Title
Genomic resources for a historical collection of cultivated two-row European spring barley genotypes.

Authors

Miriam Schreiber[1,§], Ronja Wonneberger[2,§], Allison Haaning[3,§], Max Coulter[1], Joanne Russell[4], Axel Himmelbach[2], Anne Fiebig[2], Gary J. Muehlbauer[3,$], Nils Stein[2,$], Robbie Waugh[1,4,$]

Affiliations

1. Division of Plant Sciences, University of Dundee at The James Hutton Institute, Invergowrie, Dundee, Scotland DD2 5DA, UK
2. Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Seeland, Germany
3. Department of Agronomy and Plant Genetics, The University of Minnesota, St. Paul, MN 55108
4. Cell and Molecular Sciences, The James Hutton Institute, Invergowrie, Dundee, Scotland DD2 5DA, UK.

§ These authors contributed equally to the work.
$ These authors have jointly supervised the work.

corresponding author(s): Robbie Waugh (Robbie.Waugh@hutton.ac.uk)

Abstract

Barley genomic resources are increasing rapidly, with the publication of a barley pangenome as one of the latest developments. Two-row spring barley cultivars are intensely studied as they are the source of high-quality grain for malting and distilling. Here we provide data from a European two-row spring barley population containing 209 different genotypes registered for the UK market between 1830 to 2014. The dataset encompasses RNA-sequencing data from six different tissues across a range of barley developmental stages, phenotypic datasets from two consecutive years of field-grown trials in the United Kingdom, Germany and the USA; and whole genome shotgun sequencing from all cultivars, which was used to complement the RNA-sequencing data for variant calling. The outcomes are a filtered SNP marker file, a phenotypic database and a large gene expression dataset providing a comprehensive resource which allows for downstream analyses like genome wide association studies or expression associations.

Background & Summary

Barley is one of the most important crops worldwide (5th in 2020 on area harvested, FAOSTAT[1]) and has a high value in the European agricultural sector underpinning the beer and whisky industries[2]. New barley cultivars are introduced to the market every year, after being evaluated for multiple traits e.g., disease resistance, yield, and malting quality traits[3]. Barley breeding and the introduction of barley cultivars started at the beginning of the 19th century in the UK and by the end of the 19th century all over Europe[4]. Instead of seeds being grown by the farmer with some saved for subsequent sowing the following year, breeding institutes were established, with the mission to develop improved seed stocks. Early cultivars were developed through mass selection and later followed by line selection from landraces. Initial breeding efforts focused on increasing yield[5]. Due to the considerable success of these breeding efforts, seed stocks soon became distributed across the continent and each country started their own breeding program by incorporating local landraces in crosses with these generally higher yielding genotypes. This cross-breeding technique of simple crosses

followed by selection quickly led to an increase in yield as shown for spring barley in Germany with a doubling of yield from 1800 to 1900[6]. Breeding developed further by intentionally mutating seeds with chemicals or radiation to induce higher genetic variation in the offspring[7]. One of the most notable results from mutation breeding were the dwarfing genes which were critical for the green revolution[8]. Shorter stature cultivars provided the advantage of preventing lodging which was crucial for the development of high-yielding cultivars with heavy spikes. Complementing traditional to cross- and mutation-breeding, molecular technologies developed further and were quickly adopted. One of the most successful advances was marker-assisted selection (MAS) which deploys molecular markers to detect allelic variations within a genome. The most common markers used in breeding nowadays are single nucleotide polymorphisms (SNPs)[9]. MAS is used for rapid and high-throughput selection of new genotypes and has matured from single marker analysis to genome-wide selection approaches. While SNPs are a key component of the genotyping platforms used in plant breeding purposes, they can also be used for gene discovery. Quantitative trait locus (QTL) mapping and genome wide association studies (GWAS) are valuable to identify alleles for genes underpinning genetically complex traits[10-13]. High throughput genetic markers are however only one of a number of genetic and genomic resources that have effectively revolutionised genetics and breeding. Next generation sequence data formed the basis of the first barley genome published in 2017 from the cultivar Morex[14] which has been followed quickly by additional genomes from other cultivars[15]. The availability of "reference genome sequences" has both simplified the process and allowed a more precise identification of the causative genes controlling phenotypic traits.

