Whole-Genome Resequencing of the Wild Barley Diversity Collection: A Resource for Identifying and Exploiting Genetic Variation for Cultivated Barley Improvement

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Abstract

To exploit allelic variation in *Hordeum vulgare* subsp. *spontaneum*, the Wild Barley Diversity Collection was subjected to paired-end Illumina sequencing at ~9X depth and evaluated for several agronomic traits. We discovered 240.2 million single nucleotide polymorphisms (SNPs) after alignment to the Morex V3 assembly and 24.4 million short (1-50 bp) insertions and deletions. A genome-wide association study of lemma color identified one marker-trait association (MTA) on chromosome 1H close to *HvBlp,* the cloned gene controlling black lemma. Four MTAs were identified for seedling stem rust resistance, including two novel loci on chromosomes 1H and 6H and one co-locating to the complex RMRL1-RMRL2 locus on 5H. The whole-genome sequence data described herein will facilitate the identification and utilization of new alleles for barley improvement.

Keywords: *Hordeum vulgare* subsp. *spontaneum*; whole genome sequence data; genome-wide association study; agronomic traits

Introduction

Barley (*Hordeum vulgare* L. subsp. *vulgare*) was one of the first crops domesticated in the Near East ~10,000 years ago (Zohary et al., 2012) and is currently cultivated over 47 million hectares worldwide (FAO, 2017). Its main uses include animal feed, malt for various alcoholic beverages, and human food. Through the domestication process and modern plant breeding, the genetic diversity of barley has been eroded (Civáň et al., 2024; Milner et al., 2019; Russell et al., 2016), leaving the crop vulnerable to various biotic and abiotic threats and limiting further improvements for key traits. The primary gene pool of barley includes varieties, breeding lines, landraces, and wild barley (*H. vulgare* L. subsp. *spontaneum* C. Koch. Thell.), the latter of which can readily hybridize with the cultivated forms (Harlan & Zohary, 1966; Liu et al., 2024). Studies aimed at identifying unexploited genes for use in barley breeding programs typically include panels more closely related to elite germplasm, thereby preserving the genetic linkages of favorable alleles for yield, quality, and agronomic traits. When a particular trait cannot be found in the cultivated forms of the primary gene pool, researchers often seek the desired alleles in the wild progenitor. To capture the allelic variation in wild barley, an ecogeographically diverse collection, known as the Wild Barley Diversity Collection (WBDC), was assembled (Steffenson et al., 2007). The WBDC comprises 318 accessions from across the range of *H. vulgare* subsp. *spontaneum* and has been evaluated for various agronomic, morphological, nutritional, and disease/pest resistance traits. These evaluations revealed a high level of variation for all the characterized traits, leading to subsequent genetic and genome-wide association studies (GWAS) based on various molecular marker technologies (Mahalingam et al., 2020; Roy et al., 2010; Sallam et al.,

2017; Walling et al., 2022). Here, we describe the whole-genome resequencing of 281 WBDC accessions with ~9X coverage and demonstrate its utility for identifying both previously described and novel genes in *Hordeum vulgare* using an association genetic approach.

Methods

Wild barley germplasm

Collection site data for longitude and latitude, elevation, high and low temperature, rainfall, and soil type (**Table S1; Figure 1A**) were used to assemble the WBDC at the International Center for Agricultural Research in the Dry Areas (ICARDA) (Steffenson et al., 2007). The proportion of samples included was generally reflective of the density of populations in the Fertile Crescent, Central Asia, North Africa, and Caucasus regions. Of the 318 WBDC accessions selected initially, 37 were not included in resequencing due to failed genotyping or sequencing, duplication, or seed admixtures. The final sequenced panel comprises 281 accessions from 19 countries. Single plant selections were initially made from each accession and then selfed for five successive generations in the greenhouse before being used for DNA extraction and sequencing.

DNA extractions

The first and second leaves of each accession were harvested, flash-frozen in liquid nitrogen, and stored at -80℃ until the DNA extractions were performed. For the extractions, tissue was first ground to a fine powder in liquid nitrogen using a mortar and pestle. Then, genomic DNA was extracted using a modified CTAB protocol (Yu et al.,

2017). Agarose gel electrophoresis was used to confirm that the genomic DNA was of high molecular weight (>10 kb). DNA quality was assessed using a NanoDrop spectrophotometer.

Library preparation and whole-genome sequencing (WGS)

WGS libraries were prepared using the 'Illumina Nextera DNA Flex Library Preparation Kit' (workflow for 100-500 ng DNA input, 5 PCR-cycles for the addition of indexes) according to manufacturer's instructions (Illumina, Inc., San Diego, CA, USA). The final library pool was quantified by qPCR (Mascher et al., 2021). The pool was sequenced (XP workflow, paired-end, 2 x 151 cycles) using the Illumina NovaSeq6000 device and standard protocols from the manufacturer.

