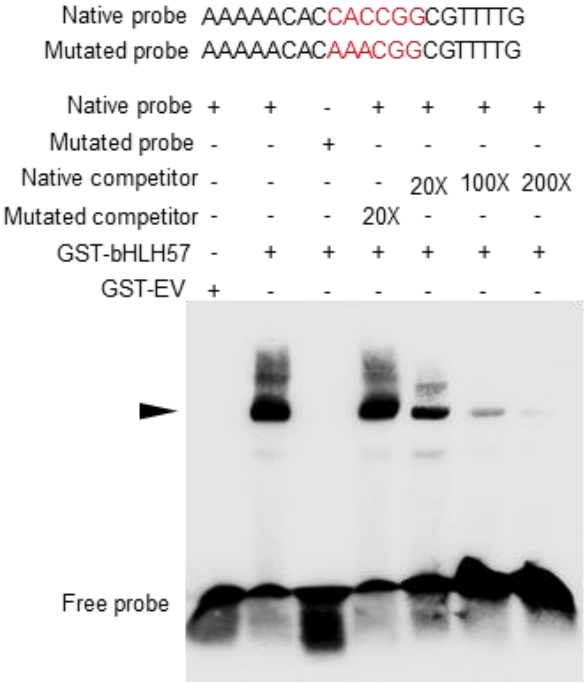


Fig. 5b, EMSA assay to confirm the direct binding of bHLH57 to the CACCGG motif. A slower migrating band for the 21-bp native probe carrying the CACCGG motif was detected when incubated with a GST-bHLH57 fusion protein. Unlabeled wildtype competitors, but not mutated competitors, at 20-fold greatly reduced the binding to the probe, indicating specific binding to this sequence.

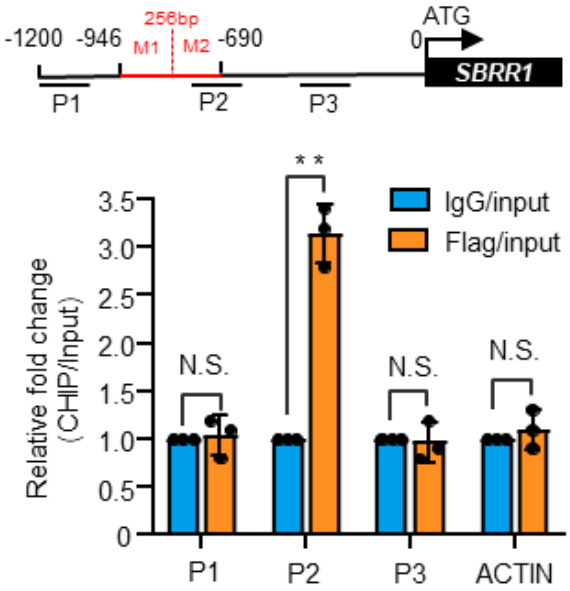


Fig. 5c, ChIP-qPCR assay showed that the P2 region of the *SBRR1-R* promoter is significantly enriched by transcription factor bHLH57. Enriched values are normalized to the input. An unrelated *OsActin1* intron region was used as a negative control. IgG-immunoprecipitated DNA was used as a control. The values represent mean ± s.d (n=3). Statistical significances were determined using a Student's two-sided t-test (**: $P < 0.01$). N.S. indicates no significant differences.

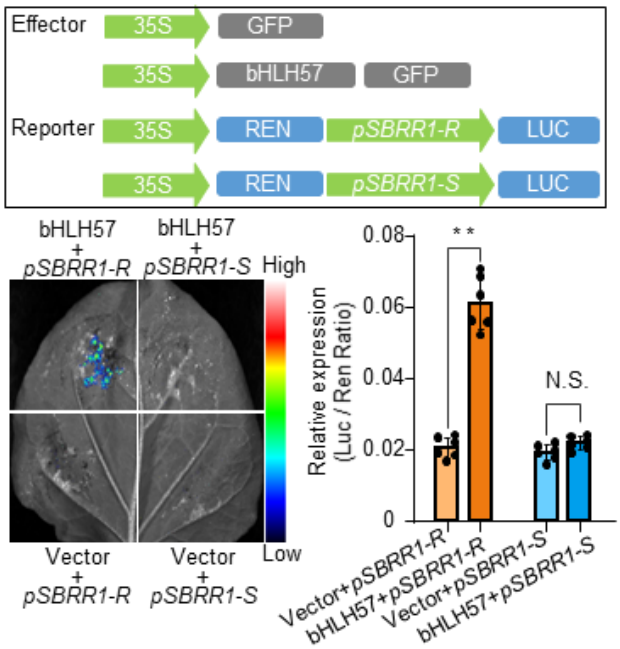


Fig. 5d, Dual-luciferase reporter assay to study the effects of bHLH57 on *SBRR1-R* expression. The values represent mean ± s.d (n=6). Statistical significances were determined using a Student's two-sided t-test (**: $P < 0.01$).

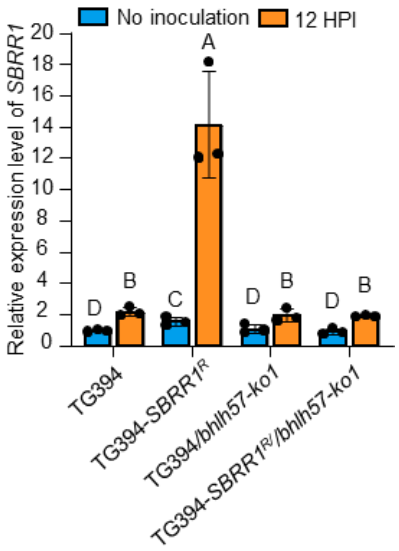


Fig. 5e, Relative RNA expression levels of the *SBRR1* gene in TG394, T394-SBRR1^{R} and their bhlh57-ko plants. The values represent mean ± s.d (n=3). Different letters indicate significant differences according to *Duncan's* multiple range tests ($P < 0.01$).

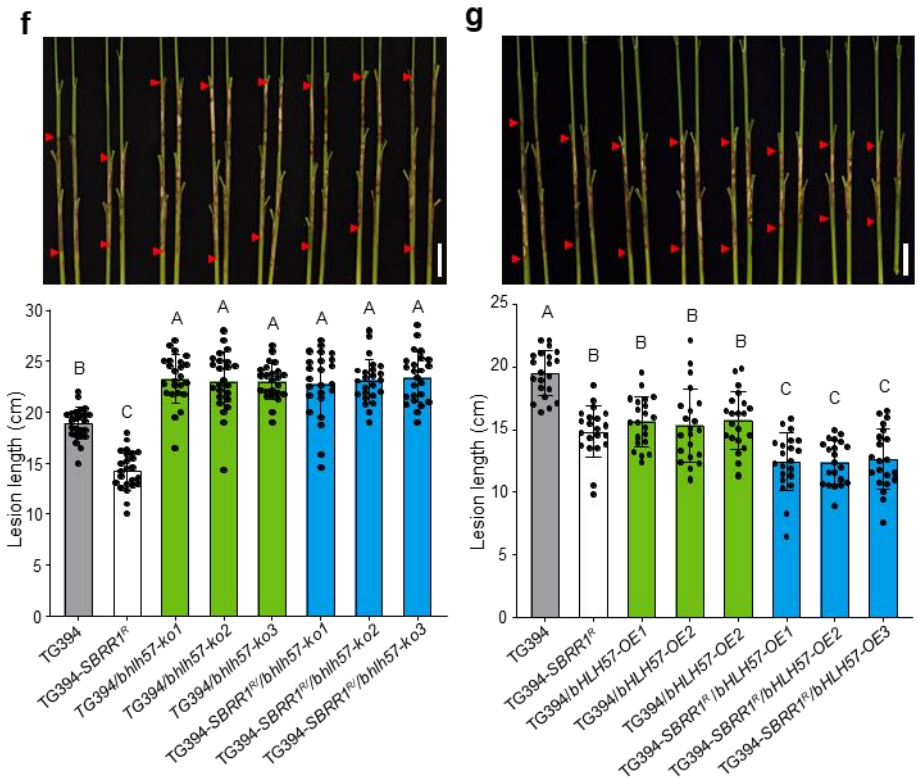


Fig. 5f and 5g, ShB lesions of *bHLH57* knockout lines (5f) and *bHLH57* overexpression lines (5g) in TG394 and T394-SBRR1^{R} backgrounds, respectively, scored 14 days after *R. solani* inoculation in greenhouse (n=21~24). The values in f and g represent mean ± s.d. Different letters indicate significant differences according to *Duncan's* multiple range tests ($P < 0.01$). Scale bar, 5 cm.

3. It is interesting that SBRR1 regulates downstream chitinase genes-associated events for sheath blight resistance. However, there is gap on how plasma membrane located SBRR1 protein regulates the transcription of the chitinase genes in nucleus?

Response: Thanks for the comment. Our genetic evidence strongly supports that the plasma membrane localization of SBRR1 is required for its function (Fig.6e), and chitinase 3 and 4 genes account for the majority of SBRR1-mediated resistance (Fig.8c, d). However, because we have not been able to identify the substrate(s) of SBRR1 or unknown kinase protein(s) that phosphorylate SBRR1 in response to pathogen infection, we have no way to investigate how SBRR1 protein transduces the signal into the nucleus. Without the identification of SBRR1 substrates and downstream signaling components, we are not in any way to fill the gap between plasma membrane-localized SBRR1 and induction of chitinase gene expression in the nucleus at the present time. Generally, this will take many years, even with luck, to elucidate how a membrane-localized receptor kinase regulates expression of downstream effector proteins (like chitinases) upon pathogen infection, because phosphorylation signals usually go through many intermediate regulators, often kinases and transcription factors. We have tried yeast two-hybrid screens and Immunoprecipitation-Mass Spectrometry (IP-MS) assays to identify interactors of SBRR1, but had no luck so far, except for SIP1. We have also tested many known rice RLCKs and found none that are involved in SBRR1 signaling, suggesting that SBRR1 signaling is very different from those of other known receptor kinases. Therefore, it is beyond our current capacity and can only be treated as a long-term goal so far.