Here we introduce new genetic and genomic datasets assembled from a European two-row spring barley population that is representative of pan-European breeding progress across the years from 1830 to 2014. A total of 209 50K SNP-array[16] genotyped barley cultivars were selected and grown in replicated field trials across three contrasting environments and for two years to score agronomic traits. Six different tissues from each cultivar were harvested and RNA was isolated for the collection of tissue and genotype specific transcript abundance (RNA-seq) data. Using both this RNA-seq data and whole genome shotgun sequence data from all individuals in the population, an exhaustive collection of high confidence SNP markers was assembled. We describe these datasets and provide examples of how they can be used.

Methods

Barley material and field trials

We assembled a collection of 209 European two-rowed spring barley cultivars (Supplemental Table 1), which is a representative subset of previously described two-row spring European barley populations[10,11,17-19] that show a significant increase in yield over time. Pedigree data was collected from publications[17,20], and the following two websites: https://grinczech.vurv.cz/gringlobal/search.aspx and https://www.lfl.bayern.de/mam/cms07/ipz/dateien/abst_gerste.pdf. Field experiments were conducted at the Leibniz Institute of Plant Genetics and Crop Plant Research (**IPK**) in Gatersleben, Germany, the James Hutton Institute (**JHI**) in Dundee, UK and the University of Minnesota (**UMN**) in St. Paul, USA in 2019 and 2020. At IPK and UMN, 100 grains of each genotype were sown in 1 m long double-rows in a completely random design with three replications in both years. At JHI, a seed density estimated to produce 350 plants per m^2 for plot sizes of 2m x 1.5 m was established. In 2019 a single replicate was grown and in 2020 a completely random design with two replicates. In addition, a polytunnel trial was included at JHI in 2019. Plant material was grown in 7 litre sized pots, 4 seeds per pot, in 3 replicate sets in a completely random design. Each replicate set had 8 columns and 30 rows and contained a replicate of each of the 209 genotypes.

Phenotyping

In total, 29 phenotypes were recorded on a per-plot basis in the field trials or on a per-pot basis in the polytunnel experiment. Developmental traits, growth habit and plant height measurements were recorded in the trials as described in Table 1. To measure spike and grain traits, ten to 15 main tiller spikes were harvested at full maturity (Zadoks stage 92) per plot, excluding the outermost plants of each row to avoid edge effects. After recording of all spike traits, spikes were hand-threshed, and grains were subjected to size and weight measurements on a Marvin SeedAnalyzer 6 (MARViTECH GmbH, Germany). Samples were first weighted and then added on to the Marvin tray for optical measurements of the grain size.

Table 1: A summary of the phenotypic traits and a description on how they were scored.

Trait	**Method**
Developmental traits	
Days to awn tipping	50% of the main tiller awns per plot have emerged up to 1 cm out of the flag leaf sheath. Recorded as days since sowing
Days to heading	50% of the main tiller spikes per plot have emerged halfway out of the flag leaf sheath. Recorded as days since sowing
Days to senescence	50% of the main tiller peduncles per plot are senescent (yellow). Recorded as days since sowing
Days from awn tipping to heading	Derived from days to awn tipping and days to heading
Days from awn tipping to senescence	Derived from days to awn tipping and days to senescence
Days from heading to senescence	Derived from days to heading and days to senescence
Growth habit (GH)	Visual evaluation using a scale of 1 (erect), 2 (intermediate) and 3 (prostrate). Recorded at the onset of stem elongation
Height and length traits	
Peduncle base height	Height of the base of the peduncle in cm
Flag leaf blade height	Height of the flag leaf sheath in cm
Culm height	Height of the base of the spike in cm
Plant height	Height of the top of the spike in cm
Awn tip height	Height of the tip of the awns in cm
Spike base to flag leaf	Calculated distance from base of spike to flag leaf sheath (auricle) in cm
Peduncle length	Calculated distance from base of spike to base of peduncle in cm
Awn length	Calculated distance from tip of awns to top of spike in cm
Spike culm ratio	Spike length divided by culm height
Spike traits (recorded on 10-15 main tiller traits per plot after harvest)	
Rachis node number	Number of rachis nodes
Spike length	Spike length in cm
Spike density	Rachis node number divided by spike length
Grain traits	
Grain traits (recorded on 10-15 main tiller traits per plot after harvest) using a Marvin Seed Analyzer 6	
Grain area	Area of all kernels per spike in mm^2. Recorded using the automatic grain area calculation function in the Marvin SeedAnalyzer 6 software
Kernel roundness	Roundness of all kernels per spike. Recorded using the automatic kernel roundness calculation function in the Marvin SeedAnalyzer 6

