

Supplementary Files

Dissecting the population structure, diversity and genetic architecture of rust resistance in wild emmer wheat (*Triticum turgidum* subsp. *dicoccoides*)

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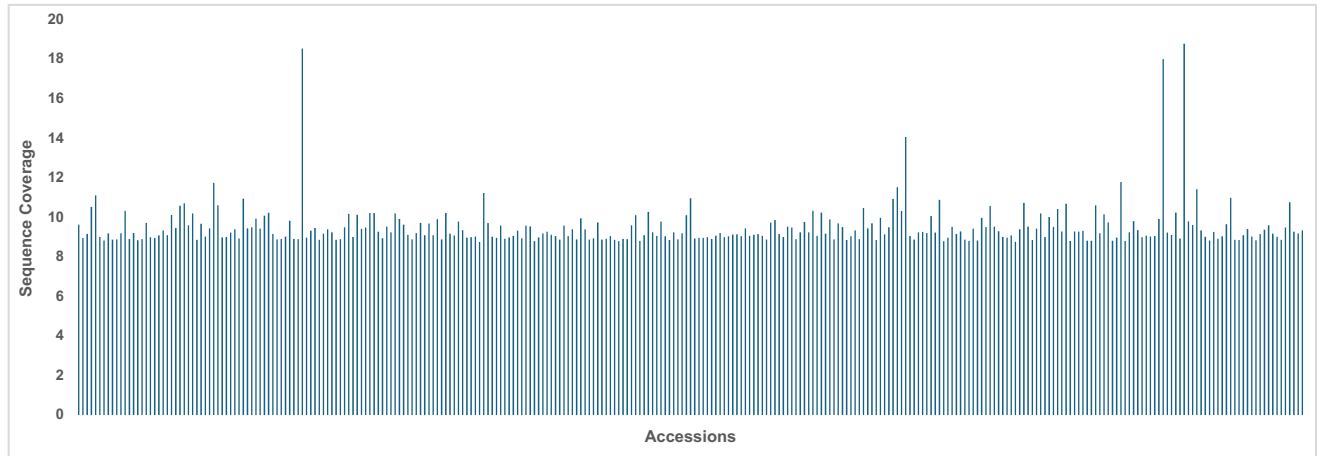
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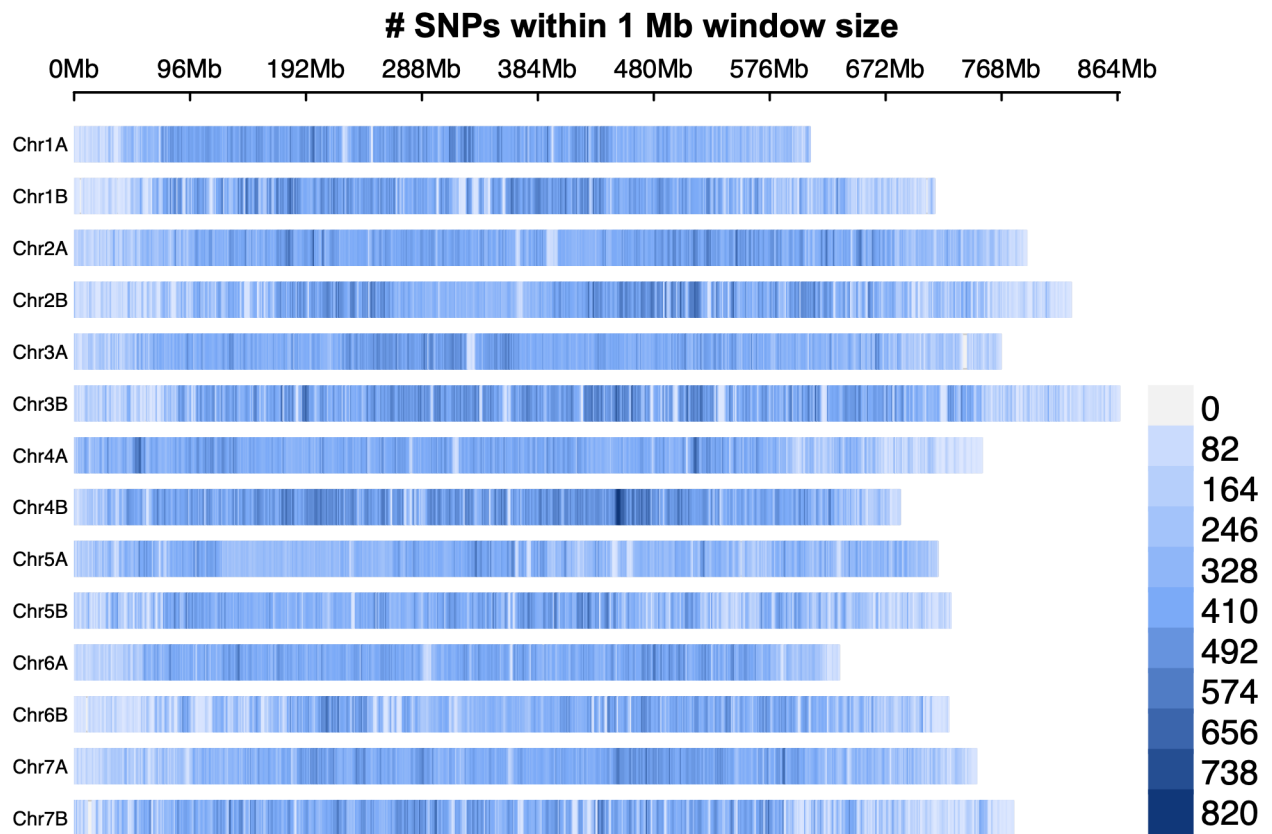
Supplementary Tables