Variant calling

Quality assessment, read mapping, deduplication, and coverage estimation utilized scripts in the RepAdapt pipeline (https://github.com/RepAdapt/snp_calling_simple). This involved quality assessment and adapter trimming with FASTP (Chen et al., 2018), read mapping with BWA MEM (Li, 2013), read deduplication with Picard (Broad Institute, 2019) and coverage estimation with samtools (Danecek et al., 2021; Li et al., 2009). Both SNP and indel variants were called using GATK version v4.1.2 (McKenna et al., 2010), with recommended GATK filtering as follows: SNP filtering "QD < 2.0 || FS > 60.0 || MQ < 45.0 || MQRankSum < -12.5 || ReadPosRankSum < -8.0 || DP > 4654.61"; and Indel Filtering "QD < 2.0 || FS > 200.0 || ReadPosRankSum < -20.0". Heterozygous and multiallelic sites were retained in the data set, and no frequency filter was applied to variants. The general feature format (GFF3) descriptions of both high and low confidence genes from the Morex V3 assembly

(Mascher, 2020) were used to create a BED file defining "gene space." Based on the descriptions in the GFF, gene space here comprises 5' and 3' UTRs, exons, and introns.

Variant statistics

Variant statistics, including sample size estimation and read depth per SNP, level of missingness, and related statistics, were calculated using the bcftools "+fill-tags" plugin (Danecek et al., 2021). Sample-level statistics were calculated with bcftools stats. The Variant Effect Predictor (VeP) (McLaren et al., 2016), along with GFF3 annotations for Morex V3, was used to annotate all variants. VeP results were used to parse variants by class for calculating the site frequency spectrum.

The folded site frequency spectrum (SFS) was estimated based on the minor allele frequency for biallelic SNPs genome-wide and for synonymous sites. The expectation for the SFS under a neutral coalescent history was generated using msprime (Baumdicker et al., 2022) based on nucleotide sequence diversity estimated as $\theta = 4N_e\mu = 0.008$ (Morrell et al., 2006; Schmid et al., 2018) and recombination rate of $\rho/\theta = 1.5$ (Morrell et al., 2006). We simulated 281 haploid samples with 1,000 replicate simulations with a locus length sufficient to generate ~100 SNPs per simulation or 100,000 variants that could be compared to the folded SFS for the empirical datasets.

Cluster analysis

The SNP dataset was filtered prior to analyses by setting heterozygote calls to missing and retaining biallelic sites with ≤10% missing data and ≥5% minor allele frequency. The SNP dataset was further pruned by discarding sites with r^2 >0.2 in windows of 50 sites. Principal component analysis was performed in TASSEL v5.0 (Bradbury et al., 2007). *K*-means clustering was used to partition the wild barley panel

into subpopulations (**Table S1**). Based on our previous knowledge of the panel (Sallam et al., 2017), seven subpopulations were assigned to the cluster analysis in JMP 17 (JMP Statistical Discovery LLC, Cary, NC, USA). JMP was used to plot the PCA results and create the map for the WBDC panel (**Figure 1**).

Phenotyping

To demonstrate the utility of the WGS dataset for identifying trait-associated loci in wild barley, we selected two important traits for study: 1) lemma color and 2) stem rust resistance. Lemma color was assessed by taking digital images of mature seeds and then analyzing each color channel using the Fiji package (Schindelin et al., 2012). The average color channel value (CCV) of two representative seeds of each accession for each color channel in the RGB color model was measured. Each RGB value was converted into a single 24-bit integer for GWAS analysis using the formula: Color = $(R \times 256^2) + (G \times 256) + B$. Stem rust assays on seedlings were performed with two races (MCCFC and QCCJB) of the wheat stem rust pathogen (*Puccinia graminis* f. sp. *tritici* (*Pgt*) and one isolate (92-MN-90) of the rye stem rust pathogen (*P. graminis* f. sp. *secalis*) (*Pgs*) as described in Sallam et al. (2017).

Genome-wide association mapping

To identify markers associated with the three traits, GWAS was conducted for 281 WBDC accessions using the following methods: 1) Mixed Linear Model (MLM) that accounts for population structure (Q) + kinship (K) (Yu et al., 2006), 2) Fixed and random model Circulating Probability Unification (FarmCPU) (Kusmec & Schnable, 2018) that utilizes fixed and random effects iteratively to improve association power, and 3) a

Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway (BLINK) (Huang et al., 2019) that utilizes Bayes and linkage disequilibrium to improve both association power and computation efficiency. The SNP dataset was filtered prior to GWAS by setting heterozygote calls to missing and retaining biallelic sites with ≤10% missing data and ≥5% minor allele frequency. The SNP dataset was further pruned by discarding sites with r^2 >0.2 in windows of 50 sites. All association mapping methods were executed in the R package GAPIT v3.5 using ~1.3 million SNP markers (Wang & Zhang, 2021). Marker trait associations (MTAs) identified using two or more methods or those detected with a single method but across two different datasets are presented. The Bonferroni test was performed to declare significant associations.

Results and Discussion

Variant calling with GATK in the sequenced 281 wild barley lines of the WBDC (~9X coverage, **Figure S1**) resulted in the identification of 240.2 million SNPs and 24.4 million indels (**Table 1**). In addition to these reported indels, there were a further 3.2 million sites where one of the variants at an indel site had a single base pair difference from the reference. These are among 6.5 million multiallelic variants, with 26.6% of indel sites called as multiallelic.

