

Reporting Summary

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Statistics

For all statistical analyses, 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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ 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. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P 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 |
| ☒ | ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

- | | |
|-----------------|--|
| Data collection | The ImageJ software v1.8.0.345 (https://imagej.net/ij/) was used to quantify the percentage of leaf surface covered with sporulating uredinia. The Epson Expression 11000XL scanner was used to evaluate the grains width and length. |
| Data analysis | The prediction of protein conserved domains was carried out using online CD-Search interface (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) and LRR domain prediction tool (https://lrrpredictor.biochim.ro/). Structure prediction: AlphaFold v3.0. ClustalW in MEGA software (v11) was used for multiple sequence alignments. Phylogenetic tree was visualized using iTOL (https://itol.embl.de/). Statistical analysis and data visualization were performed using SPSS 25.0 (SPSS, Inc., Chicago, IL) and GraphPad Prism v9.0. |

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](#)

Data supporting the findings of this work are available within the paper and its Supplementary Information. The wild emmer reference genome assembly WEW_v2.1 was collected from National Center for Biotechnology Information (NCBI) database under BioProject PRJNA310175 (ref. 52). Transcriptome raw data for this project are available as BioProject PRJNA1221773. The genomic sequence of ptg005881l contig for YrTD121 region is available as accession number PV110151. The genomic DNA sequences of TdNLR1 and TdNLR2 from TD121 are accessible under NCBI GenBank accessions OP948046 and PP874936. The genomic DNA sequences of other TdNLR haplotypes in this project are available as accession numbers OP948047–OP948060, OR472555 for TdNLR1; and PP874937–PP874945, PQ412542 for TdNLR2. Source data are provided with this paper.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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](https://www.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	The sample size for all experiments was determined based on sample availability and the requirements of statistical analysis. 145 F2 plants of LDN × TD121 were used to genetic analysis and preliminary mapping of YrTD121. A total of 5,256 F2 plants of LDN × TD121 were further used to construct a high-resolution genetic map of YrTD121. The population size is bigger than most population sizes in similar studies (Periyannan et al. 2013, Science, 341,786-788; Saintenac et al. 2013, Science, 341, 783-786; Fu et al. 2009, Science, 323, 1357-1359; Li et al. 2023, Nat. Commun., 14, 6072).
Data exclusions	No data was excluded from analyses.
Replication	All experiments were repeated at least three times and multiple biological replicates were used in all experiments as indicated. Conclusions drawn in this study are derived from at least three consistent independent experiments.
Randomization	We consistently selected samples at random according to genotype for our experiments. EMS populations and controls were randomly selected for phenotyping as normal practice to identify susceptible mutants. Homozygous recombinant lines and transgenic plants were grown randomly with in the same experiment plot to minimize unexpected environmental variation during experimentation. It is not relevant to other experiments as no subjects were allocated to experimental groups.
Blinding	Blinding was performed when phenotyping the mutant lines/transgenic plants, gene expression analysis. It is not performed when carrying out phylogenetic analysis, gene haplotype sequencing analysis, protein structure analysis, and functional validation as required by the experiments.

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

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☐	☒ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Commercial antibodies: anti-GFP (Sangon Biotech, Shanghai, D110008; 1:5000 dilution) and HRP-conjugated goat anti-mouse IgG (Sangon Biotech, D110087; 1:10000 dilution).

Validation

Anti-GFP and goat anti-mouse IgG were used in our immunoblot analysis in *Nicotiana benthamiana* expressing TdNLR1-YFP, TdNLR1_CC-YFP, TdNLR1_CCdel-YFP, TdNLR1_CCh4-YFP, TdNLR1_NB-YFP, TdNLR1_LRR-YFP, TdNLR1_CC-NB-YFP, TdNLR1_NB-LRR-YFP, TdNLR2_Un-YFP, TdNLR2_NB-YFP, TdNLR2_LRR-YFP, TdNLR2_Un-NB-YFP, TdNLR2_NB-LRR-YFP, and TdNLR2-YFP. The results showed that the antibody allow detection of the specific corresponding protein with expected size.

Dual use research of concern

Policy information about [dual use research of concern](#)

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes
☒	☐ Public health
☒	☐ National security
☒	☐ Crops and/or livestock
☒	☐ Ecosystems
☒	☐ Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes
☒	☐ Demonstrate how to render a vaccine ineffective
☒	☐ Confer resistance to therapeutically useful antibiotics or antiviral agents
☒	☐ Enhance the virulence of a pathogen or render a nonpathogen virulent
☒	☐ Increase transmissibility of a pathogen
☒	☐ Alter the host range of a pathogen
☒	☐ Enable evasion of diagnostic/detection modalities
☒	☐ Enable the weaponization of a biological agent or toxin
☒	☐ Any other potentially harmful combination of experiments and agents

Seed stocks	The wild emmer wheat accessions used in this study were kept in Wild Cereal Gene Bank, the University of Haifa, Israel. The genetically modified seeds and YrTD121 introgression lines are produced by Triticeae Research Institute, Sichuan Agricultural University and preserved in our lab.
Novel plant genotypes	The TdNLR1 and/or TdNLR2 transgenic plants were generated in common wheat cultivar Fielder using the PureWheat technique. Positive transgenic plants were identified and self-pollinated. For functional verification, three(TdNLR1+TdNLR2), two(TdNLR1), two (TdNLR2) independent T1 complementary transgenic lines were analyzed. Three(TdNLR1+TdNLR2), three(TdNLR1), three (TdNLR2) overexperssion T1 lines were analyzed. For CRISPR/Cas9-mediated gene editing, the guide RNA (ACGTCAGCGACCGGCGCACCAGG/TACGGGGTCGAGAACCTTACAG target TdNLR1; ATGCCCTGGCTGAACGTACAGGG/AGATACAGGCATCTTCTGCTGGG target TdNLR2) targets the exons, and three (TdNLR1) and two (TdNLR2) independent lines were produced. Primers are listed in the Supplementary Table 3 file.
Authentication	Gene and vector specific primers are paired to detect presence of the exogenous vectors in the transgenic lines. PCR based sequencing was used to identify mutations in the target genes.