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 Editorial Policies and the Editorial Policy Checklist.

## 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<br>*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. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted<br>*Give P values as exact values whenever suitable.* |
| ☒ | ☐ | 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

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|---|---|
| Data collection | Seedling images for germination phenotyping were captured using a standard digital camera mounted on a calibrated tripod. Root and shoot length measurements were made using ImageJ. No custom software was used for data collection. Raw sequencing data were generated using standard manufacturer protocols on PacBio Revio (HiFi reads), Illumina NovaSeq X Plus (Hi-C, 2x150 bp), Illumina NovaSeq 6000 (RNA-Seq, skim-sequencing, IBSpy WGS, 2x150 bp) and PacBio Sequel II (Iso-Seq) platforms. |
| Data analysis | Genome size estimation: Jellyfish v2.2.10, GenomeScope v2.0. Genome assembly: hifiasm v0.19.5. Hi-C trimming: Trimmomatic v0.39. Hi-C scaffolding: YaHS v1.2a.2. Manual curation: PretextView v0.2.5. Contaminant removal: TIARA v1.0.2. Organelle assembly: Oatk pipeline. Repeat annotation: EIRepeat pipeline, LTR_retriever (LAI calculation). Transcript alignment: HISAT2 v2.2.1, minimap2. Transcript assembly: StringTie2 v2.1.5, Scallop v0.10.5. Gene annotation: REAT pipeline, Mikado, AUGUSTUS, EVidenceModeler, Liftoff v1.5.1. Protein alignment: Spaln v2.4.7, Miniprot v0.3. Functional annotation: EIFunAnnot pipeline, InterProScan v5.22.61, BLASTp (BLAST+ v2). Assembly quality: Merqury v1.3, BUSCO v5.4.3. Phylogenomics: OrthoFinder v2.5, IQ-TREE v2.1.3, ASTRAL-III, MCMCtree (PAML v4.9), Gblocks v0.91b, FigTree v1.4.3. k-mer analysis: KMC v3.1.2, IBSpy. Synteny: MCScanX, JCVI toolkit. Skim-sequencing introgression mapping: HISAT2 v2.2.1, Picard, SAMtools v1.16, BEDTools; custom Python scripts available at https://github.com/Surbhigrewal/Introgression_mapping. Statistical analysis for salt tolerance phenotyping: SciPy (Python); one-sided Mann-Whitney U tests. |

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

Raw HiFi, Hi-C, RNA-Seq and IsoSeq sequencing reads for Thinopyrum bessarabicum accession PI531712 are deposited in the European Nucleotide Archive (ENA) under study accession PRJEB106499. Raw whole-genome sequencing reads for decaploid Th. elongatum and Th. ponticum are deposited in the ENA under study accession PRJEB112780. The chromosome-scale nuclear and organelle genome assembly and gene annotations are publicly available at Figshare (https://doi.org/10.6084/m9.figshare.32188491); an ENA genome accession has been requested and will be provided upon acceptance. The genome browser is accessible at GrainGenes (https://malt.pw.usda.gov/jb/?data=/ggds/thi-bessarabicum). The skim-sequencing introgression mapping pipeline is available at GitHub (https://github.com/Surbhigrewal/Introgression_mapping). All other data supporting the findings of this study are available within the paper and its supplementary information.

## 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.

| | |
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| Reporting on sex and gender | *Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.*<br>*Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.*<br>*Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.* |
| Reporting on race, ethnicity, or other socially relevant groupings | *Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).*<br>*Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)*<br>*Please provide details about how you controlled for confounding variables in your analyses.* |
| Population characteristics | *Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."* |
| Recruitment | *Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.* |
| Ethics oversight | *Identify the organization(s) that approved the study protocol.* |

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.

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| Sample size | No formal sample size calculation was performed. For germination-stage phenotyping, five seeds per genotype per NaCl concentration were used. For hydroponic phenotyping, 2-5 plants per genotype per concentration were used, reflecting the availability of seed from homozygous introgression lines. |
| Data exclusions | Two data exclusions were applied. First, Th. bessarabicum accession PI 531712 (the assembly accession) was excluded from germination phenotyping due to poor seed germination under controlled conditions, precluding sufficient replication. Second, CIMMYT-derived wheat introgression lines carrying chromosome 5J segments were excluded from hydroponic phenotyping because they are maintained in a Prinia background, which has shown evidence of salt tolerance and would confound attribution of any phenotypic effect to the introgressed J- |

| | |
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| | chromosome segment. Both exclusions were determined prior to analysis. |
| Replication | Germination and hydroponic phenotyping experiments were conducted as independent experiments using different plant cohorts and NaCl concentration ranges. Consistent directional effects of chromosome 5J on salt tolerance were observed across both vegetative traits (DWSTI and TNSTI) and across multiple NaCl concentrations in the hydroponic experiment, supporting reproducibility of the Chr5J tolerance effect. Germination-stage tolerance of Th. bessarabicum accessions relative to bread wheat was consistent across both RLSTI and SLSTI measures. |
| Randomization | In the hydroponic experiment, mesh pots were placed in randomised positions within hydroponic tanks to minimise positional effects. In the germination experiment, Petri dishes were assigned randomly to incubator positions. |
| Blinding | Blinding was not applied to phenotypic data collection or analysis. Genotype identity was known to the investigator during seedling measurement and data collection. |