The vast majority of detected indels were one bp deletions; the second most abundant class was one bp insertions, with roughly half as many one bp insertions identified relative to deletions (**Figure S2**). The majority of variants identified were annotated as intergenic variants, including 219.2 million SNPs and 20.8 million indels (**Table 2**). Among coding SNPs, 53.3% (807,753) were missense changes, 45.2%

(685,452) were synonymous changes, and 1.2% (18,571) were stop-gained. Among indels affecting coding regions, 61.9% (74,148) were frameshift variants, 20.7% (24,768) were inframe deletions, 13.1% (15,642) were inframe insertions, and 1.9% (2,329) were stop-gained.

We also partitioned the data set into variants found within gene space as defined by Morex V3 annotations. SNPs within genic regions showed much lower rates of multiallelic polymorphisms at 2.6% and a lower missingness rate at 0.068 (±0.158) with a median of 0.007, consistent with the relative ease of read alignment and variant calling within gene space (**Table 1**).

The transition to transversion ratio (Ts/Tv) can vary among classes of variants and is a potential metric of variant call quality. Values in partitions for the dataset ranged from 1.07 for indels to 1.90 for variants in gene space. These values align with prior reported values of 1.7 for Sanger sequencing in wild barley (Morrell et al., 2006) and Illumina exome capture sequencing from domesticated barley (Kono et al., 2016).

There were 59.5 million biallelic SNPs in the dataset that occurred outside of indels and thus were unique mutations. The folded site frequency spectrum in Figure 2 includes all biallelic SNPs. We compared frequencies with expectations under a standard coalescent model of a panmictic population with constant population size. The SFS shows that a large proportion of variants reside in the rarest frequency class, here <2.5% frequency. While this was consistent with expectations under a standard coalescent model (Tajima, 1989), rare variants at the whole-genome level were more abundant than expected based on neutral coalescent simulations (**Figure 2**). The frequency spectrum for synonymous sites more closely resembles the expectation for neutral variants based

on coalescent simulations but again demonstrate more variants in the rarest frequency classes. This result likely reflects both the challenges of variant calling in a highly repetitive genome and an excess of rare variants at most wild barley loci, consistent with a recent population expansion in the species' coalescent history (Morrell et al., 2006).

Prior to GWAS, population structure was assessed by *k*-means clustering and principal component analyses (**Figure 1B**). Consistent with previous results on the population structure of wild barley (Fang et al., 2014; Russell et al., 2016; Sallam et al., 2017), genetic relatedness mirrored geographic distance: the distribution of population centers roughly traced a path from the North African coast and the Southern Levant along the Fertile Crescent to Central Asia (**Figure 1A**). A detailed analysis of population structure in wild barley and its relationship to domesticated accessions was undertaken by Guo et al. (2025) using the present data set.

Lemma color

Lemma color in the WBDC ranged from pale yellow (straw-colored) to brown and dark black based on visual inspection (**Figure 3A**). Converted RGB values from digital images of pale yellow and dark black seed generally ranged from 10,750,000-13,550,000 and 4,350,000-6,570,000, respectively **(Table S1; Figure S3)**. Black lemma is a classic morphological trait in barley and is controlled by the *Blp* locus, which is composed of different alleles contributing to the intensity and distribution of color (Franckowiak and Lundqvist 1997). GWAS identified one association (WBDC_LC_1H_499.0) by a single SNP (S1H_499023721) on chromosome 1H using all three models (MLM, FarmCPU and BLINK) (**Figure 3B**, **Table 3**). This SNP explained 17.5% of the phenotypic variation and lies in close proximity to *HvBlp*, the recently cloned gene controlling black lemma color

positioned between 498.5 to 499.0 Mbp on 1H in the Morex V3 assembly (Li et al., 2024) (**Table 3**). Due to the complexity of the locus and a duplicated fragment of *HvBlp*, it is difficult to state with certainty the physical relationship of the identified SNP marker and this gene.

Stem rust

Based on a coefficient of infection threshold of 2.7, only 15 (5.0%), 39 (14.0%), and 54 (19.0%) of the sequenced WBDC accessions were classified as resistant to *Pgt*-MCCFC, *Pgt*-QCCJB and *Pgs*-92-MN-90, respectively (**Table S1; Figure S4**). Four MTAs (WBDC_SR_1H_11.7, WBDC_SR_1H_67.4-71.5, WBDC_SR_5H_562.9, and WBDC_SR_6H_501.8) were identified for stem rust resistance. WBDC_SR_1H_11.7 was novel and mapped to chromosome 1H in response to both *Pgt*-MCCFC and *Pgt*-QCCJB, explaining 20.0% and 14.9% of the variation, respectively (**Figure 4**, **Table 3**). WBDC_SR_6H_501.8 was also novel and positioned on 6H in response to both *Pgt*-MCCFC and *Pgt*-QCCJB, explaining 15.4%-15.8% of the variation (**Table 3**). WBDC_SR_1H_67.4-71.5 was mapped on chromosome 1H in response to races *Pgt*-MCCFC and *Pgt*-QCCJB (**Table 3**). The two different SNPs (S1H_67388912 and S1H_71536803) identified in the MTA were in moderate linkage disequilibrium (r^2 = 0.565). The position of this MTA is close to S1H_71499376, a genotyping-by-sequencing (GBS)-derived marker that was found significantly associated with resistance to both *Pgt*-MCCFC and *Pgt*-QCCJB based on 314 individuals (Sallam et al. 2017). This MTA explained 18.7-31.0% of the variation in this study (**Table 3**). WBDC_SR_5H_562.9 was identified on chromosome 5H (S5H_562922829) after challenge with all three *P. graminis* cultures using all three models and explained 22.4-32.3% of the variation (**Table 3**). It co-