	software
Thousand kernel weight	Calculated from the number and weight of the kernels using the Marvin SeedAnalyzer 6 software
Grain length	Length of all kernels per spike in cm
Grain width	Width of all kernels per spike in cm
Spike traits (recorded on 10-15 main tiller traits per plot after harvest)	
Infertile florets at top and bottom (=edges) of spike	Number of infertile florets at the top of spike down to first fertile floret + number of infertile florets at the base of spike up to first fertile floret
Infertile florets in the middle of the spike	Number of infertile florets in the centre of the spike
Number of fertile grain	Total number of fertile florets per spike
Percent of fertile florets	Total number of fertile florets per spike divided by rachis node number
Number infertile florets	Total number of infertile florets per spike

Tissue sampling for RNA-seq

Six different tissues were sampled for RNA-seq analysis: crown, root, inflorescence, peduncle, spikelet and grain. At UMN, crown and root tissues were sampled from seven-day-old seedlings (GRO:0007060, first leaf unfolded). Ten seeds per genotype were surface sterilized and planted in moist vermiculite in individual Cone-tainers (6000 RLC3 size, Ray Leach, Tangent, OR). The Cone-tainers were put into a dark cold room for four days to achieve more consistent germination. Then they were moved into a growth chamber at 20°C with 16 hours of light for seven days. Tissues were harvested within three hours, starting at 9:00 am USA Central Time Zone to reduce the circadian effect on gene expression. Roots were sampled by cutting the longest root from each seedling adjacent to the germinated seed, and crowns by removing the roots and keeping the 1 cm shoot tissue immediately above. For each individual genotype five plants were combined and snap frozen in liquid nitrogen.

At JHI, the barley plants grown in 2019 under polytunnel conditions were used for tissue sampling. When plants reached the booting stage, which was 84-85 days after germination, 3 – 5 cm whole developing inflorescence tissue was taken, two from each replicate per genotype per sample. Whole peduncles were taken at 2 – 5 cm in length, three from each replicate per genotype per sample when plants were 88 – 90 days old. Samples were snap frozen in liquid nitrogen and stored at -80°C.

At IPK, barley plants grown in the 2019 field trial were monitored daily by dissecting single spikelets and recording the date of green anther stage and flowering stage on paper tags attached to the spikes. Sampling was limited to a two-hour period between 10:00 and 12:00 am Central European Summer Time each day to reduce the circadian effect on gene expression. Three spikes per plot of one repetition were selected at green anther stage and two central spikelets from the centre of each spike were sampled. At 5 days post anthesis, three spikes per plot of one repetition were selected and six developing grains per spike were sampled from the central region of each spike. All samples were snap-frozen in liquid nitrogen and stored at -80 °C until RNA extraction.