Supplementary Tables S1–S15 are provided in a separate Excel file titled “Supplementary_WEW_Tables”

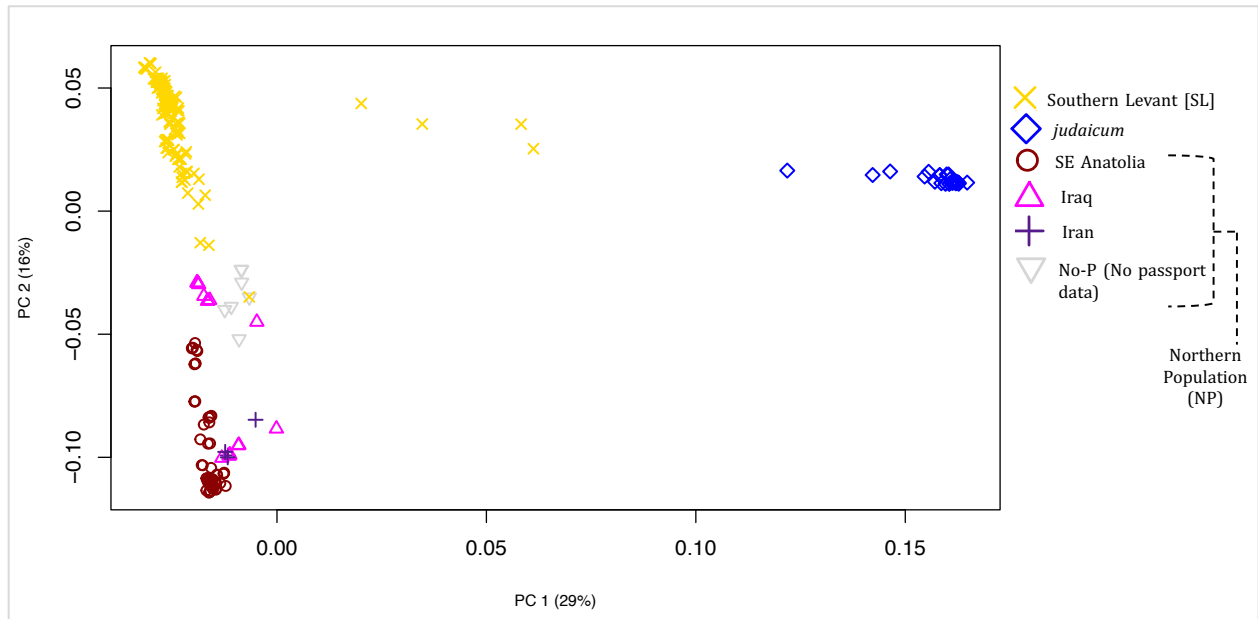
Supplementary Figures



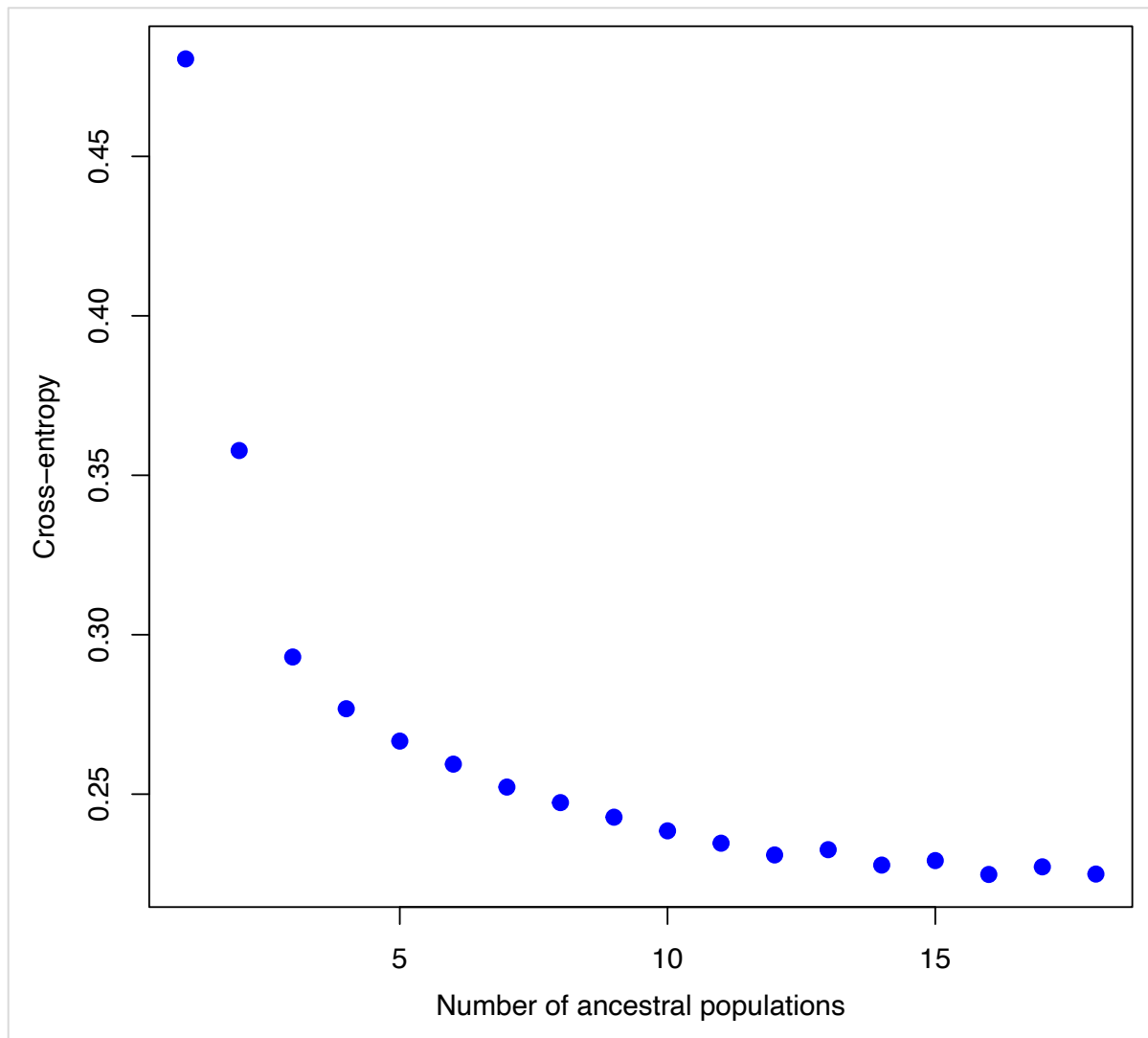
Supplementary Figure S1. Bar chart displaying the sequence coverage for the 291 wild emmer wheat whole-genome sequencing (WGS) panel, which averaged 9.5x across all accessions. Each bar indicates a single accession. The FASTQ files containing this sequencing data are available on NCBI-SRA under BioProject accession # PRJNA1007489.