located to the position of the complex RMRL1-RMRL2 loci (Wang et al., 2013) from which several component resistance genes (e.g. *rpg4* and *Rpg5*) were cloned (Arora et al., 2013; Brueggeman et al., 2008).

WGS data for diverse accessions of a crop and its wild relatives are essential for population genomic studies, the informed selection of genotypes for full genome sequence assembly (pangenomics), and the isolation of agronomically important genes. Our dataset complements similar short-read datasets for 1,315 domesticated barleys (Jayakodi et al., 2020, 2024) and 100 wild barleys from another collection (Jayakodi et al., 2020). Chromosome-scale genome assemblies of nine WBDC accessions have been completed (Jayakodi et al., 2024) with more accessions to follow in the future. Applying GWAS to the WBDC, we demonstrated the utility of high-coverage sequence data for identifying novel genetic variation that may be useful in barley improvement. Additionally, we also validated major genes controlling key traits in barley such as *Blp* for black lemma color and RMRL1/RMRL2 for stem rust resistance. Thus, this dataset may serve as a starting point for the identification of candidate genes underlying other important traits. In a companion paper, Guo et al. (2025) demonstrated the utility of WBDC sequence data in a population genomic study. They analyzed this dataset together with sequence data from other diverse wild and domesticated barley accessions to reconstruct the evolutionary history of wild barley and elucidate the origin of haplotypes in cultivated barley. The sequenced WBDC genomes will help connect target phenotypic traits to chromosome positions. Reference genome positions, as identified by HORVU I.D.s in the Morex V3 assembly (Mascher, 2020), serve as anchors to protein-protein interactome

hubs (Velásquez-Zapata et al., 2022) and the potential for engineering the molecular and cellular mechanisms by which key phenotypes are expressed.

Data Availability Statement

Seed of the complete WBDC (N=318) can be obtained from the USDA-ARS National Small Grains Collection as accessions PI 681726 to PI 682043. Raw sequence data are deposited in the European Nucleotide Archive (ENA) under project ID PRJEB56087. The variant data are deposited in the GrainGenes database (Yao et al. 2022) at https://graingenes.org/snpversity/. SNP names from previous barley genotyping platforms (Bayer et al., 2017; Close et al., 2009; Comadran et al., 2011, 2012) are added as annotations. Scripts used for variant calling, filtering and other analyses can be found in GitHub repository: https://github.com/SteffensonLab/Barley_IPK_variant_calling. We used stem rust reaction type data from a previously published G3 paper (Sallam et al. 2017): https://doi.org/10.1534/g3.117.300222. These data are also included in Table S1. Figures S1-S4 and Table S1 are available to download at G3 online.

Web Resources

https://graingenes.org/snpversity/

Acknowledgments

A.H. acknowledges Ines Walde for expert technical assistance in the sequencing operation. B.J.S. thanks Harold Bockelman, Matthew Martin, and Tamas Szinyei for technical assistance in the handling of the wild barley materials. We acknowledge the University of Minnesota Supercomputing Institute (MSI) for providing computing resources.

Conflict of Interest

The authors declare no competing interests.

Funding

The research of B.J.S. was supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, Hatch project (#MIN-22-085 “Exploiting Wild Relatives for Cultivated Wheat and Barley Improvement”); Lieberman-Okinow Endowment at the University of Minnesota; Binational Agriculture Research and Development Fund (BARD US-5089-18 “Cloning and comparative sequence analysis of powdery mildew and leaf rust resistance gene complements in wild barley”); and American Malting Barley Association. Barley genome research in the laboratory of M.M., U.S. and N.S. is supported by a grant from the German Ministry of Research and Education (BMBF, SHAPE-P3, 031B0190). The wild barley research of M.M. is supported by a grant from the German Research Foundation (DFG, 460265804). A.T. was supported by project RECOBAR (Recovering and Exploiting Old and New Barley Diversity for Future-Ready Agriculture). B.K. was supported by Biodiversity for Opportunities, Livelihoods and Development (BOLD) Project funded by the Government of Norway [grant number: QZA-20/0154]. B.B.H.W. was supported by funding from King Abdullah University of Science and Technology. The research of C.D. is supported by the Carlsberg Foundation (CF15-0236). J.D., A.P. and J.Š. were supported from the project TowArds Next GENeration Crops, reg. no. CZ.02.01.01/00/22_008/0004581 of the ERDF Programme Johannes Amos Comenius. For R.P.W., research was supported

in part by the National Science Foundation – Plant Genome Research Program grant 13-39348, and USDA-Agricultural Research Service projects 3625-21000-067-00D and 5030-21220-068-000-D. For C.H., R.M., M.A.V., and J.G.W, research was supported by USDA-Agricultural Research Service projects 5090-21430-011-000D and 5090-43440-007-000D. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA, ARS, or the National Science Foundation. USDA is an equal opportunity provider, employer and lender. M.J.M. was supported by United States Department of Agriculture-Agricultural Research Service CRIS #5062-21220-025-000D.