RNA extraction and RNA sequencing

RNA was extracted using the RNeasy Plant Mini Kit (Qiagen) and treated with DNaseI following the manufacturer's instructions. Buffer RLC was used for seedling root extractions, and Buffer RLT was used for all other tissue extractions. To ensure a high purity of spikelet and grain samples, a more rigorous cleanup using 700µl RW1 and three wash steps with RPE was performed. The integrity of samples was determined using an Agilent 2100 Bioanalyzer, an Agilent 4200 TapeStation or a 1% agarose gel. All tested samples had a RIN factor of >=8

154 and were suitable for further processing. Paired-end libraries with a read length of 150 bp
155 were constructed from spikelet and grain samples (IPK Gatersleben) and seedling root and
156 crown samples (University of Minnesota Genomics Center, Minneapolis, MN, USA) using the
157 Illumina TruSeq Stranded Total RNA Library Prep Plant with Ribo-Zero Plant kit and
158 sequenced on the NovaSeq 6000 platform. For the inflorescence and peduncle samples (JHI)
159 Illumina RNA-seq library preparation and RNA-seq was carried out by Novogene (Company
160 Limited, Hong Kong). The libraries were prepared using NEBNext® Ultra™ Directional RNA
161 Library Prep Kit and sequenced using Illumina NovaSeq 6000 (PE 150).
162
163 **Bioinformatics**
164 Read quantification
165 We generated 77.95 billion raw reads from RNA-seq of the six different tissues
166 (Supplemental Table 2). Raw reads were trimmed with Trimmomatic 0.39[21] to remove
167 adapters and reads shorter than 60 bp. Salmon 1.3.0[22] was used for expression
168 quantification including the gcBias setting to align trimmed reads to the transcriptome. We
169 followed the approach of selective alignments by generating a decoy-aware transcriptome
170 from the barley reference transcript dataset V2 (BaRTv2)[23] and the reference genome of *cv*
171 Barke[15]. This approach is recommended[24] to reduce inaccurate transcript quantification
172 caused by unannotated genomic loci that have a high sequence similarity to annotated
173 transcripts.
174
175 Expression analysis
176 Tissue-specific genes were identified using different R packages[25]. For each tissue the raw
177 counts were imported and combined to gene expression counts using tximport[26]. Raw counts
178 were normalised (calcNormFactor), and log transformed to counts per million (cpm) using
179 edgeR[27]. The tissue-specific expressed genes were identified by filtering for an average cpm
180 of above 1 across all samples in this tissue and an average cpm of below -1 for all the other
181 tissues. In addition, gene expression for two and more tissues were filtered with the same
182 parameters to build the intersection sets required to create an UpSet[28,29] plot of expressed
183 genes in the different tissues. Gene ontology (GO) enrichment for the identified genes and
184 visualisation was done as previously described[30].
185
186 Variant calling
187 For variant calling, the trimmed RNA-seq reads were mapped to the reference genome of *cv*
188 Barke[15] using the two-pass mode implemented in STAR v. 2.7.5[31] allowing 6% mismatches
189 normalized to read length, intron lengths between 60 and 15000 bp, a maximum distance of
190 2000 bp between mates and a maximum number of 30,000 transcripts per window. Due to
191 the high number of reads in the grain and spikelet tissue the splice junction files were
192 filtered for at least one uniquely-mapped read in more than one sample, with non-canonical
193 splice sites removed and then used to generate a new genome index for the second mapping
194 run. For the other tissues, the splice junction files from the first pass were provided as part
195 of the input for the second mapping step. Duplicated reads were marked with Picard
196 2.18.29[32] followed by filtering with bamtools 2.5.1[33] to remove reads with 2% mismatches
197 and a mapping quality <= 50 .The legacy algorithm of Freebayes 1.3.2[34] was used to call
198 variants with a minimum fraction of alternate allele observations of 20%, a minimum
199 alternate allele count of 2, a minimum coverage of 4, and minimum base and mapping
200 qualities of 30.
201
202 Whole genome shotgun (WGS) approach
203 DNA was extracted from snap-frozen second leaves of greenhouse-grown (21°C/18°C
204 day/night temperature) two-week old seedlings using a guanidinium thiocyanate-NaCl-based
205 method as described[35]. DNA quality and quantity was assessed by agarose gel

