nature portfolio

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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>.

Stat	15 LI	CS
For all	stat	istic

For	all st	atistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a	Cor	nfirmed
	X	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
	X	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	x	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.
X		A description of all covariates tested
X		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)
	x	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>
x		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
x		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
x		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.

Software and code

Policy information about availability of computer code

Data collection

No software used

Data analysis MutantHunter pipeline: https://github.com/steuernb/MutantHunter

MuTrigo package: https://github.com/TC-Hewitt/MuTrigo

BioKanga package v4.4.2: https://github.com/csiro-crop-informatics/biokanga

FGENESH: http://www.softberry.com/berry.phtml

CodonCode Aligner v8.0: https://www.codoncode.com/aligner/

NLR-Parser: https://github.com/steuernb/NLR-Parser

SMART: http://smart.embl.de

R package edgeR GeneOntology AgrigoV2 ShinvGo

MEGA X: www.megasoftware.net

AlphaFold3: https://golgi.sandbox.google.com/

AlphaFold2: https://colab.research.google.com/github/sokrypton/ColabFold/blob/main/beta/AlphaFold2_advanced.ipynb

PyMOL v2.5.5

CLC Sequence Viewer v8

Weblogo: https://weblogo.berkeley.edu/logo.cgi

For manuscripts utilizing 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 reviewers. We strongly 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

The data generated in this study are openly available in NCBI, the mRNA sequence of Sr6 have been deposited at NCBI GenBank with accession number PP949235. Sequencing read data for MutRenSeq is deposited at DDBJ/EMBL/GenBank under BioProject PRJNA1127689 under accessions SRX28382285-SRX28382292. Other data generated in this study are provided in the Supplementary Information/Source Data files. Source data are provided with this paper.

Research involving human participants, their data, or biological material

Policy information about studies with <u>human participants or human data</u>. See also policy information about <u>sex, gender (identity/presentation)</u>, <u>and sexual orientation</u> and <u>race</u>, <u>ethnicity and racism</u>.

Reporting on sex and gender	None
Reporting on race, ethnicity, or other socially relevant groupings	None
Population characteristics	None
Recruitment	None
Ethics oversight	None

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 sel	lection.
X Life sciences	Behavioural & social sciences	Ecological, ev	volutionary & 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	All the information regarding sample size were specifically described in the manuscript.
Data exclusions	No data were excluded from the analyses.
Replication	Mutant plants were identified in M2 generation and were confirmed by progeny test in M3 generation.

	For qPCR, there are both biological replicates and technical replicates. When phenotyping plants, we randomized mutant, wild-type, and control plants as is normal practice. Blinding was performed when phenotyping the mutant lines for their resistance to Pgt, candidate gene confirmation by wheat transformation, qPCR for determining transgene expression. It is not performed when carrying out RenSeq, Sequencing analysis, phylogenetic analysis, protein structure predictions as required by the experiments.			
Randomization				
Blinding				
e require informa	tion from authors about some type	materials, systems and methods es of materials, experimental systems and methods used in many studies. Here, indicate whether each material are not sure if a list item applies to your research, read the appropriate section before selecting a response.		
Materials & e	xperimental systems	Methods		
/a Involved in	,	n/a Involved in the study		
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Eukaryoti	ic cell lines			
Animala a	ology and archaeology and other organisms	MRI-based neuroimaging		
X Clinical d	· ·			
Number of	research of concern			
▼ Plants	research of concern			
lants				
Seed stocks	, , ,	Egyptian 2D, Chinese Spring, and Westonia (PBI) are maintained at the Plant Breeding Institute, University of		

Sydney; Westonia (CSIRO) is maintained at CSIRO Canberra; LMPG, LMPG-Sr6, LMPG-Sr13, and LMPG-Sr21 are maintained at the Cereal Disease Laboratory, USDA-ARS, St. Paul.

2,700 seeds of wheat line Chinese Spring*5/Red Egyptian 2D were mutagenized with EMS. Seven independent mutants were

Novel plant genotypes

identified in M2 generation using the Sr6-avirulent race 21-2,3,7.

Authentication

The seven mutants obtained were progeny tested and confirmed at the M3 generation.