Author Contributions

B. J. Steffenson and M. Mascher designed and led the study. A. H. Sallam, G. Bethke, and J. Simmons grew and harvested the plant materials and extracted DNA. A. Himmelbach, and A. Fiebig performed the sequencing operations. R. Spanner, A. H. Sallam, Y. Lee, M. Jayakodi, M. Mascher, Y. Qiu, P. L. Morrell, L. W. Pacheco Arge and Y. Guo analyzed the data. B. J. Steffenson, R. Spanner, A. H. Sallam, Y. Lee, P. L. Morrell, M. Mascher, and Y. Guo drafted the manuscript and made the revisions. All authors reviewed and edited the manuscript.

Ethics declarations

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Tables

Table 1. Summary statistics for variants obtained after aligning whole-genome sequencing reads from 281 individuals of the Wild Barley Diversity Collection to the Morex V3 reference genome. Biallelic sites include positions with unique mutations and no overlapping indels. Gene space includes coding regions and UTRs from Morex V3 gene annotation.

Data set	Variant #	Multiallelic	Ts/Tv	Proportion missing
SNPs	240,171,785	9,214,159	1.39	0.094 (+0.177) -0.018
Indels	24,387,195	6,493,811	1.07	0.148 (+0.217) - 0.039
Biallelic	59,520,067	–	1.47	0.083 (+0.166) -0.014
SNPs - Gene space	6,385,855	166,712	1.9	0.068 (+0.158) - 0.007

Table 2. Variant Effect Predictor (VeP) results for genome-wide SNPs and indels.

	SNPs		Indels	
Variant	Count	Proportion (%)	Count	Proportion (%)
Splice acceptor	2,007	0.0008	986	0.00404
Splice donor	1,747	0.0007	1,201	0.00492
Stop gained	18,571	0.0077	2,329	0.00955
Frameshift variant	-	-	74,148	0.30404
Stop lost	1,520	0.0006	190	0.00078
Start lost	1,507	0.0006	267	0.00109
Inframe insertion	-	-	15,642	0.06414
Inframe deletion	-	-	24,768	0.10156
Missense	807,753	0.3363	334	0.00137

Protein altering variant	-	-	1,163	0.00477
Splice donor 5th base	4,472	0.0019	1,248	0.00512
Splice region	47,271	0.0197	7,983	0.03273
Splice donor region	12,910	0.0054	2,225	0.00912
Splice polypyrimidine tract	46,258	0.0193	11,488	0.04711
Start retained	-	-	9	0.00004
Stop retained	989	0.0004	98	0.0004
Synonymous	685,452	0.2854	71	0.00029
Coding sequence variant	-	-	781	0.0032
5'UTR	96,094	0.04	36,888	0.15126
3'UTR	229,979	0.0958	56,752	0.23271
Intron	2,757,606	1.1482	554,672	2.27444
Upstream gene	9,135,842	3.8039	1,560,586	6.3992
Downstream gene	7,049,038	2.935	1,194,674	4.89878
Intergenic	219,000,000	91.2983	20,838,692	85.44932

1

Table 3. Single nucleotide polymorphism (SNP) markers significantly associated with lemma color and stem rust resistance in 281 *Hordeum vulgare* subsp. *spontaneum* accessions of the Wild Barley Diversity Collection.