In the revised version, we have also added a description for *SBRR1-R* in Discussion: "However, how SBRR1 is phosphorylated and then transduces the signal into the nucleus to activate the expression of chitinase genes and other genes remains to be further elucidated."

Minors

4. As Sbrr1 affects many chitinase genes expression (Chit3, Chit4, Chit7, Chit12 and Chit17), why only chitinase genes Chit3 and Chit4 are most important for sheath blight resistance? Only because the expression of these two genes induced more higher by pathogen Rhizoctonia solani infection?

Response: Thanks for the scientific comment. Investigating the expression of chitinase genes was mainly due to the finding in RNAseq data that suggests that chitinase genes are associated with the SBRR1-mediated downstream signaling (Supplementary

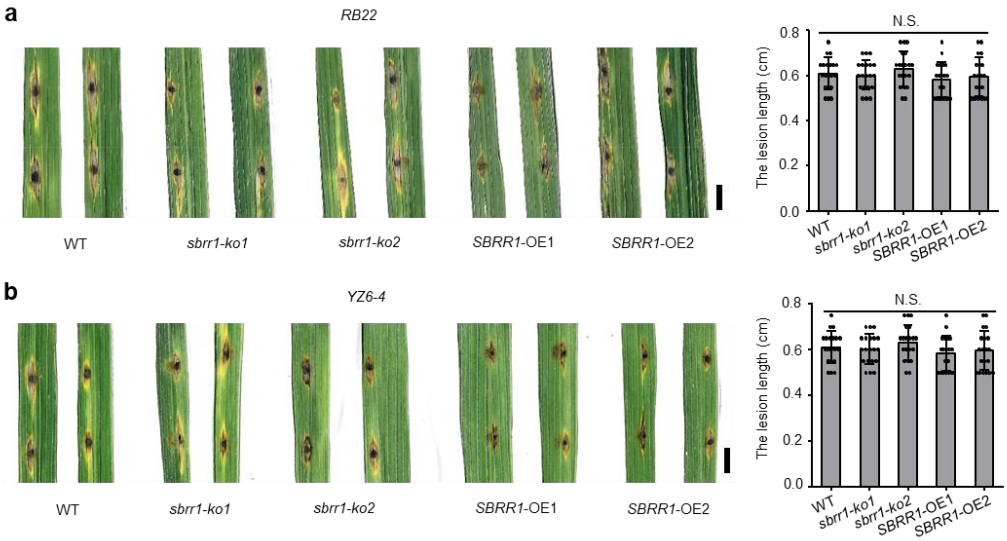
Fig.25). Furthermore, the chitinase activities in SBRR1-OE and SIP1-OE plants are significantly higher than in sbrr1-ko and sip1-ko plants at 24 and 48 HPI (Fig. 8b). So, we further detected all chitinase genes using RT-qPCR (Fig.8a; Supplementary Fig.26). We found that the expression levels of several chitinase genes, including those mentioned by the reviewer, were affected in sbrr1-ko plants when compared with WT. However, **we only chose Chit3 and Chit4 for further study because these two genes were not only clearly affected in sbrr1-ko plants, but also dramatically affected in sip1-ko plants** (Fig.8a; Supplementary Fig.26). SIP1 acts as a molecular chaperone to assist efficient SBRR1 accumulation on PM where SBRR1 functions, but SIP1 itself has no ShB resistance effect. For instance, SIP1 overexpression in sbrr1-ko plants did not increase ShB resistance (Fig.6e). Therefore, we reasoned that the chitinase genes commonly affected in both sbrr1-ko and sip1-ko plants are the best candidates that are responsible for the SBRR1-mediated downstream resistance response. And so, we chose Chit3 and Chit4 for subsequent study. This is in addition to the fact that *R. solani* infection highly induces their expression.

In our subsequent experiments on transgenic lines (Fig.8c, d), including SBRR1-OE, SBRR1-OE/chit3chit4-ko, Chit3-OE, sbrr1-ko, sip1-ko, sbrr1-ko/Chit3-OE and sip1-ko/Chit3-OE, we confirmed that Chit3 and Chit4 account for the majority of SBRR1-mediated ShB resistance. Also, the chitinase activities in TG394 (contain *SBRR1-S*) are significantly lower than in near-isogenic line TG394-SBRR1^{R} (Supplementary Fig.27), further supporting that the SBRR1-mediated resistance depends on chitinase genes to a great extent.

5. Since chitinase activity is also involved in rice blast resistance, does SBRR1 regulates blast resistance? Is this molecular regulation of SBRR1 in blast resistance same as in sheath blight resistance?

Response: Thanks for the valuable suggestion. In fact, we have previously tested whether SBRR1 regulates blast resistance by using two blast strains *RB22* and *YZ6-4*. *RB22* is a well-known strain that has been widely used in previous blast disease-related studies (Park et al., The Plant Cell, 2012, 24:4748-4762; Kang et al., Mol Plant Pathol, 2016, 17:959-972). *YZ6-4* is a strain collected from rice field in Jiangsu province. Using the punch inoculation method (NP-Fig.2a, b), we found that both strains could infect WT plants, but no significant differences on lesion lengths among WT, *sbrr1-ko* and *SBRR1*-OE plants were observed. This suggests that SBRR1 does not regulate blast resistance. We reason that *SBRR1-R* (and thus chitinase genes) may be only greatly

induced by *R. solani* infection. Given that these data are not necessary for the current manuscript, we did not include them in the revised version.



NP-Fig. 2, Disease phenotypes and lesion lengths of WT, *sbrr1-ko* and *SBRR1*-OE plants after punch inoculation with *M. oryzae* isolates *RB22* (a) and *YZ6-4* (b). RB22 is a well-known blast strain which has been widely used previously. YZ6-4 is a strain collected from rice field in Jiangsu province. Blast punch inoculation followed the method described by Li et al. (Cell, 2017, 170, 114-126). A 10 μL drop of spore suspension (5×10^5 spores/mL) was applied to the wounded site, which was then wrapped with Scotch tape. Disease symptoms were evaluated at 10~12 days after inoculation, and lesion lengths were measured.

Reviewer #2:

Remarks to the Author:

This study identifies the first gene underlying sheath blight resistance in rice, along with some mechanistic insights. Given the importance of this pathogen, I think the manuscript would appeal to a broad audience.

The genomic and evolutionary analyses of the MS appear to be done well. This is not my primary area of expertise, so I focus my comments more on the molecular and biochemical experiments.

Response: Thanks for the reviewer's recognition on the study.

1. The transgenic line phenotypes are convincing. I am missing the generation tested. Are they all homozygous or earlier generations? This information should be provided.

Response: Thanks for the scientific suggestion. We apologize for not providing the information in details in the original manuscript. Since we have conducted this project for over 8 years, the transgenic rice lines used in the current study were all homozygous lines of at least T_2 generation, except for *bhlh57-ko* (homozygous line, T_1 generation) and *bHLH57*-OE lines (T_1 generation) in Fig. 5f-g in the revised version. Although the *bhlh57-ko* and *bHLH57*-OE lines were T_1 generation, we believe that they are sufficient for the phenotypic test. This is because all *bhlh57-ko* T_1 lines tested in the study were selected from T_0 plants carrying the homozygous mutation based on the sequencing data. The bHLH57-OE plants in phenotypic tests were all measured using RT-qPCR and Western blotting, which ensured overexpression of the gene in these transgenic T_1 plants as well as T_0 plants. In the revised version, we have added these in details in the Figure legends.

2. claims of increased yield, etc should be toned down. The data rely on one greenhouse and one smaller field trial. See: https://www.nature.com/articles/d41586-023-02895-w

Response: Thanks for the kind and professional suggestion. We have actually conducted two field trials to evaluate the yield and grain quality for two pairs of rice lines, T394 and T394-*SBRR1*^{R}, DJ and SBRR1-OE1 (DJ background), respectively. Both field trials contained two disease conditions/treatments: slight disease treatment controlled by spraying with fungicide and severe disease treatment conducted by artificial inoculation with *R. solani* on 6 tillers per plant to ensure severe disease development (Fig.4c, d). Each treatment consisted of 5/6 replications/plots and each plot was 2.37 m in length and 1.38 m in width. Each plot comprised 10 rows with 12 plants per row. In order to reduce marginal effects, only the 6 inside rows with middle 8 plants in each row (48 plants covering around 1.32 m^2 in the field) were disease-rated and collected for grain yield and quality analysis.

(1) The first field trial consisted of T394 and T394-SBRR1R, and each treatment contained 6 replications/plots. Based on the disease scores (Fig. 4c), we believe that our

serious disease treatment was successful. We found that under the slight disease condition, no significant differences in grain yield between the two lines were observed, suggesting that *SBRR1-R* was not a grain yield-related gene. On the contrary, we found that TG394-*SBRR1*^{R} had significantly lower grain yield loss than TG394 by approximately 9.54% under severe disease condition (Fig.4d), demonstrating a great value for *SBRR1-R* under serious disease condition. The yield increase under severe disease conditions can be attributed to the reduction of plant disease due to *SBRR1-R*, which is reasonable and is different from those yield-related genes previously reported that were easily affected by genetic backgrounds.

(2) the second field trial comprised DJ and SBRR1-OE1/DJ, and each treatment contained 5 replications/plots. We found that overexpression of *SBRR1* rescued yield losses up to 13.2% under severe disease condition (Supplemental Fig. 16b-c).

