nature portfolio

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Last updated by author(s):	May 27, 2023

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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For	all statistical ar	alyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.		
n/a	Confirmed			
	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement			
	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly			
	The statistical test(s) used AND whether they are one- or two-sided Only common tests should be described solely by name; describe more complex techniques in the Methods section.			
	A description of all covariates tested			
	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons			
	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)			
	For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>			
	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings			
	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes			
	\square Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated			
		Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.		
So	ftware an	d code		
Poli	cy information	about <u>availability of computer code</u>		
D	ata collection	The gene sequences of insect or plant are collected from Genbank.		
D	ata analysis	All quantitative data presented in figures were analyzed using two-tailed t-tests in GraphPad Prism 7 software.		
		s custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.		

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All data are available in the main text or the supplementary materials.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	This study did not involve human research.
Population characteristics	This study did not involve human research.
Recruitment	This study did not involve human research.
Ethics oversight	This study did not involve human research.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.			
∑ Life sciences	Behavioural & social sciences Ecological, evolutionary & environmental sciences		
For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf			

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

- 1. To examine the cleavage pattern of NcVg, protein was extracted in equal amounts from the ovaries of 30 female leafhoppers, as well as the salivary glands from 30 nonviruliferous or viruliferous leafhoppers.
- 2. To investigate the release of salivary proteins in rice plants, approximately 50 adult leafhoppers were allowed to feed on a single rice seedling.
- 3. To detect the release of target proteins in rice plants, 15 female adults were allowed to feed on a region of a single rice seedling in a small cage.
- 4. To trace target proteins of leafhoppers in rice plants, approximately 30 viruliferous or nonviruliferous leafhoppers were allowed to feed on a region of a single rice seedling.
- a region of a single rice seedling.

 5. To determine the RNAi efficiency, RT-qPCR and western blot assays were performed on salivary glands from 30 dsRNA-treated leafhoppers.
- 6. To test the presence of target proteins in plant samples using western blot assays, approximately 50 dsRNA-treated leafhoppers were allowed to feed on one rice seedling.
- 7. To test the effect of GW4869 treatment on NcVg release from salivary glands, salivary glands from 30 GW4869-treated leafhoppers were analyzed in RT-qPCR and western blot assays. Approximately 50 GW4869- or DMSO-treated leafhoppers were allowed to feed on one rice seedling.
- 8. To test the effect of RDV infection on NcVg release into rice plants, salivary glands from 30 viruliferous or nonviruliferous leafhoppers were dissected and analyzed.
- 9. To examine the release of target proteins into rice plants, 30 viruliferous or nonviruliferous leafhoppers were allowed to feed on one rice seedling.
- 10. To test the content of JA, SA and H2O2, as well as related metabolites, approximately 50 female adults were starved for 2 hours and then fed on two rice seedlings.
- 11. For the relative quantification of target gene expression in salivary glands, total RNAs were extracted from the salivary glands of leafhoppers (nonviruliferous, viruliferous, dsGFP-treated, or dsNcVg-treated) obtained from 30 leafhoppers.
- 12. To examine the relative expression of genes in the JA or SA pathway, 30 leafhoppers (nonviruliferous or viruliferous) were fed on one rice seedling.
- 13. To conduct EPG technique, each leafhopper was continuously recorded for 3 hours.
- 14. To test the effect of NcVg knockdown of viruliferous leafhoppers on insect resistance of rice and viral transmission, salivary glands of 30 dsGFP- or dsNcVg-treated viruliferous leafhoppers were dissected and analyzed in RT-qPCR and western blot assays. Thirty dsGFP- or dsNcVg-treated viruliferous leafhoppers were then fed on one rice seedling, which were analyzed using western blot assays, or determine the contents of rice H2O2, MDA, GSH, and GSSG, as well as the activities of GST, POD, and CAT.
- 15. To test GST activity, GSH and GSSG contents, approximately 15 female adult leafhoppers (nonviruliferous, viruliferous, dsGFP- or dsNcVg-treated) were allowed to feed on a rice seedling. Alternatively, 30 female adult leafhoppers were allowed to feed on a region of a leaf of WT or NcVg2-OE plants.
- $16. \ To \ determine \ the \ effect \ of \ NcVg \ knockdown \ on \ OsGSTF12 \ suppressing \ H2O2, \ approximately \ 80 \ dsNcVg- \ or \ dsGFP-treated \ leafhoppers \ were \ allowed \ to \ feed \ on \ rice \ plants.$
- 17. To examine the effect of NcVg knockdown on the suppression of H2O2 by OsGSTF12, approximately 80 dsNcVg- or dsGFP-treated leafhoppers at 4 days post-microinjection were allowed to feed on rice plants.
- 18. To determine contents of GSH and GSSG in OsGSTF12-KO plants, approximately 30 leafhoppers were allowed to feed on a region of one leaf of OsGSTF12-KO and WT plants.
- 19. Approximately 50 viruliferous newly emerged female adults were starved for 2 hours, then fed on leaves of NcVg2-OE, OsGSTF12-KO, or WT plants.

Data exclusions

No data were excluded from the analyses.

Replication	All experiments of RT-qPCR, western blot, contents of Jrice H2O2, MDA, GSH, and GSSG, as well as the activities of GST, POD, and CAT were performed for three biological replicates at least. All EPG recorded at least 20 valid biologically independent replicates.	
Randomization	Leafhopper or rice samples were randomly collected for experimental groups.	
Blinding	We were blinded to group allocation during data collection and analysis.	

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems Methods		thods	
n/a	Involved in the study	n/a	Involved in the study
	X Antibodies	\boxtimes	ChIP-seq
\boxtimes	Eukaryotic cell lines	\boxtimes	Flow cytometry
\boxtimes	Palaeontology and archaeology	\boxtimes	MRI-based neuroimaging
	Animals and other organisms		
\boxtimes	Clinical data		
\boxtimes	Dual use research of concern		

Antibodies

Antibodies used

Rabbit polyclonal antisera against RDV antigens was provided by Dr. Toshihiro Omura of the National Agricultural Research Center, Japan. Polyclonal antibodies against P8 were obtained from ABclonal, China, while polyclonal antibodies against NcRab27a and NcRab5 were sourced from Beyotime, China. Genscript Biotech Corporation, Nanjing, China, prepared polyclonal antibodies against NcVg2 and OsGSTF12, and the process was approved by the Science Technology Department of Jiangsu Province of China.

Validation

Genscript Biotech Corporation, Nanjing, China, prepared polyclonal antibodies against NcVg2 and OsGSTF12, and the process was approved by the Science Technology Department of Jiangsu Province of China.

Animals and other research organisms

Policy information about <u>studies involving animals</u>; <u>ARRIVE guidelines</u> recommended for reporting animal research, and <u>Sex and Gender in</u> Research

Laboratory animals	Rice green leafhopper Nephotettix cincticeps.		
Wild animals	Nonviruliferous individuals of N. cincticeps were collected from rice fields in Fujian Province, southeastern China.		
Reporting on sex	Not applicable.		
Field-collected samples	Nonviruliferous individuals of N. cincticeps were collected from rice fields in Fujian Province, southwestern China, and propagated for several generations in the laboratory. The initial source of RDV-infected rice plants was also collected from rice fields in Fujian Province and propagated via transmission by N. cincticeps under greenhouse conditions.		
Ethics oversight	No ethical approval or guidance was required because the materials were insects and plant.		

Note that full information on the approval of the study protocol must also be provided in the manuscript. $\frac{1}{2} \int_{\mathbb{R}^{n}} \left(\frac{1}{2} \int_{\mathbb{R}^{$