Phenotype	Association designation[a]	Treatment or Trait[b]	SNP[c]	Chr[d]	Pos[e]	MAF[f]	GWAS detection method[g]	*p*-value range[h]		R^{2i}
Lemma color	WBDC_LC_1H_499.0	Lemma color	S1H_499023721	1H	499.0 Mbp	0.06	MLM/FarmCPU/BLINK	9.78×10^{-17}	1.36×10^{-9}	17.51%
Stem rust resistance	WBDC_SR_1H_11.7	MCCFC	S1H_11651434	1H	11.7 Mbp	0.07	MLM/FarmCPU	9.83×10^{-11}	1.81×10^{-10}	20.02%
		QCCJB	S1H_11651434	1H	11.7 Mbp	0.07	MLM/FarmCPU	2.17×10^{-14}	5.76×10^{-9}	14.86%
	WBDC_SR_1H_67.4-71.5	MCCFC	S1H_67388912, S1H_71536803	1H	67.4 – 71.5 Mbp	0.07	MLM MLM/FarmCPU/BLINK	1.50×10^{-24}	1.87×10^{-8}	22.46 – 31.03%
		QCCJB	S1H_67388912	1H	67.4 Mbp	0.07	BLINK	5.77×10^{-14}		18.71%
	WBDC_SR_5H_562.9	MCCFC	S5H_562922829	5H	562.9 Mbp	0.07	MLM/FarmCPU/BLINK	3.25×10^{-31}	3.49×10^{-12}	22.38%
		QCCJB	S5H_562922829	5H	562.9 Mbp	0.07	MLM/FarmCPU/BLINK	7.98×10^{-34}	3.77×10^{-13}	24.08%
		92-MN-90	S5H_562922829	5H	562.9 Mbp	0.07	MLM/FarmCPU/BLINK	2.89×10^{-42}	5.09×10^{-17}	32.28%
	WBDC_SR_6H_501.8	MCCFC	S6H_501789703	6H	501.8 Mbp	0.08	MLM	1.84×10^{-9}		15.80%
		QCCJB	S6H_501789703	6H	501.8 Mbp	0.08	MLM/FarmCPU	9.49×10^{-10}	2.60×10^{-8}	15.43%

[a] Association designation is based on the germplasm (WBDC), trait abbreviation (e.g. lemma color), chromosome location (1H), and physical position from the Morex (V3) genome assembly.

[b] Treatment or Trait includes lemma color, reaction to races MCCFC and QCCJB of the wheat stem rust pathogen (*Puccinia graminis* f. sp. *tritici*) and reaction to isolate 92-MN-90 of the rye stem rust pathogen (*P. graminis* f. sp. *secalis*).

[c] SNP designation is based on the chromosome and physical position from the Morex (V3) genome assembly.

[d] Barley chromosome and arm designation: S = short or L = Long.

[e] Physical position based on the Morex (V3) genome assembly.

[f] Minimum allele frequency

[g] Only associations detected with two or more methods or with a single method but in more than one dataset are shown. MLM denotes Mixed Linear Model (MLM); FarmCPU denotes fixed and random model Circulating Probability Unification; and BLINK denotes Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway.

[h] Range of p-values for the associations identified through different models.

[i] R^2 values for the SNP marker found significantly associated with the trait.

Figure Legends

Figure 1. A) Geographic distribution of 281 *Hordeum vulgare* subsp. *spontaneum* accessions of the Wild Barley Diversity Collection (WBDC) and **B**) Principal component analysis determined from ~1.3 million single nucleotide polymorphisms (SNPs). Accessions are color-coded by sub-population, defined by *k*-means clustering.

Figure 2. Folded site frequency spectrum for genome-wide biallelic SNPs. The plot includes all biallelic sites, synonymous sites, and variants simulated under a neutral coalescent history.

Figure 3. A) Examples of different lemma colors in the Wild Barley Diversity Collection; from left to right: yellow (straw) from WBDC045, brown from WBDC204, diffuse black from WBDC014, and dark black from WBDC355. **B)** Manhattan plots displaying single nucleotide polymorphism (SNP) markers significantly associated with lemma color in the Wild Barley Diversity Collection. Three models were used in the analysis: 1) a Mixed Linear Model (MLM), 2) a Fixed and random model Circulating Probability Unification (FarmCPU), and 3) a Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway (BLINK). Bonferroni significance threshold is shown with a horizontal solid green line.

Figure 4. Manhattan plots displaying single nucleotide polymorphism (SNP) markers significantly associated with resistance to the wheat stem rust (*Puccinia graminis* f. sp. *tritici, Pgt*) and rye stem rust (*P. graminis* f. sp. *secalis, Pgs*) pathogens: (**A**) race *Pgt*-MCCFC, (**B**) race *Pgt*-QCCJB, and (**C**) isolate *Pgs*-92-MN-90 in the Wild Barley Diversity Collection. Three models were used in the analysis: 1) a Mixed Linear Model (MLM), 2) a Fixed and random model Circulating Probability Unification (FarmCPU), and 3) a Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway (BLINK). The Bonferroni significance threshold is shown with a horizontal solid green line. The vertical blue, purple, yellow and green lines show the significant associations consistently identified for resistance to two cultures of *P. graminis* with at least one or two models or

to one culture with all three models. RMRL1/RMRL2 is a complex of several stem rust resistance genes.