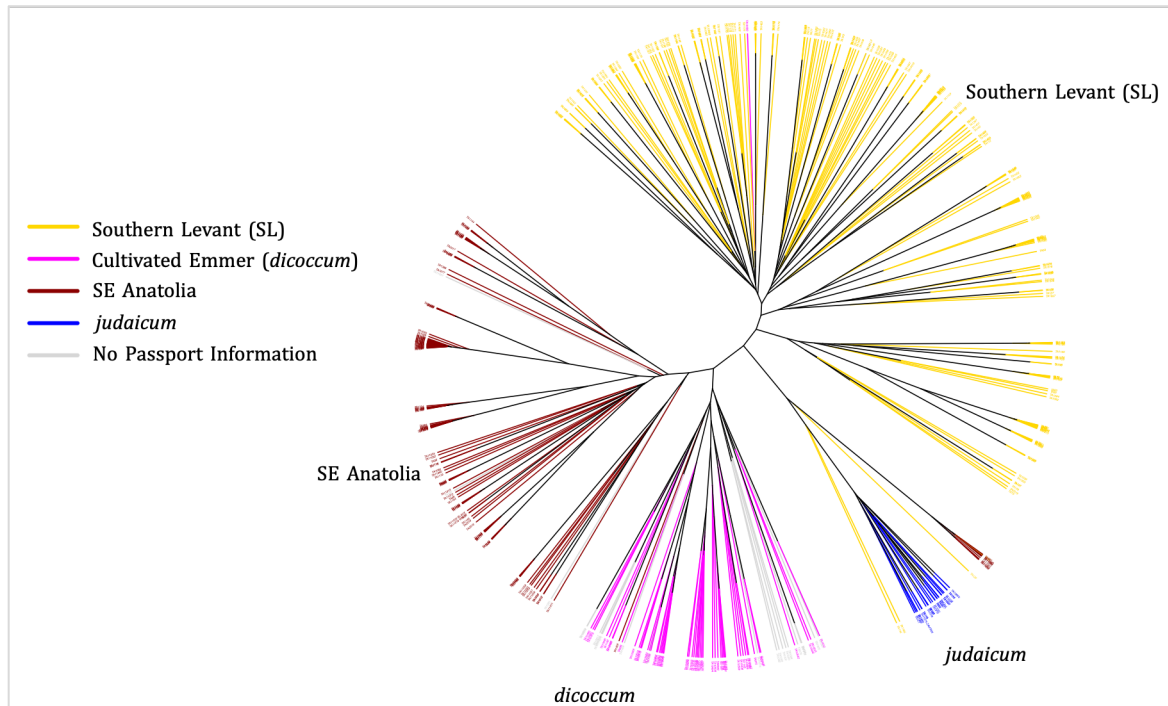
Supplementary Figure S2. The SNP density as the number of SNPs within a 1 Mb window size. Each horizontal bar represents one of the 14 different chromosomes of wild emmer wheat and extends to its physical length as shown on the x-axis at the top. The colors of the bars indicate the level of SNP density.