Together, this means that we tested the effect on rescuing yield loss in the field in two different rice genetic backgrounds. Especially, TG394 is a commercial rice variety that has been widely grown in Jiangsu Province, China. Therefore, we are confident with our data and believe that *SBRR1-R* has a great value in developing ShB resistant varieties.

Certainly, considering your concern, we have toned down this description by removing this value in rescuing yield loss from the Abstract, but still kept them in the Results and Discussion sections in the revised version. We have also added these field designs in details in Materials and Methods section in the revised version. Thanks again for your professional suggestion.

3. phosphorylation: blot in fig 2e is heavily cropped, positive and negatives should be run on the same gel. Samples should be treated with a phosphatase to show removal of the phosphorylation signal.

Response: Thank you very much for your valuable suggestions. Accordingly, we have repeated this phosphorylation assay by using His-SBRR1-ICD and His-SBRR1-ICD^{K553E} proteins side by side, and have used the new Fig. 2c in the revised version to replace the original Fig.2e. The results show that His-SBRR1-ICD, but not His-SBRR1-ICD^{K553E}, possesses auto-phosphorylation activity, which is consistent with our previous result in the original Fig.2e. In addition, a phosphatase treatment is also included to confirm this finding (Fig.2c).

The new Fig.2c is listed as below.

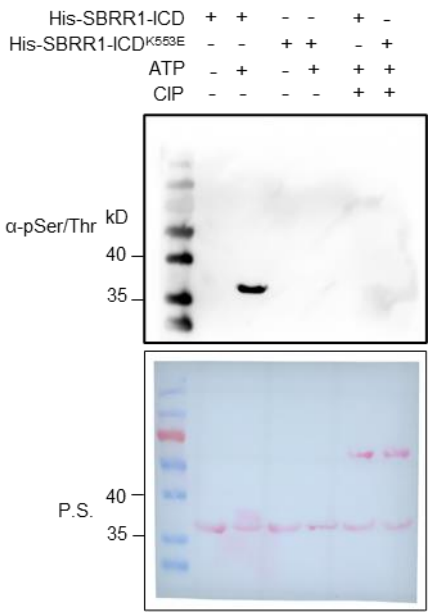


Fig.2c, Immunoblotting assay using a pSer/Thr antibody to detect autophosphorylation of His-SBRR1-ICD and His-SBRR1-ICD^{K553E}. CIP, calf intestinal alkaline phosphatase. Ponceau S stains His-SBRR1-ICD and His-SBRR1-ICD^{K553E} proteins. P.S., Ponceau S staining.

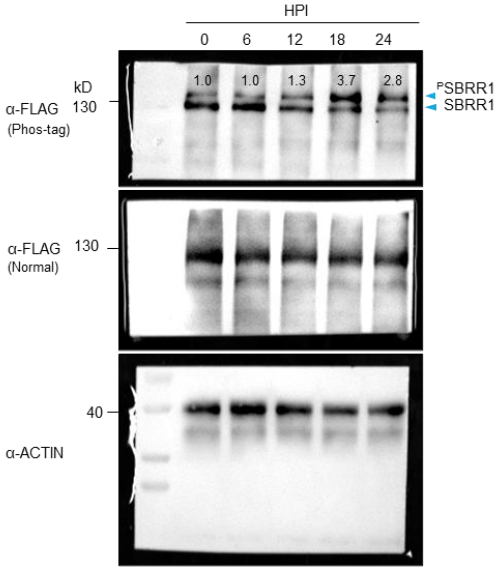
4. Is SBRR1 phosphorylated in planta? Can it phosphorylate SIP1 (in vitro or in vivo)? The size shift in fig 5c for SIP1 indicates it could be phosphorylated. One can check phosphorylation after IP using a gel shift assay + phosphatase if anti pS/pT is not strong enough.

Response: Thanks for raising these concerns. We have tested SBRR1 phosphorylation level in planta under normal condition as well as upon pathogen attack (Fig.2e; Supplemental Fig.9a, b). We found SBRR1 protein can be separated into two bands when running on Phos-tag gels, suggesting that SBRR1 is phosphorylated under normal condition. We also observed clear increases in the upper SBRR1 protein band from 12 to 24 HPI with *R. solani* with a peak (~4-fold) at 18 HPI compared to 0 HPI, indicating that phosphorylation of SBRR1 is induced by pathogen attack (Fig.2e; Supplementary Fig.9a, b).

Regarding the second point "Can it phosphorylate SIP1 (in vitro or in vivo)? The size shift in fig 5c for SIP1 indicates it could be phosphorylated": We have tested these using the pSer/Thr antibody assay (NP-Fig.3) and an isotope-labelling assay (NP-Fig.4). Both results indicate that SBRR1 can self-phosphorylate, but cannot phosphorylate SIP1. In addition, considering the fact that the SBRR1-SIP1 interaction occurs at the extracellular domain of SBRR1 (Supplementary Fig.22b, d), and SIP1 functions intracellularly to facilitate SBRR1 accumulation on PM (Fig.7c, f), it is logical and anticipated that no phosphorylation of SIP1 by SBRR1 kinase occurs. Therefore, these

data (NP-Fig.3 and 4) are not necessary for the current study and not included in the revised version.

Some new data in the revised version are listed as below.



Supplementary Fig. 9a, Comparison of phosphorylation levels of SBRR1 protein in *SBRR1*-OE1 plants at different time points post *R. solani* inoculation. Total protein was fractionated by SDS-PAGE containing Phos-tag and normal SDS-PAGE. CIP, calf intestinal alkaline phosphatase. ACTIN was used as a loading reference.

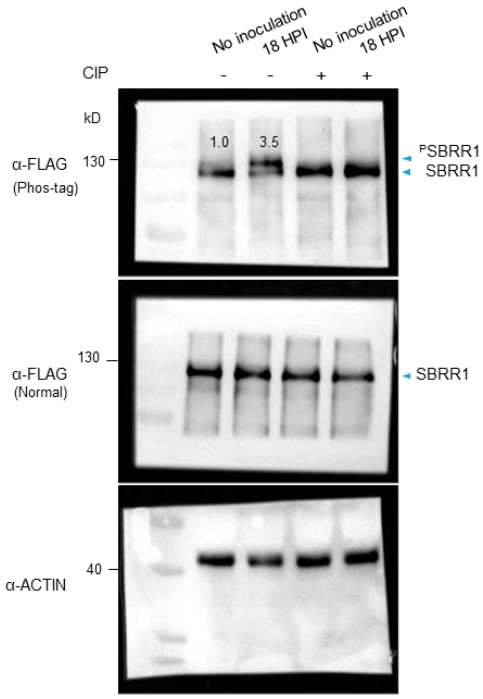
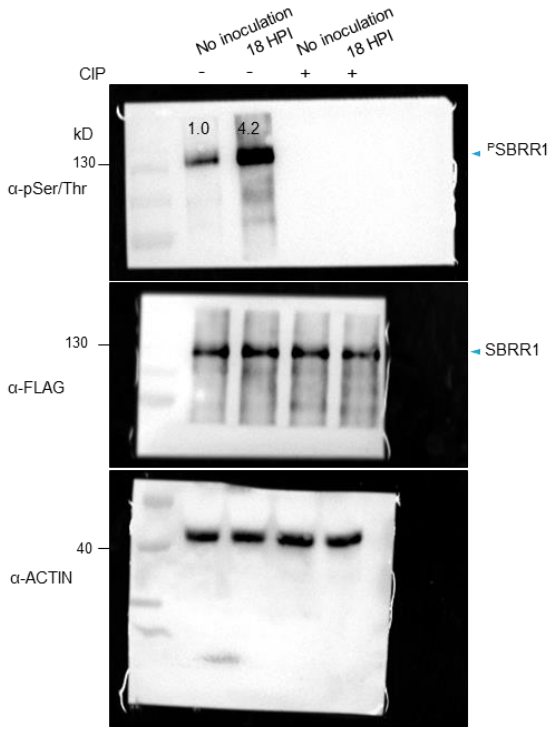
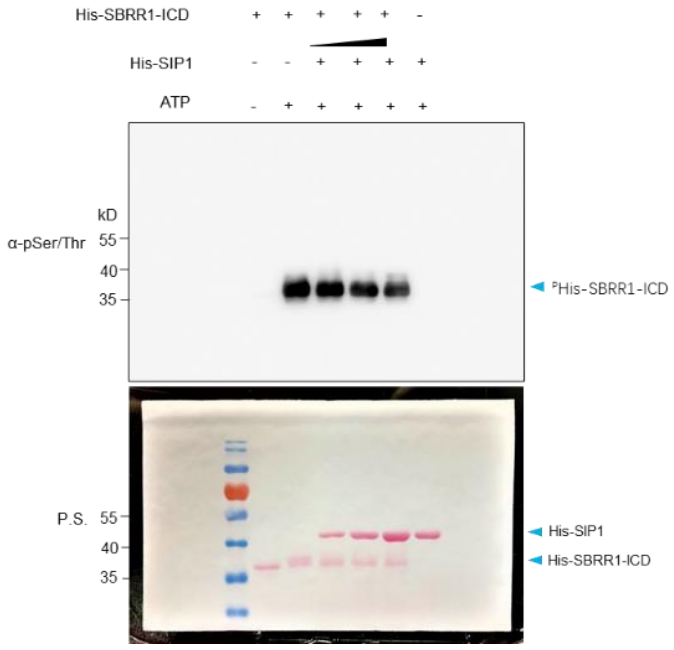


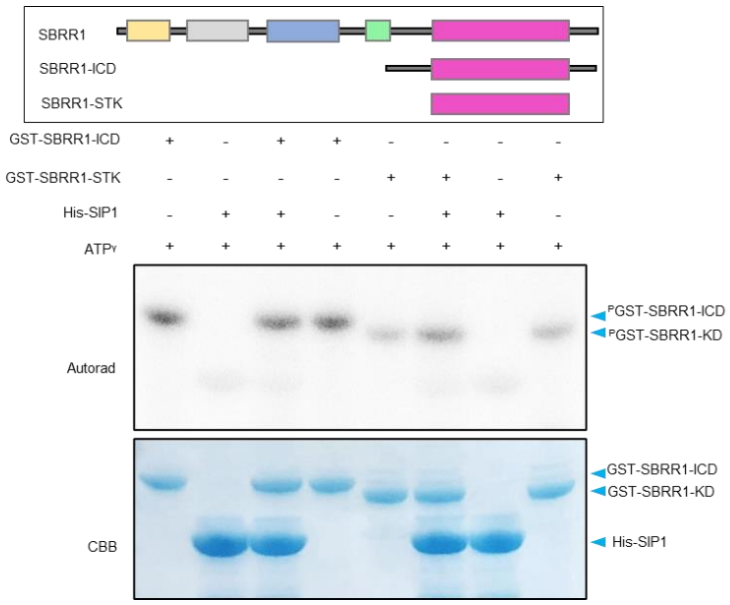
Fig. 2e, Comparison of phosphorylation levels of SBRR1 protein in *SBRR1*-OE1 with or without *R. solani* infection. Total protein was fractionated by SDS-PAGE containing Phos-tag and normal SDS-PAGE. CIP, calf intestinal alkaline phosphatase. ACTIN was used as a loading reference.