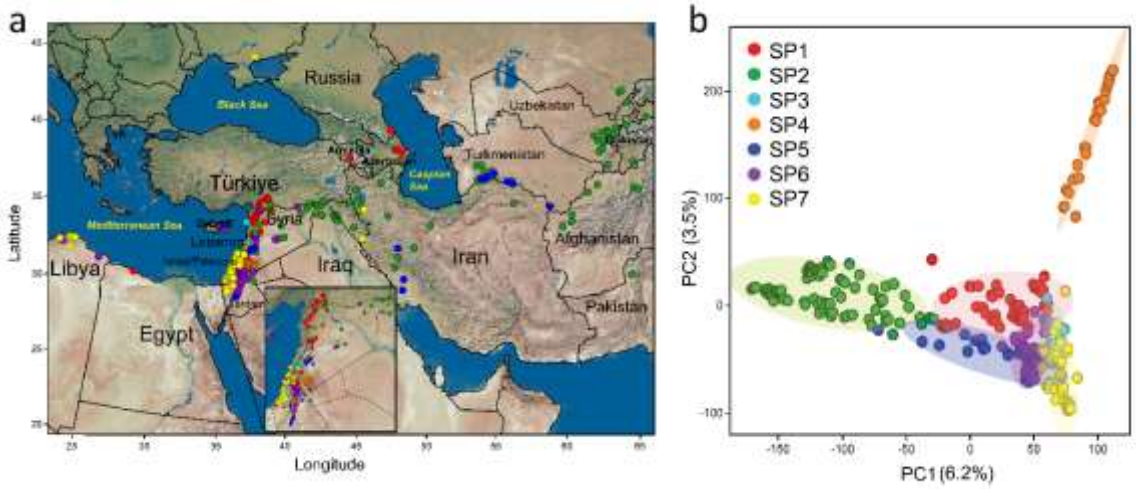

Figure 1
155x67 mm (x DPI)

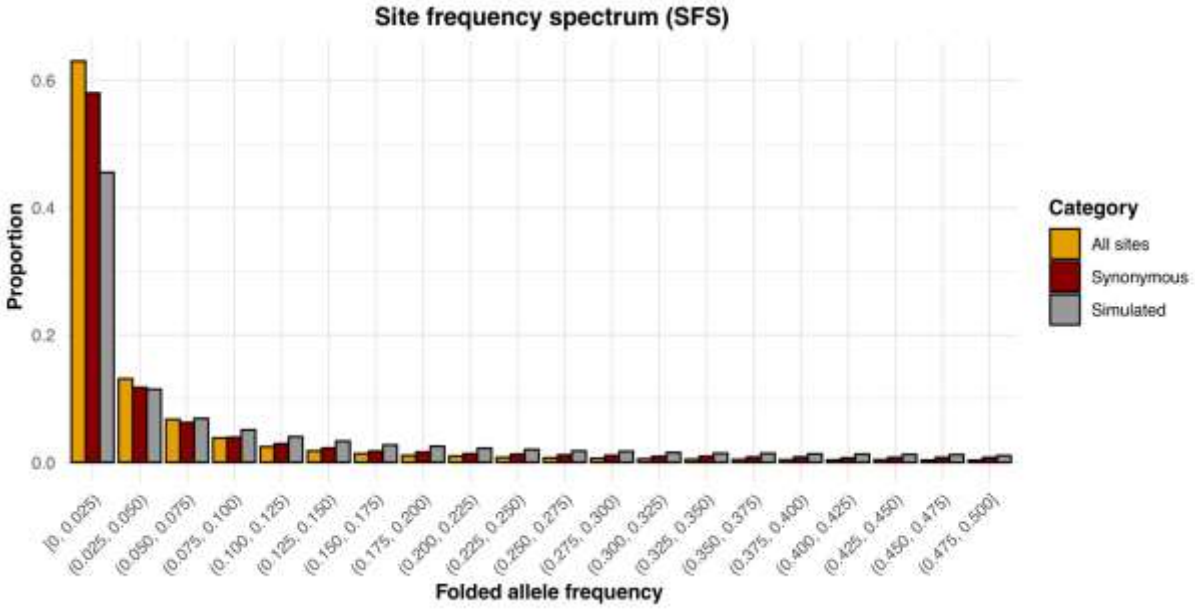

Figure 2
229x114 mm (x DPI)