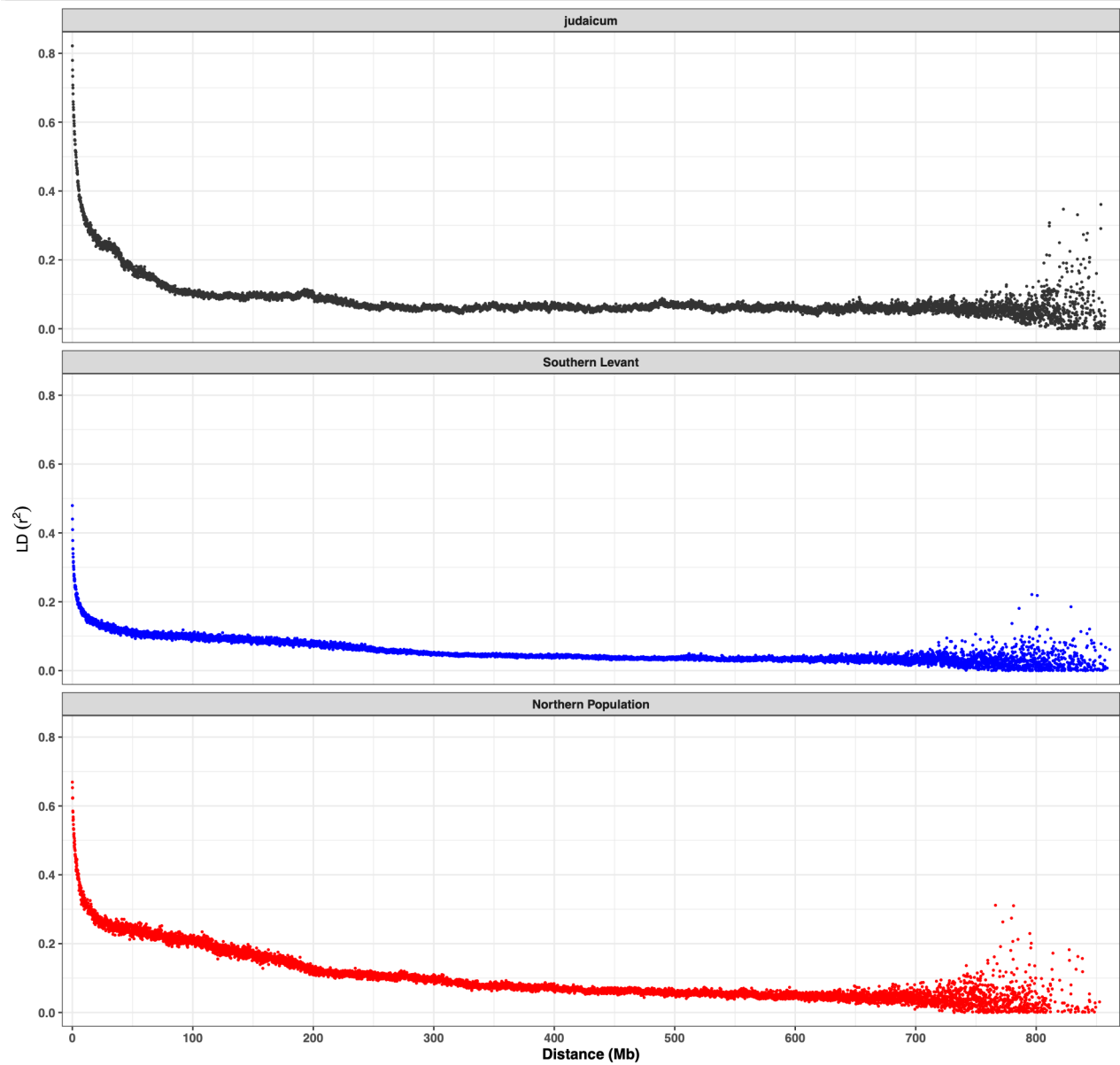
Supplementary Figure S3. Principal component analysis (PCA) plot for wild emmer wheat WGS panel with the first two principal components (PCs).