Supplementary Fig. 9b, Immunoblotting assay using a pSer/Thr antibody to detect phosphorylation levels of SBRR1 protein in *SBRR1*-OE1 with or without *R. solani* infection. CIP, calf intestinal alkaline phosphatase. ACTIN was used as a loading reference.



NP-Fig. 3, Phosphorylation assay using a pSer/Thr antibody to test whether SBRR1 can phosphorylate SIP1. His-SIP1 recombinant protein was incubated with His-SBRR1-ICD in kinase reaction buffer supplemented with ATP and then separated by SDS-PAGE for western blotting. Ponceau S stains His-SBRR1-ICD and His-SIP1 proteins. P.S., Ponceau S staining.



NP-Fig. 4, Phosphorylation assay using an isotope-labelling method to test whether SBRR1 can phosphorylate SIP1. His-SIP1 recombinant protein was incubated with GST-SBRR1-ICD or GST-SBRR1-STK in kinase reaction buffer supplemented with 32P-γ-ATP and then separated by SDS-PAGE for autoradiography. Upper panel, autoradiogram; bottom panel, CBB-stained gel.

5. Fig 2 b-h could be labeled better for easier interpretation.

Response: Thanks for the suggestion. In revised version, we have labeled each part for easier interpretation.

6. Fig 4 – several of the graphs indicate significant differences but error bars are overlapping. Statistics should be double-checked or raw data provided (Fig 4f, 4k, 4i).

Response: Thanks for the scientific suggestion. We have carefully double-checked the raw data and statistics analyses of Fig.4 and other Figures, and confirmed the accuracy. Also, in the revised version we have added raw data points to all bar charts including Fig 4f, 4k and 4i. It is common that some data points may overlap when the overall statistics shows a significant difference.

7. Fig.5-5b the signal for GST input looks stronger than GST IP. Were samples diluted after IP? There should be a stronger signal after IP for GST. 5c-there is no negative control for IP, the authors need to use an antibody that localizes to the ER/PM in rice to demonstrate a specific interaction. The PIP1;1 aquaporin antibody could be used (Fig.6).

Response: Thanks for these valuable comments. Yes, in the GST pull-down assay, the IP protein samples were diluted 6 times, otherwise they would show very strong signals. During revision, we repeated the GST pull-down assay and used CBB-stained gels as

the reference to represent protein loadings and quality, and consequently diluted these IP proteins 2 times. The results show that the 2-fold diluted IP protein presents a stronger signal than the input (Fig.6b).

As suggested, we also repeated the Co-IP assay for SBRR1 and SIP1 interaction using PIP1;1 as a negative control. The results (Fig.6c) show that SIP1, but not PIP1;1, is immunoprecipitated by SBRR1, which is consistent with our previous Fig. 6.

The new Fig.6b and 6c are listed as below.

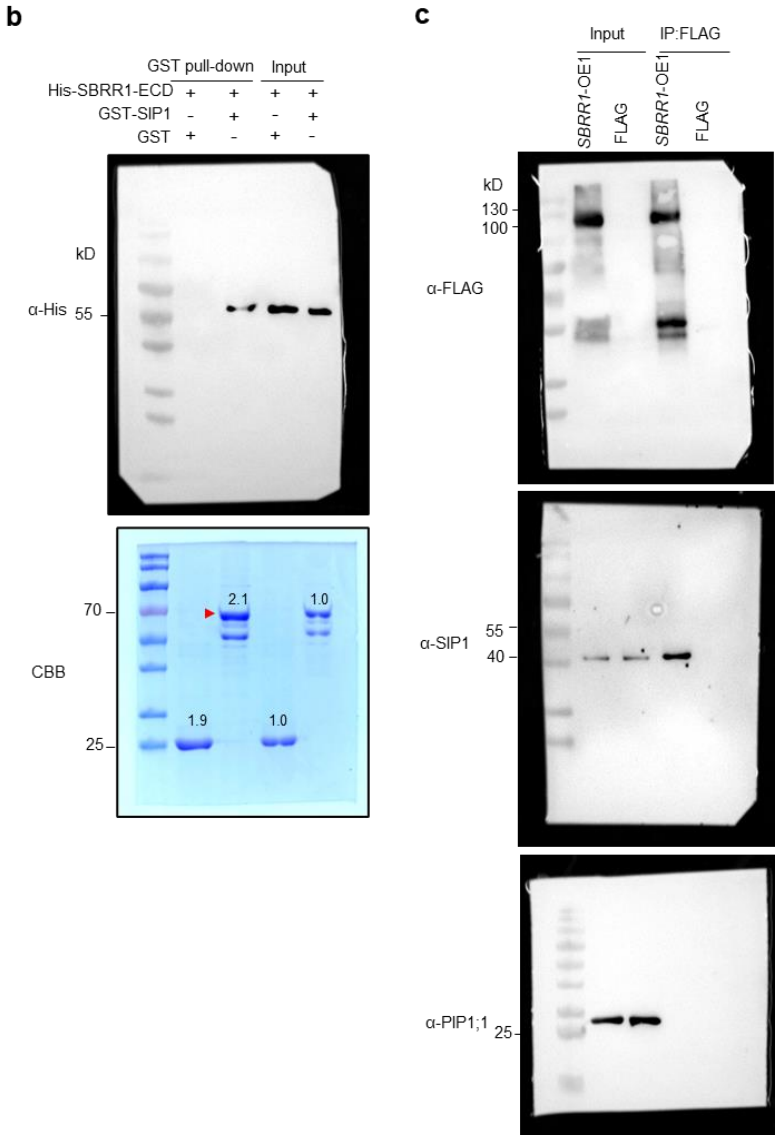


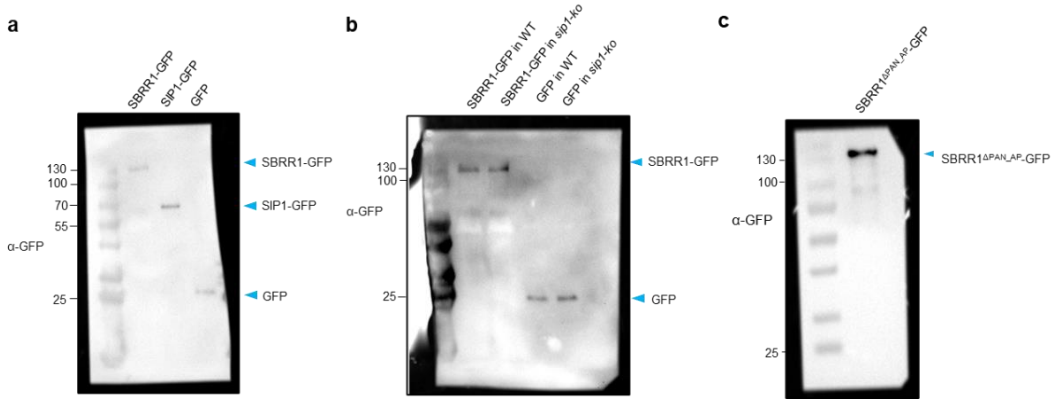
Fig. 6b, c, *in vitro* GST pull-down assay (b) and *in vivo* co-immunoprecipitation assay using *SBRR1*-OE1 transgenic plants (c) for SBRR1 and SIP1 interaction. Lower panel in b shows the protein abundance stained by CBB in SDS-PAGE gel. FLAG, representing transgenic plants expressing a FLAG tag alone, and anti-PIP1;1 were used as negative controls.

8. Localization: western blots should be provided to demonstrate no GFP cleavage in rice protoplasts (Fig 6). Western blots should also be provided to demonstrate expression in Y2H in the main and supplemental figures.