electrophoresis. The Nextera DNA kit (Illumina) was used for library preparation of 150 bp paired-end reads. Libraries were multiplexed and sequenced on a NovaSeq 6000 platform at IPK Gatersleben. 12.16 billion raw paired-end raw reads (Supplemental Table 2) were trimmed with Cutadapt 1.15[36] to remove adapters and reads shorter than 30 bp. Trimmed reads were mapped to the reference genome of *cv* Barke using Minimap2 2.11[37]. The resulting alignment files were sorted and duplicate-marked using Novosort 3.06.05[38] and converted to cram files using samtools 1.8[39,40]. Bcftools[40] call was used to call variants using genotype likelihoods calculated from alignments with a minimum quality score of 20 with bcftools mpileup. Variants were re-called based on read depth ratios using a custom awk script similar to the one at https://bitbucket.org/ipk_dg_public/vcf_filtering/src/master/ with the following parameters modified: dphom = 1, dphet = 2, minhomn = 10, tol = 0.249, minmaf = 0.1, minpresent = 0.01.

Genotype marker file

The final genotype file was generated by filtering and merging multiple files. First all RNA-seq vcf files from the six tissues were filtered to remove insertions and deletions (Indels). RNAseq SNPs from individual tissues were then merged, prioritizing homozygous calls by retaining heterozygous calls only if no homozygous calls were present in any of the tissues. The merged RNAseq SNPs were then merged with WGS and 50K array SNPs[16], prioritizing homozygous calls in the same manner. The resulting unfiltered dataset contained 209 cultivars and 32,484,981 bi-allelic SNPs. The merged SNP dataset was filtered using TASSEL5[41] to remove SNPs with more than 20% missing data, minor allele frequency (MAF) < 0.01, heterozygosity > 0.02, and only keeping bi-allelic SNPs. Missing data was imputed using the FILLIN plugin[42] in TASSEL5 by first identifying haplotypes. For haplotype identification each chromosome was split into 500 blocks. The number of markers per haplotype block (-hapSize) was the total number of markers per chromosome divided by 500 and rounded to be divisible by 64 (TASSEL5 software requirement). Haplotypes were identified for each block with a maximum number of haplotypes of 20 (-maxHap 20) and at least five different genotypes per haplotype (-minTaxa 5). Haplotype information was used as input for the imputation. Further filtering removed seven lines that had more than 30% missing data after imputation (Aramir, Balder J, Dallas, KWS Irina, Power, Proctor and Spey), and one line was removed that had more than 2% heterozygosity (Rika). In a last filtering step, we removed SNPs which still had more than 20% missing data, MAF < 0.025 and heterozygosity > 0.02. SNPs were LD pruned with PLINK (v1.9)[43] using a window size of 5000, a step size of 50 and an r^2 threshold of 0.99. The final SNP dataset after pruning contained 201 cultivars and 1,509,447 SNPs.

Variant effect using SnpEff

To identify the effect of variants on the protein, we filtered the raw vcf files in a different way to generate an input file for SnpEff[44]. The aim for the genotype marker set explained above was to reduce the number of SNPs with pruning to a size which can be used for association analysis. For the variant effect, on the other hand, we need all the available SNP information and more importantly do not want to lose any SNPs due to pruning in gene space. For SNPs, the merged unfiltered vcf file containing RNA-seq, WGS and 50k data was filtered by removing heterozygous calls, removing SNPs with missing data in more than 20% of the samples and a minor allele frequency of < 0.025. In addition, a dataset containing Indels was created by using the six vcf output files from the RNA-seq data after variant calling with Freebayes. All were filtered to keep Indels only, remove heterozygous calls, removing variants with missing data in more than 20% of the samples and a MAF of < 0.025. The six Indel vcf files were combined into one. A SnpEff database was built based on BaRTv2 and the Barke reference genome.

Statistical analysis of phenotypic data, calculation of Best linear unbiased predictions (BLUPs) and heritability

Statistical analysis was performed using R 3.6.1[25]. The Pearson correlation coefficient between experiments was calculated for each phenotypic trait and datasets showing an insignificant correlation ($p>0.05$) with at least one other dataset of the same trait were removed before calculating BLUPs. The datasets being used in each of the BLUP calculations are listed in Supplemental Table 3. BLUPs were calculated across experiments using a randomized complete block model in META-R with experiments set as a random factor following formula 3 in Alvarado *et al.*[45].