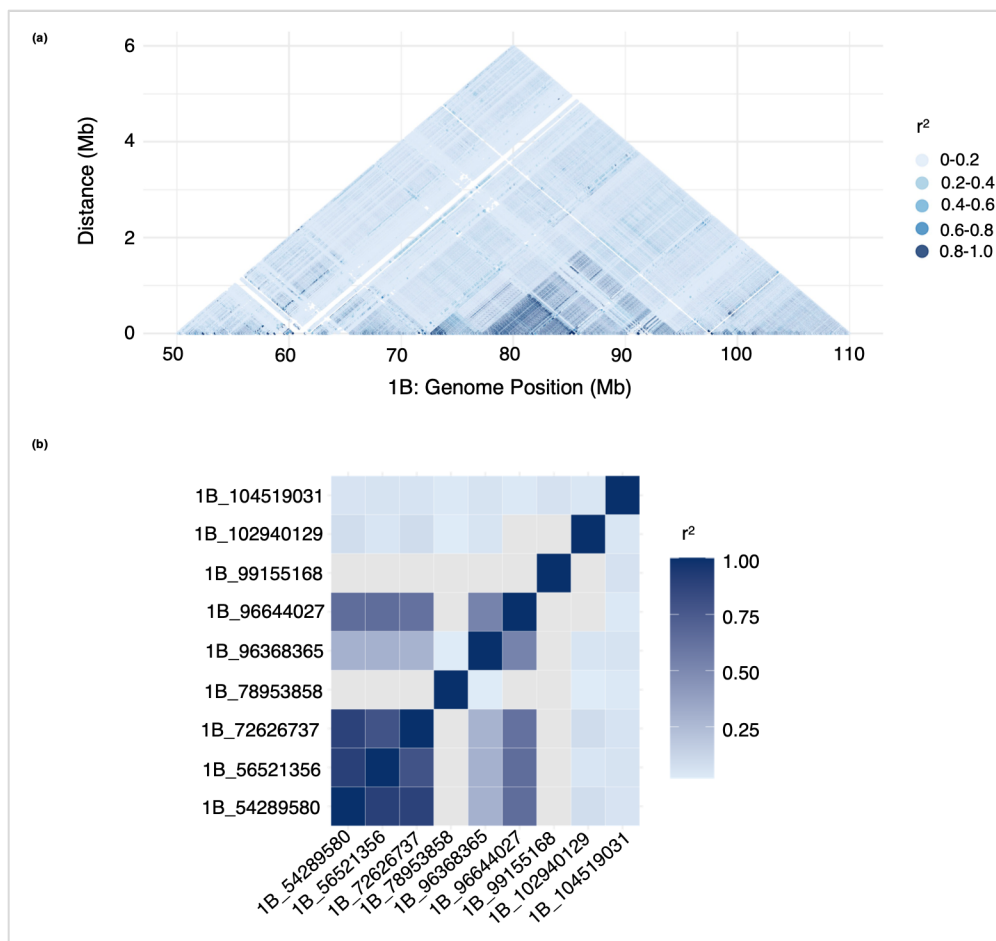
Supplementary Figure S4. Cross-entropy scree plot from LEA showing model fit for different numbers of ancestral populations (K) in wild emmer wheat.