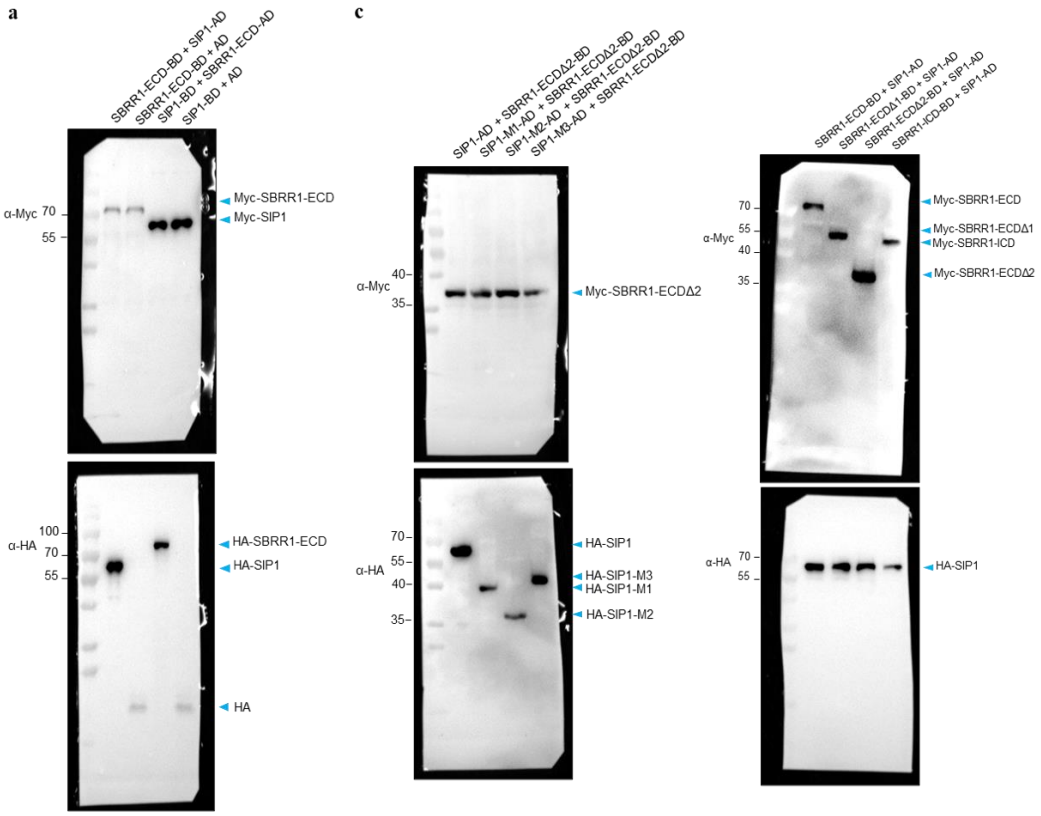
Response: Thanks for these suggestions. During revision, we have repeated expression of all GFP fusion proteins in rice protoplasts, and performed Western blotting to confirm that the major band contains no GFP cleavage in rice protoplasts. Using GFP antibodies, we found that each of the GFP fusion proteins showed a clear single major band, consistent with the theoretical molecular weight (Supplementary Fig.23).

We also re-transferred all yeast two hybrid related constructs in the study into yeast for checking their protein expression. The western blot results showed that all fusion proteins were expressed as a single major band, consistent with their theoretical molecular weights, suggesting the correct expression of these constructs in yeast cells (Supplementary Fig.22a, c). In the revised version, we have provided these new data as Supplementary Figures.

Some new data are provided as below.



Supplementary Fig. 23, Western blot detection of the expression of full-length GFP fusion proteins in rice protoplasts. The proteins (labeled in the figures) used in Figure 7a-d were probed by using a GFP antibody. Each of the GFP fusion proteins was detected essentially as a single major band representing the full-length GFP fusion protein in rice protoplasts, consistent with the predicted protein size.

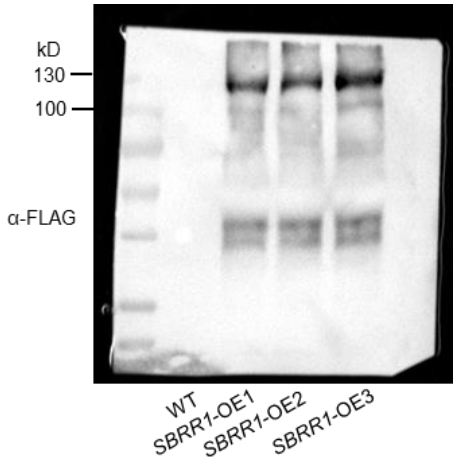


Supplementary Fig. 22a, c, Western blot detection of the expression of Myc/HA fusion proteins in yeast. These proteins (labeled in each of the figures) were used in Fig. 6a (a) and Supplementary Fig. 22b (c). By using Myc/HA antibodies, each of the Myc/HA fusion proteins was detected essentially as a single major band representing the full-length GFP fusion protein in rice protoplasts, consistent with the predicted protein size.

9. Fig S13 – western blots are closely cropped. Is SBRR1 cleaved in planta or is only full length detectable?

Response: Thanks for this scientific comment. We did find other weak bands below the SBRR1-FLAG band, in addition to the full-length SBRR1-FLAG band, in the SBRR1-FLAG overexpression lines, (Supplementary Fig.6c). This is seen more clearly after immunoprecipitation using anti-Flag agarose (Fig.6c-upper panel). These bands might represent partially degraded SBRR1-FLAG protein that occurred during protein extraction or incubation, or natively. Nevertheless, the major band representing the full-length SBRR1-FLAG protein is always detected easily and clearly in all these plants.

Some new data in the revised version are listed as below.



Supplementary Fig. 6c, Western blot analysis for SBRR1 protein in *SBRR1* overexpression lines. In these overexpression lines (*SBRR1*-OE), the C terminal of SBRR1 protein was fused with a 3×FLAG tag.

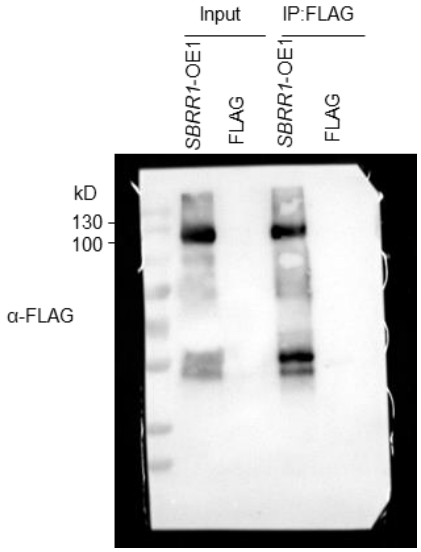


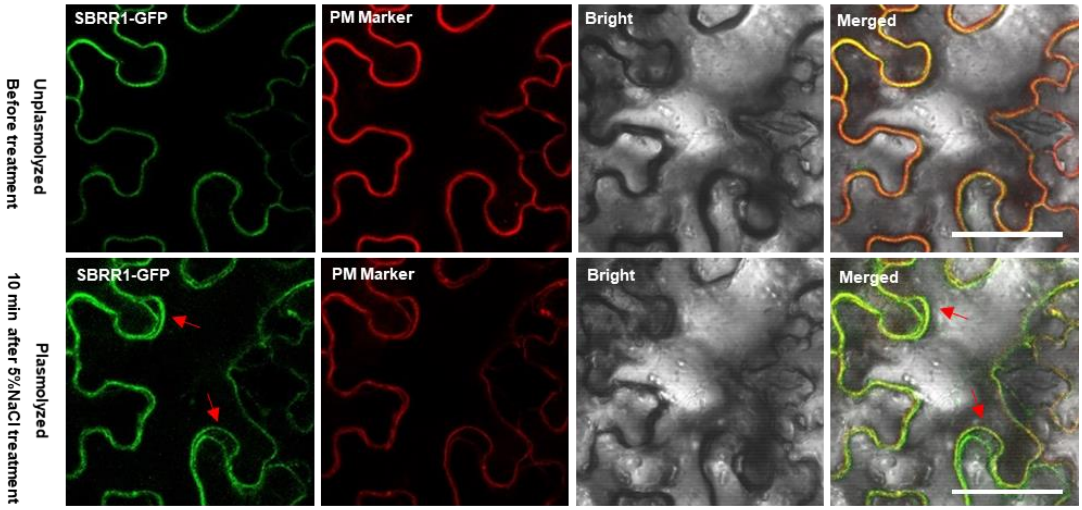
Fig. 6c-upper panel: *in vivo* co-IP assay for detection of SBRR1 protein using *SBRR1*-OE1 transgenic plants. FLAG, transgenic plants expressing a FLAG tag alone. Rice total proteins were extracted from SBRR1-OE1 and FLAG plants, separately, then the level of the SBRR1-FLAG fusion protein was detected using a FLAG antibody (Input). Anti-Flag agarose was used to immunoprecipitate the SBRR1-FLAG fusion protein, and then the SBRR1-FLAG fusion protein was detected using the FLAG antibody (IP:FLAG).

10. The domain architecture of both SBBR1 and SIP1 should be provided in a main figure.

Response: Thanks for the suggestion. As suggested, we have included the domain architecture of SBRR1 and SIP1 in revised Fig. 6a, which shows the interaction between SBRR1-ECD and SIP1 in the yeast two hybrid (Y2H) assay.

11. Fig S15 – plasmolysis should be used to demonstrate PM localization after transient expression in Nicotiana. Or the PM localization should be strengthened for the rice protoplasts as described above.

Response: Thanks for the constructive suggestion. As suggested, we have introduced the SBRR1-GFP construct into *N. benthamiana* leaf epidermal cells and performed plasmolysis treatment by incubating the leaf epidermal cells in 5% NaCl. After treatment for 10 minutes, we observed plasmolysis and found that the green fluorescence of the SBRR1-GFP protein was still present on PM, merging well with the red signals of the PM marker (Supplementary Fig.24). This result further confirms the PM localization of the SBRR1-GFP protein. We have added these data in the revised version. Thanks again for this scientific suggestion.



Supplementary Fig. 24, Subcellular localization of SBRR1-GFP proteins in *N. benthamiana* leaf epidermal cells. Plasmolysis was achieved by incubating cells in 5% NaCl. The red arrows indicate positions of shrinking plasma membranes. Scale bar, 50 μm.