# Behavioural & social sciences study design

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

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| Study description | Not applicable. |
| Research sample | Not applicable. |
| Sampling strategy | Not applicable. |
| Data collection | Not applicable. |
| Timing | Not applicable. |
| Data exclusions | Not applicable. |
| Non-participation | Not applicable. |
| Randomization | Not applicable. |

# Ecological, evolutionary & environmental sciences study design

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

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| Study description | Quantitative comparative genomics study. Phylogenomic analysis of genome relationships across 29 Triticeae taxa using single-copy orthologue supermatrices and k-mer-based genome similarity scores. Transposable element dynamics compared between two diploid Triticeae genomes (J and E). Gene family copy number compared across 29 species using orthogroup inference. No field-based ecological sampling was conducted; all plant material was grown under controlled glasshouse conditions. |
| Research sample | Accessions of Thinopyrum bessarabicum (PI531712, PI531711), Th. elongatum (PI401007) and Th. ponticum (PI547312) obtained from the USDA National Plant Germplasm System. Published genome assemblies and sequencing data for 25 additional Triticeae species were obtained from public repositories. |
| Sampling strategy | No statistical sampling procedure was used. Species included in comparative analyses were selected based on availability of high-quality genome assemblies and their phylogenetic relevance to the J genome lineage. Sample sizes for phenotyping are described in the Life Sciences study design section. |
| Data collection | Genomic sequencing data were collected by Novogene UK using PacBio Revio and Illumina NovaSeq platforms as described in the methods. Phenotypic data were collected by a single investigator (J.W.) using ImageJ for seedling measurements and direct counts for tiller number. |
| Timing and spatial scale | All plant material was grown under controlled glasshouse conditions at the University of Nottingham Sutton Bonington Campus. Germination phenotyping and hydroponic experiments were conducted between 2023 and 2024. Genome sequencing was conducted between 2023 and 2024. All comparative genomic analyses used publicly available assemblies deposited prior to 2025. |
| Data exclusions | No exclusions were made from the comparative genomic or phylogenomic analyses. |
| Reproducibility | Phylogenomic analyses were conducted with multiple complementary methods (maximum likelihood concatenation with IQ-TREE and coalescent-based species tree with ASTRAL-III) yielding concordant topologies. IBSpy k-mer analysis was performed using six independent k-mer sets and results were averaged, providing internal reproducibility assessment. Phenotyping results were consistent across two independent traits within each experiment. |
| Randomization | N/A |
| Blinding | N/A |

Did the study involve field work? ☐ Yes ☒ No

# 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 |

## Plants

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| Seed stocks | Thinopyrum bessarabicum accessions PI531712 and PI531711, Th. elongatum accession PI401007, and Th. ponticum accession PI547312 were obtained from the USDA National Plant Germplasm System. Triticum urartu, Aegilops speltoides and Ae. tauschii used as genomic DNA probe sources for GISH are maintained at the Nottingham Wheat Research Centre. Wheat cultivars Paragon and Chinese Spring are also maintained at the Nottingham Wheat Research Centre. Shiraz (GRU accession W9438) and Wembley (GRU accession W3588) were obtained from the Germplasm Resource Unit at the John Innes Centre. Genebank Th. bessarabicum introgression lines used for skim-sequencing characterisation were obtained from the GRU at the John Innes Centre, from CIMMYT, and from the Nottingham Wheat Research Centre (WRC-coded lines); full details are provided in Supplementary Table S21. TWB-SIL and DAS used for hydroponic phenotyping were obtained from the GRU at the John Innes Centre. |
| Novel plant genotypes | Five homozygous wheat-Th. bessarabicum introgression lines (WRC22-Bess1 to WRC22-Bess5) were generated by crossing hexaploid wheat cv. Paragon ph1/ph1 as female parent with Th. bessarabicum PI531712, followed by recurrent backcrossing to Paragon Ph1/Ph1. Marker-assisted selection using the Axiom Wheat-Relative Genotyping Array at BC1, BC2 and KASP markers from BC4 onwards was used to identify and fix introgression-carrying plants. Lines were selfed to homozygosity prior to characterisation. No transgenic approaches, gene editing or chemical/radiation mutagenesis were used. |
| Authentication | All Th. bessarabicum accessions were authenticated by genomic in situ hybridisation (GISH) confirming the expected diploid karyotype (2n = 2x = 14, JJ). Novel introgression lines WRC22-Bess1 to WRC22-Bess5 were authenticated by dual-reference skim-sequencing against the bread wheat RefSeq v2.1 and the Th. bessarabicum assembly, confirming the J chromosome of origin and introgression boundaries at 1 Mb resolution, and by multicolour GISH confirming the presence of J-genome chromatin in a wheat background (Supplementary Fig. S12). Genebank introgression lines were authenticated by the same dual-reference skim-sequencing pipeline, which corrected several previously catalogued designations (Supplementary Table S21). Chromosome counts were not performed on all lines but ploidy was inferred from skim-sequencing coverage patterns. No secondary insertion effects are applicable as no transgenic or gene-editing approaches were used. |