Supplementary Figure S5. Clustering of WEW accessions and the cultivated emmer wheat (*Triticum turgidum* ssp. *dicoccum*) within a neighbor-joining (NJ) tree as constructed using genotyping-by-sequencing (GBS) SNP markers. The SE Anatolia subgroups within the wild population clustered closer to the cultivated emmer.



Supplementary Figure S6. Genome-wide linkage disequilibrium (LD) decay curves for three subpopulations: *judaicum*, Southern Levant (SL), and the Northern Population (NP) of wild emmer wheat. The y-axis represents pairwise LD (r^2), while the x-axis represents physical length (Mb). Genome-wide LD was computed for all SNP pairs (20K SNPs for each subpopulation), and mean LD (r^2) was computed using a 100 kb window, then plotted as a dot plot. LD decayed rapidly in all three subpopulations, with a marked decline observed at approximately 250 kb for each.



Supplementary Figure S7. Pairwise linkage disequilibrium (LD) patterns among loci and across the 50–110 Mb region on chromosome 1B. (a) Triangular LD plot showing LD among all markers within the region. Color intensity reflects LD strength, with darker blue indicating higher r^2 values. (b) LD heatmap showing pairwise LD (r^2) among significant loci.

Supplementary Notes

Supplementary Note S1

2.6 Pathogen isolates

The WEW panel was phenotyped at the seedling stage against five races each of the stem rust (*Puccinia graminis* f. sp. *tritici*), leaf rust (*Puccinia triticina*), and stripe rust (*Puccinia striiformis* f. sp. *tritici*) pathogens of wheat (Supplementary Table S2). The selection of races of *P. graminis* f. sp. *tritici* aimed to cover the four major genetic groups described for this pathogen and capture a broad virulence spectrum. Race TTKSK (isolate 04KEN156-04, genetic clade I) has the same virulence spectrum as the original isolate Ug99 identified in Uganda in 1998 [1]. Races JRCQC (isolate 09ETH08-3, clade II) and TTRTF (isolate 13GEO189-1, clade III) are virulent on the 'b' allele of the *Sr13* gene [2], the most important stem rust resistance gene in durum wheat [3]. Race TKTTF (isolate 13ETH18-1, clade IV) is a widely distributed race that caused significant epidemics in East Africa [4] and outbreaks in Europe [5]. Race TTTTF (isolate 02MN84A-1-2) is the most widely virulent race reported in the U.S., producing high infection types (ITs) on all but *Sr24* and *Sr31* in the 20-line wheat stem rust differential set [1]. Among the races of the leaf rust pathogen screened in the study, the race BBBQD (isolate) is highly virulent to durum wheat cultivars but mostly avirulent to bread wheat [6]. The remaining four races (MFPSB, TBBGS, TFBGQ, and TNBGJ) are bread-wheat-type races predominant in the U.S. The race TBBGS, virulent on *Lr21* and *Lr39*, was one of the predominant races in the U.S. from 2018 to 2020 [6]. The race TNBGJ, virulent on *Lr2a*, *Lr24*, and *Lr39*, was a common phenotype in the southern and northern Great Plains in the early 2010s [6]. For *Puccinia striiformis* f. sp. *tritici*, we used four races of the U.S. origin (Pst-14, Pst-37, Pst-143, and Pst-221) and one race 38E134 from Israel. The race 38E134 represents the *Pst* isolate PST5006 (PST5006 hereafter). Races Pst-14 and Pst-37 were selected based on their high virulence phenotype as they possess virulence to 12 and 10 out of the 18 lines of the stripe rust differential set [7]. Pst-37 is also a predominant race in the United States [7, 8]. Races Pst-143 and Pst-221 have been identified more recently in the U.S. and have a lower virulence phenotype [8]. The PST5006 is a prevalent race in Israel with a high virulence profile [9, 10].