Reviewer #3:

Remarks to the Author:

The fungal pathogen Rhizoctonia solani can infect various staple crops such as rice, wheat, maize, soybean, resulting in severe crop diseases, which not only poses a threat to global food security, but also leads to significant economic losses. To the best of my knowledge, so far, there has been no successful cloning of an effective gene against sheath blight from natural rice varieties. In this study, the authors employed comprehensive genetic analysis combined with biochemical evidence to successfully clone the first major quantitative disease resistance gene, SBRR1-R, against sheath blight in rice. They elucidated the mechanism behind its disease resistance and demonstrated the significant practical value of their research findings. A few comments listed below I think may further improve the manuscript.

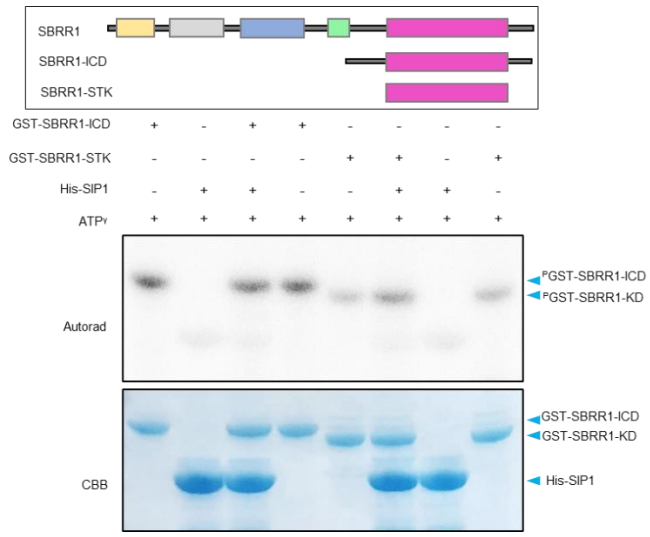
Response: Thanks for the reviewer's recognition on the study.

1. Fig. 2e, the kinase activity of His-SBRR1 looks prominent. However, it would be more convincing to use the isotope labeled the ATP to analysis the kinase activity of His-SBRR1. Also, In Fig. 2, the paper presents the disease resistance phenotype of sheath blight through the measurement of disease lesion length. It is better to utilize fungal biomass and cell staining data as additional criterions to validate the observed disease resistance phenotype for some critically transgenic plants.

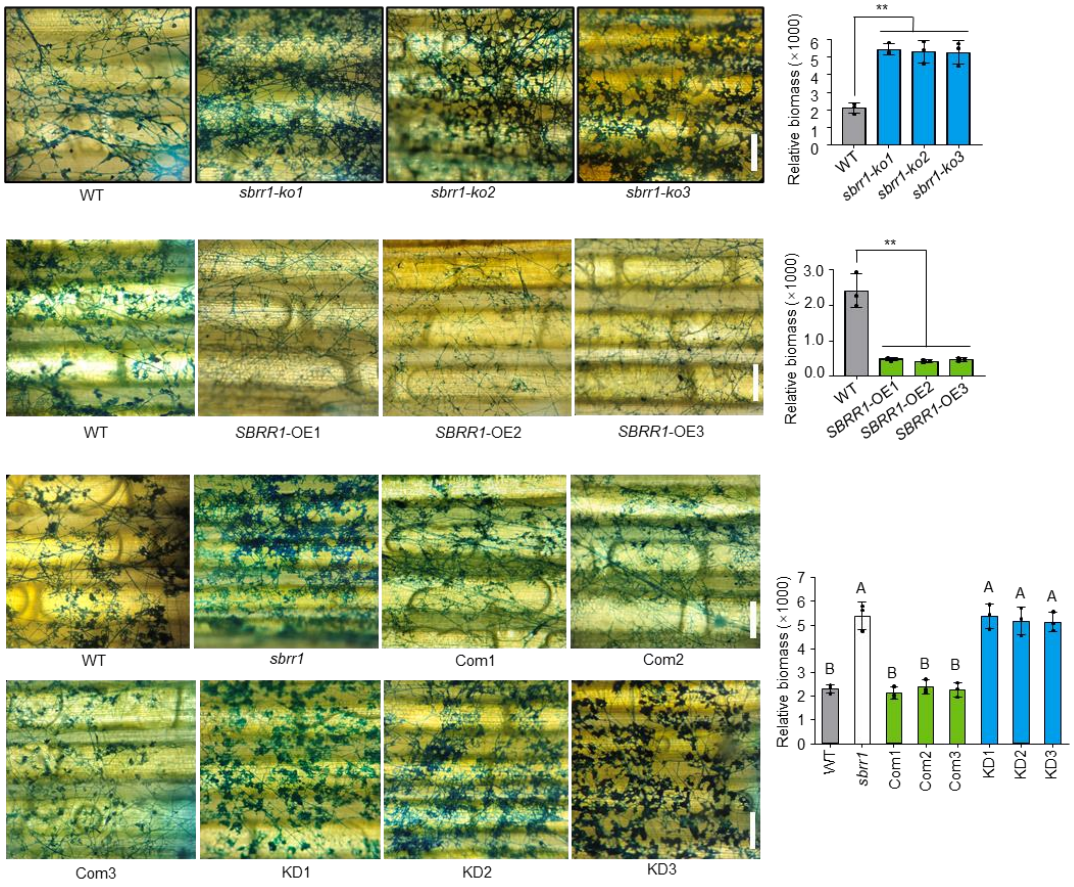
Response: Thanks for these scientific comments. Yes, we have previously employed an isotope-labelling method to test whether SBRR1 can phosphorylate SIP1 after we confirmed the interaction between SBRR1 and SIP1. The results indicate that both GST-SBRR1-ICD and GST-SBRR1-STK can self-phosphorylate, further confirming the kinase activity of SBRR1, but cannot phosphorylate SIP (NP-Fig.4).

For the point "It is better to utilize fungal biomass and cell staining data as additional criteria to validate the observed disease resistance phenotype for some critical transgenic plants"**:** During revision, we have conducted hyphal staining and fungal biomass measurement for several critical *SBRR1* transgenic lines at 48 hours post inoculation (HPI), and the results were all consistent with our previous sheath blight phenotypic results. We have provided these data in Supplementary Figure 7 in the revised version.

Some new data are listed as below.



NP-Fig. 4, Phosphorylation assay using the isotope-labelling method to test whether SBRR1 can phosphorylate SIP1. His-SIP1 recombinant protein was incubated with GST-SBRR1-ICD or GST-SBRR1-STK in kinase reaction buffer supplemented with ^{32}P-γ-ATP and then separated by SDS-PAGE for autoradiography. Upper panel, autoradiogram; bottom panel, CBB-stained gel.



Supplementary Fig. 7, Lactophenol cotton blue staining and relative abundance of *R. solani* biomass in *SBRR1*-related transgenic lines in Figure 2. Lactophenol cotton blue staining shows the hyphae of *R. solani* at the inoculation sites in leaf sheaths.

Relative abundance of *R. solani* biomass in the infected leaf sheath was measured by quantitative PCR using specific primers for the 18S rRNA gene of *R. solani*. The *OsActin* gene was used as the reference. The values represent mean ± s.d (n=3). **: $P < 0.01$ by Student's two-sided t-test. Different letters indicate significant differences according to *Duncan's* multiple range tests ($P < 0.01$). Bars =1mm.

2. Fig. 4b, as these are in vitro interaction data of purified recombinant proteins, I would like to suggest the authors to detect and then quantify by immunoblot (that easily saturates and is making the interaction assay less quantitative than simply quantifying on stained gels). Affinity assays would provide more convincing support for the claims made.

Response: Thanks for the scientific comments. As suggested, we repeated the GST pull-down assay for SBRR1 and SIP1 interaction, and replaced GST antibody detection with Coomassie Brilliant Blue (CBB) staining. We further conducted a quantitative analysis for the protein stained by CBB staining. The results further confirmed the interaction between SBRR1 and SIP1 *in vitro* (Fig.6b).

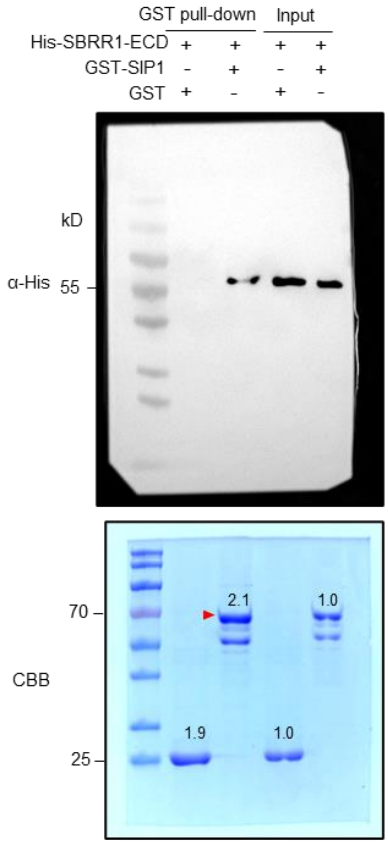


Fig. 6b, *in vitro* GST pull-down assay for SBRR1 and SIP1 interaction. Lower panel shows the protein abundance stained by CBB in SDS-PAGE gel.