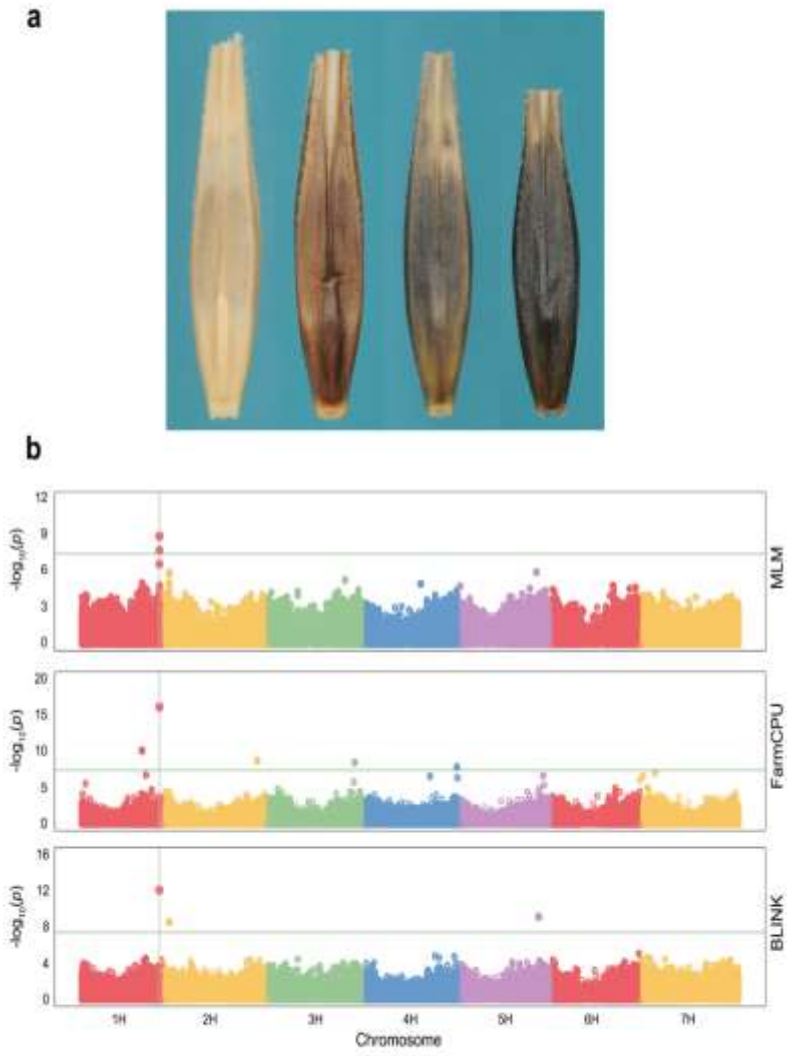

Figure 3
210x297 mm (x DPI)

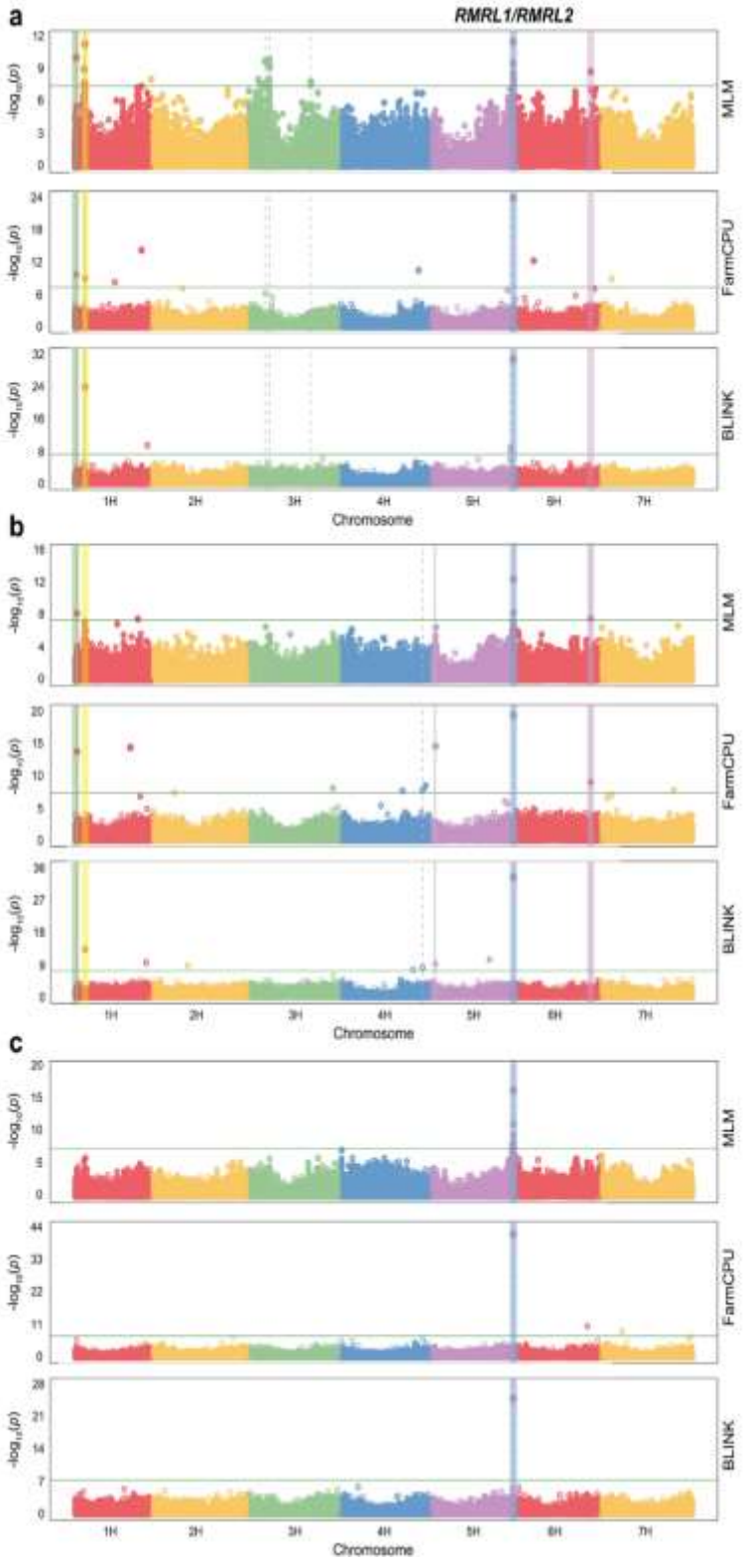

Figure 4
210x297 mm (x DPI)