2.7 Inoculation and disease assessments

Rust inoculations followed standard protocols used at the USDA-ARS Cereal Disease Laboratory and the University of Minnesota. On the day of inoculation, urediniospores of the rust pathogens were taken from the -80°C freezer, heat-shocked in a 45°C water bath for 15 minutes, and then rehydrated in an 80% relative humidity chamber for at least 4 hours. Ten milligrams (mg) of urediniospores were placed into individual size 00 gelatin capsules filled with 800 µl of mineral oil. The inoculum suspension was applied to 10-day-old plants (with the first leaf fully expanded and 2 cm of the second leaf developed) using

custom atomizers (Tallgrass Solutions, Inc., Manhattan, KS) pressurized by a pump set at 30 kPa. Approximately 0.15 mg of urediniospores were applied per plant. After inoculation, plants were left for 30 minutes to allow the oil carrier to evaporate from leaf surfaces before being placed inside a mist chamber. They were kept in darkness for 16 hours under 100% humidity conditions maintained with ultrasonic humidifiers (Vick's model V5100NSJUV; Proctor & Gamble Co., Cincinnati, OH). For stem rust evaluations, light (400 W high-pressure sodium lamps emitting $300 \mu\text{mol photon s}^{-1} \text{ m}^{-2}$) was provided for 4-6 hours after the dark period. After drying inside the dew chamber, plants were transferred to a greenhouse with a day/night temperature of 22/19°C and a 14-hour photoperiod provided by 400 W high-pressure sodium lamps emitting a minimum of $300 \mu\text{mol photons s}^{-1} \text{ m}^{-2}$.

Leaf rust evaluations followed the same protocols as stem rust, except that no light was provided during the 16-hour dew period. Stripe rust evaluations were conducted using procedures similar to those for leaf rust, with two modifications: (i) during the infection period, plants were incubated in a dew chamber at 10 °C and 100% relative humidity for 24 hours; and (ii) the incubation period was carried out in a growth chamber under a 16-hour photoperiod with a temperature regime of 18 °C (day) and 15 °C (night).

All rust phenotyping experiments were conducted using a completely randomized design and were repeated at least once over time. For stem rust and leaf rust, plants were scored for infection type (IT) 14 days after inoculation. Stem rust and leaf rust responses were evaluated using the 0–4 scale described by (Stakman et al., 1962)[11] and Long and Kolmer (1989) [12], respectively. Infection types were classified as follows: '0', no visible symptoms; ';', hypersensitive flecks; '1', small uredinia with necrosis; '2', small to medium-sized uredinia surrounded by chlorosis; '3', medium-sized uredinia with or without chlorosis or necrosis; and '4', large uredinia without chlorosis or necrosis. Variation in uredinia size within each IT was denoted by '+' (larger than expected) or '-' (smaller than expected).

Seedlings displaying ITs from 0 to 2+ or 'X' (a mixture of low and high ITs evenly distributed on the leaf surface) were classified as resistant, whereas ITs from 3– to 4 were considered susceptible. Accordingly, stem rust and leaf rust phenotypes were grouped into resistant (ITs 0–2+) and susceptible (ITs 3– to 4) categories.

For stripe rust, plants were scored 18 days after inoculation using the 0-9 scale developed by A Wan and X Chen [7]. Stripe rust infection types were defined as: '0', no visible symptoms; '1', necrotic or chlorotic flecks; '2', necrotic or chlorotic blotches without sporulation; '3', necrotic or chlorotic blotches with trace sporulation; '4', necrotic or chlorotic blotches with light sporulation; '5', intermediate

sporulation with chlorosis or necrosis; '6', moderate sporulation with chlorosis or necrosis; '7', abundant sporulation with chlorosis or necrosis; and '8–9', abundant sporulation without chlorosis or necrosis.

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