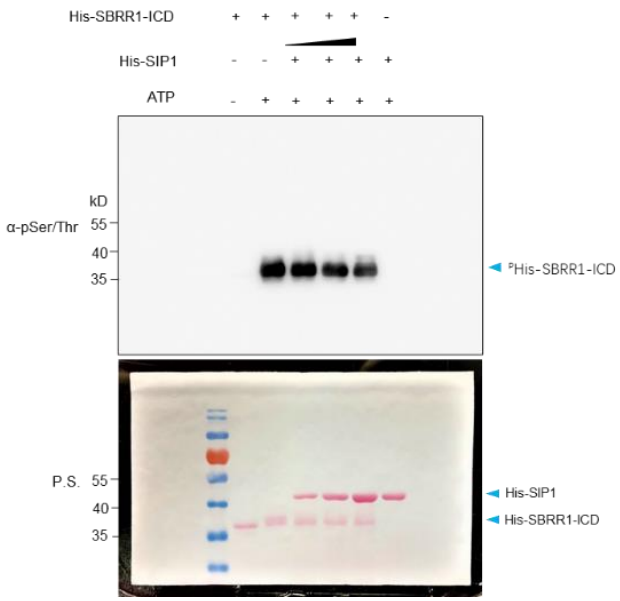
3. Fig. 5, the authors showed that the SIP1 physically interacts with SBRR1, and the data from Fig. 6 suggested that the SIP1 is required for the transportation of SBRR1 to

the plasma membrane, could the authors comment on mechanism underlying here, and have the authors checked whether the SIP1 altered SBRR1 kinase activity in vivo? In addition, considering that SBRR1 is a protein kinase, can SBRR1 phosphorylate SIP1 and is it possible that SIP1 is one of the phosphorylation substrates of SBRR1?

Response: Thanks for these scientific and constructive comments. Both our *in vitro* and *in vivo* data support that SBRR1 interacts with SIP1. Also, via subcellular localization tests, we confirmed that SIP1 mainly functions to facilitate the accumulation of SBRR1 on plasma membrane (PM). Knockout of SIP1 severely abolished the PM localization of SBRR1 protein (Fig.7b, c). Also, SBRR1OE/sip1ko plants showed lower resistance than SBRR1OE plants (Fig.6e), indicating that the correct PM localization of SBRR1 is required for its function. Therefore, we conclude that SIP1 likely functions as a molecular chaperone to assist effective SBRR1 accumulation on PM, contributing to SBRR1-mediated ShB resistance. In the revised version, we have added these in the Discussion section.

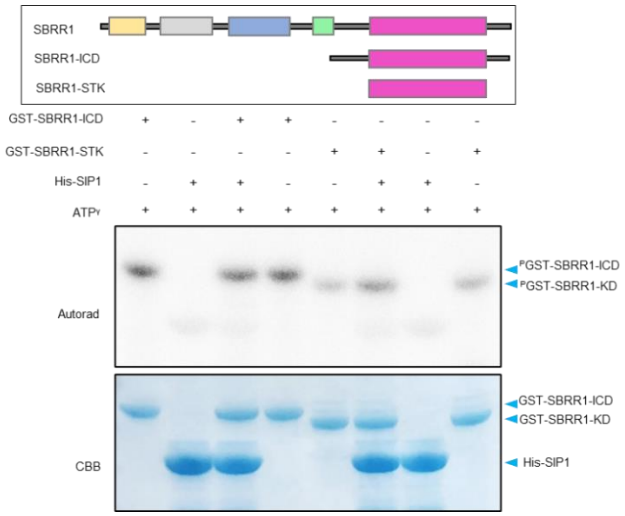
During the revision, we conducted a phosphorylation assay and found that the addition and increase in concentration of His-SIP1 protein does not significantly affect the autophosphorylation activity of SBRR1 (NP-Fig.3), indicating that SIP1 does not affect the kinase activity of SBRR1. We also tested whether SBRR1 can phosphorylate SIP1 using a pSer/Thr antibody assay (NP-Fig.3) and an isotope-labelling method (NP-Fig.4). Both results indicate that SBRR1 can self-phosphorylate, but cannot phosphorylate SIP1. Therefore, we believe that SIP1 is not a phosphorylation substrate of SBRR1.

Some new data are listed as below.



NP-Fig. 3, Phosphorylation assay using a pSer/Thr antibody to test whether SBRR1 can phosphorylate SIP1. His-SIP1 recombinant protein was incubated with His-SBRR1-ICD in kinase reaction buffer supplemented with ATP and then separated by

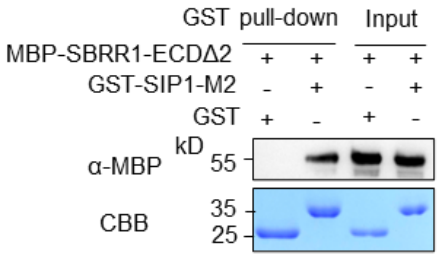
SDS-PAGE for western blot. Ponceau S stains His-SBRR1-ICD and His-SIP1 proteins. P.S., Ponceau S staining.



NP-Fig. 4, Phosphorylation assay using an isotope-labelling method to test whether SBRR1 can phosphorylate SIP1. His-SIP1 recombinant protein was incubated with GST-SBRR1-ICD or GST-SBRR1-STK in kinase reaction buffer supplemented with ^{32}P-γ-ATP and then separated by SDS-PAGE for autoradiography. Upper panel, autoradiogram; bottom panel, CBB-stained gel.

4, Fig. S14, the Y2H data suggested that, the SBRR1-ECD (366-353) is required for its interaction with SIP1, and SIP1-M2 is required for its interaction with SBRR1, have the authors used other assays to conform these interactions?

Response: Thanks for the suggestion. In the revised version, we further employed a pull-down assay to validate the interaction between SBRR1-ECDΔ2 (PAN_AP domain) and SIP-M2 (DP repeat domain) *in vitro* (Supplementary Fig.22d). The result is consistent with our previous results.



Supplementary Fig. 22d, *in vitro* GST pull-down assay for the PAN_AP domain of SBRR1 and DP repeat domain of SIP1 interaction. Lower panel shows the protein abundance stained by CBB in SDS-PAGE gel.

5. The SBRR1-R showed elevated expression after R. solani infection due to its 256 bp insertion in the promoter region. So, I would like to suggest the authors predict the

possible upstream regulators that may activate higher expression of SBRR1-R, but not SBRR1-S.

Response: Thanks for this valuable suggestion. In the revised version, we have added a new section with a subtitle "bHLH57 is a positive regulator differentiating expression of elite *SBRR1-R and SBRR1-S* " in Results. In this section, we employed both *in vitro* and *in vivo* methods to identify transcription factor bHLH57 and confirm that bHLH57 directly binds to the 256-bp insertion region in the promoter of elite *SBRR1-R*, but not *SBRR1-S*, then activates higher expression of *SBRR1-R* upon *R. solani* infection. We also generated several transgenic rice lines, including *bhlh57* knockout lines in TG394 (without *SBRR1-R*) and TG394-SBRR1^{R} backgrounds, and *bHLH57* overexpression lines in TG394 and TG394-SBRR1^{R} backgrounds. Through comparison of the phenotypic data of these transgenic lines and wild type controls, we confirmed that bHLH57 acts as a master positive regulator that promotes expression of elite *SBRR1-R*, but not *SBRR1-S*, in response to *R. solani* infection. We have also provided some of these critical data in details in Response to major concern 2 of Reviewer 1. Inclusion of these results has significantly improved this manuscript.

6. Although the data supported the chitinase genes contribute a lot to the resistance of SBRR1-R mediated resistance, the remaining resistance is unclear. The authors showed that the kinase activity of SBRR1 is required for its full resistance, so, it is better to add some explanations on how the SBRR1 mediated full resistance to the pathogen by comparing the transcriptomic data of SBRR1-OE and WT or sbrr1-ko plants.

Response: Thanks for the suggestion. Several lines of our genetic evidence strongly support that chitinase 3 and 4 genes account for the majority of SBRR1-mediated resistance. During the revision, we further confirmed that the phosphorylation level of SBRR1 is induced by pathogen infection and reaches the highest level around 18 HPI (See Fig.2e and Supplementary Fig.9a, b in Response to the first point in major concern 1 of Reviewer 1 please). According to the suggestion, we further compared the transcriptomic data of WT, sbrr1-ko and sip1-ko lines, and we did find that the antioxidative pathway (oxidation-reduction process, oxidoreductase activity) is commonly affected in both sbrr1-ko and sip1-ko plants (Supplemental Fig.25) in addition to chitinase genes. Considering the fact that the resistance of SIP1 on ShB resistance is fully dependent on SBRR1, we therefore consider that antioxidative pathway probably also contributes to SBRR1-mediated full resistance to the pathogen. Cao et al. (Plant Biotechnology Journal, 2022, 20: 335-349) previously showed that antioxidative pathway presented various changes between ShB resistant and susceptible

varieties upon the *R.solani* infection. Therefore, we speculate that antioxidative pathway could be another important downstream component involved in full SBRR1-medaited resistance to *R. solani*. However, due to lack of further evidence at the current status, we did not further elaborate in the text but only added a short sentence to the Results and Discussion sections in the revised version. Thanks again for this scientific suggestion.

7. There are still some minor errors in the text. Such as, which supplementary material does "Supplementary xxx" refer to in Line 511? The text in the horizontal axis of Figure 5e overlaps. The “promotor” in Line 213 should be “promoter”. The “timepoints” in Line 388 should be “time points”. The “Light panel” in the legend of Fig. 1 should be “Left panel”.

Response: Thanks for your carefully reviewing and pointing out these mistakes. We have corrected them accordingly in the revised version.

Point by point responses to the comments

Reviewer #1 (Remarks to the Author):
The authors have provided some data and have improved this manuscript. However, it still lacks of data on how this kinase activates chitinase genes expression. It would be better to provide these data or make some mechanism explanation on how this kinase gene regulates disease resistance against sheath blight.

Response: We sincerely appreciate your comments and suggestions. Based on our data, *chitinase* genes expression are affected by SBRR1, as revealed in Figs 8a, 8b and S27; we also present sufficient data to show the genetic link between *SBRR1* and *chitinase 3/4* (Figs 8c-d, S28 and S29), and the plasma membrane localization of SBRR1 protein. Therefore, we reason that SBRR1 relays the intracellular signaling to downstream transcription factors, which are able to directly bind to the promoters of *chitinase 3/4* genes and others defense related genes to activate their expressions.

According to published data, we knew that MPKs, RLCKs, CDPKs and small G proteins all have been widely reported to play important roles in kinase-mediated downstream signaling that, ultimately, are mostly associated with ROS production for disease resistance (*Couto et al., Nat Rev Immunol, 2016, 16:537-552; Liang et al., Annu Rev Plant Biol, 2018, 69: 267-299; Zhong et al., Nat Genet, 2024, 56:315-326; Jones et al., Cell, 2024, 187:2095-2116*). Additionally, kinases can also phosphorylate transcription factors and then allow these transcription factors to regulate expression of defense genes, like *PR* genes, for disease resistance (*Wang et al., Cell, 2022, 185:2961-2974; Zhu et al., Nat Genet, 2024, 56:2815-2826*). These findings not only provide the possibility to explain how SBRR1 regulates *chitinase* genes expression, but also shed lights on how to refine SBRR1-mediated downstream defense signaling.

Therefore, although we have no direct or reliable data to clarify how SBRR1 activates *chitinase* genes expression, based on the present data and knowledge, we have added a sentence in the Discussion section: “Considering the data in membrane localization of SBRR1 protein and the contribution of *chitinase* gene expression in SBRR1-mediated resistance, we hypothesized that SBRR1 relays the intracellular signaling directly or indirectly to downstream transcription factor(s), which in turn regulates the expression of *chitinase* and other defense genes to counteract *R. solani* infection.”

Thanks again for these valuable suggestions.

Reviewer #2 (Remarks to the Author):

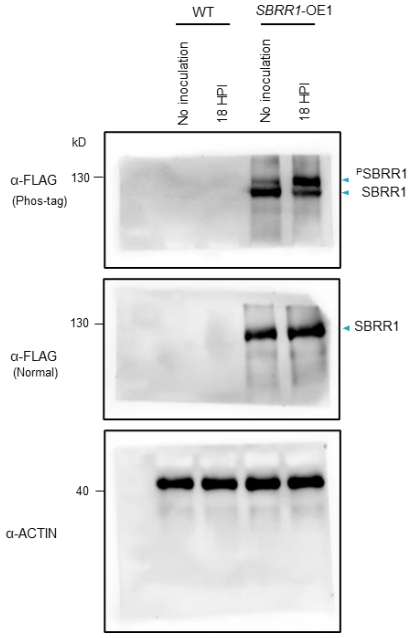
The authors have addressed most of my previous comments and the manuscript is greatly improved. I only have a few remaining suggestions, below.

Response: Thanks for the reviewer's recognition on the study.

1. For phos-tag experiments, there are critical controls missing. Thus, it is not clear if the upper band corresponds to phosphorylated SBBR1. Non-transformed rice (or kinase dead variant of SBBR1) should be used as a control. Phos-tag is not always specific for detecting phosphorylation. An experiment should also be performed demonstrating the upper signal disappears upon incubation with a phosphatase (Fig S9a, Fig S2e). This does not need to be done for each gel but can be done for one to demonstrate specificity.

Response: Thank you for these professional suggestions. For the phos-tag assay in our previous experiments, we had the data using wild-type (WT) rice as a negative control to analyze the specificity of the SBRR1-FLAG protein bands in *SBRR1-OE1* plants under normal or *R. solani*-infected conditions. In this revision, we have added this result in Supplementary Fig. 9c and inserted a short description in text file. As shown in Supplementary Fig. 9c, we found that the lower bands as well as the higher bands of SBRR1-FLAG proteins specifically exist in *SBRR1-OE1* plants, but not in WT seedlings.

Regarding the phosphatase treatment assays, we apologize for our unclear previous labeling in Fig.2e (in the comment it was written as Fig S2e). In the previous Fig.2e, the phosphatase treatment was performed and labeled as CIP (calf intestinal alkaline phosphatase). After the phosphatase (CIP) treatment, the upper band of SBRR1 in SBRR1-OE1 plants disappeared, indicating that this upper band corresponds to phosphorylated SBRR1. To clarify the labeling, we have changed "CIP" to "Phosphatase" in the revised Fig.2e.



Supplementary Fig. 9c (newly added) Comparison of phosphorylation levels of SBRR1 protein in *SBRR1*-OE1 and WT plants with or without *R. solani* infection. Total protein was separated in Phos-tag acrylamide-containing or regular SDS-PAGE gels. ACTIN was used as a loading reference. HPI, hours post inoculation.

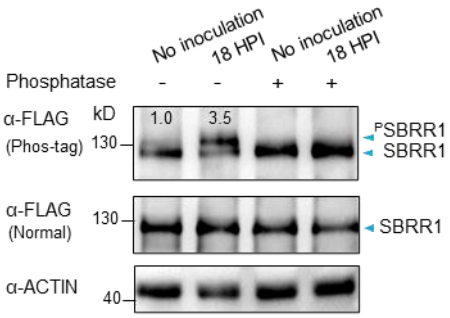


Fig. 2e Comparison of phosphorylation levels of SBRR1 protein in *SBRR1*-OE1 with or without *R. solani* infection. Total protein was separated in Phos-tag acrylamide-containing or regular SDS-PAGE gels. Phosphatase, calf intestinal alkaline phosphatase. ACTIN was used as a loading reference.

2. Fig 2g – can the authors provide a blot exposure with higher background? It appears overly edited for decreased contrast.

Response: Thanks for the valuable suggestion. In the revised version, we have adjusted the contrast of Fig. 2g (see below please). We also provided data of the other two

biological replicates as below (NP-Fig.1). They all showed that SBRR1 possesses kinase activity and the phosphorylation of TT682/683 is essential for its kinase activity.

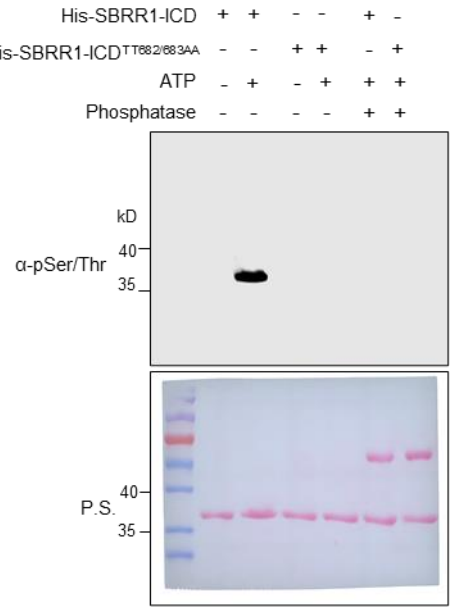
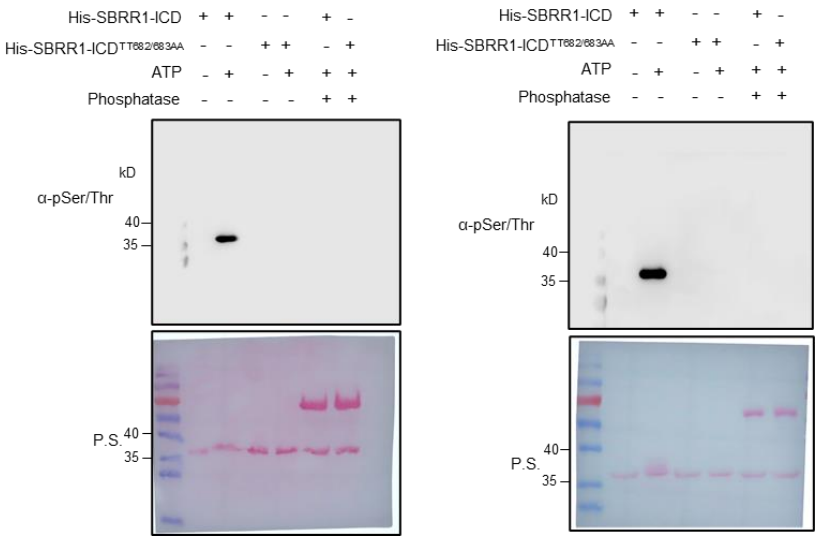


Fig. 2g Immunoblot assay using a pSer/Thr antibody to detect autophosphorylation in His-SBRR1-ICD$^{TT682/683AA}$. Phosphatase, calf intestinal alkaline phosphatase. Ponceau S stains His-SBRR1-ICD and His-SBRR1-ICD$^{TT682/683AA}$ proteins. P.S., Ponceau S staining.



NP-Fig.1 The other two biological replicates of Fig. 2g

3. For the claims of yield - although the data are promising, they are not large-scale tests with sufficient numbers to make strong claims. I suggest the authors note this limitation in the discussion and the opportunity for more extensive testing in the future.

Response: Thanks for the scientific suggestion. In the revised version, we have noted this limitation in the Discussion section and changed the previous description "Importantly, under severe ShB conditions, this elite allele was found to rescue up to 9.54% of the total 26.75% yield loss, suggesting a great potential for *SBRR1-R* in developing ShB resistant varieties" to "Importantly, under severe ShB conditions, this elite allele was found to rescue up to 9.54% of the total 26.75% yield loss. Certainly, this conclusion still needs more large-scale field tests and more evaluations in different rice varieties. However, it suggests a great potential for *SBRR1-R* in developing ShB resistant varieties".

Reviewer #3 (Remarks to the Author):

The authors appropriately addressed all of my concerns and those of the other reviewers' comments. They performed additional kinase assay experiments and analysis for comprehensive enhancements to the SBRR1 manuscript. The authors provide sufficient evidence to support their conclusion that natural variation of SBRR1 for sheath blight resistance in rice. I have no further objections to publication of this MS in Nature Genetics.

Response: Thanks for your help and efforts.