Genome wide association studies (GWAS)

Association between phenotype and genotype was done using the Mixed Linear Model (MLM)[46] with GAPIT (version 3)[47]. As input, we used the genotype marker file of 201 cultivars and 1,509,447 SNPs and the BLUP values of the Awn length phenotype were used to show an example of the process. Three principal components (PCs) were calculated within GAPIT and model selection set to TRUE to enable GAPIT to select the optimal number of PCs for the individual phenotype based on a Bayesian information criterion (BIC).

Data Records

All raw data files for both raw RNA sequencing data and whole genome shotgun data have been deposited at the European Nucleotide Archive (ENA) under the following project number: PRJEB49069 for the RNA-sequencing reads and PRJEB48903 for the whole genome shotgun sequencing reads.

Phenotypic data and the SNP marker file are available through Germinate[48]: https://ics.hutton.ac.uk/germinate-barn/

The database contains the raw data by year and by site plus the calculated BLUP dataset.

Derived datasets are available through e!Dal[49]. The datasets consist of two sets of gene expression files per tissue; one for the raw read counts and one for the TPM values mapped against BaRTv2. Further two files containing the variant identification are available. The first contains the SNPs and the second with the Indel information with SnpEff annotation.

Preview link: https://doi.ipk-gatersleben.de/DOI/815787d8-4036-408b-999e-725f7645eacf/6e532074-b4d7-4393-9221-8fe643e100f2/2/1847940088

Technical Validation

Population

The 209 two-row spring barley population was selected from previously established datasets containing 647 genotypes[10,11,17-19]. To include a wide range of genetically representative individuals, we used available BOPA SNP data (as previously described)[50] and did a multi-dimensional scaling plot (Figure 1 a). Dimension 1 showed the progression from the oldest to the newest cultivars and explains 10.7% of the genetic variation. Genotypes were then chosen to be spread across year of registration as a cultivar to the UK market. Except for the first time-range which encompassed 130 years (1830-1959) of cultivar releases, all other ranges were split into decades and each time-range is represented by a similar number of genotypes (Figure 1 b). The final population was representative of breeding progress in cultivated barley for improved yield over time.

Pedigree data showed that modern barley germplasm is highly connected with most current genotypes' descendants of a small number of "founder" genotypes (Pedigree file: Supplemental File 1, Pedigree attributes: Supplemental File 2). These supplemental pedigree files can be used as input for the pedigree visualisation tool Helium

310 (https://helium.hutton.ac.uk/)[51]. Intermediate crosses were omitted from the file to be able
311 to display the pedigree and produce a tree which is both readable and navigable. Using the
312 pedigree data within Helium allows for further analyses. For example, eigenvector centrality
313 analysis can be used to measure the importance of a node in a network. The cultivar with
314 the highest influence on the pedigree tree was Kenia, followed by Maja and Gull. In total,
315 163 out of 209 (77.6%) of the cultivars used in our population can be traced back to those
316 three genotypes, confirming the importance of the founder genotypes for the overall
317 population.
318
319 Phenotyping
320 Field trials were done in 2019 and 2020 in three different locations: Minneapolis (Lat.
321 44.987, Long: -93.258; MN, USA), Dundee (Lat. 56.462, Long. -2.971; UK) and Gatersleben
322 (Lat. 51.823, Long. 11.287; Germany). In total 29 agronomic traits were scored associated
323 with development (earliness and growth habit traits), grain and height measurements. All
324 the phenotypic data can be viewed and studied in a Germinate database:
325 https://ics.hutton.ac.uk/germinate-barn/. Across years and sites, the results were consistent
326 except for a few traits. All earliness traits showed a faster development to awn tipping all the
327 way to peduncle senescence in Minnesota and slowest in Dundee, with for example the days
328 from sowing to senescence differing by more than 50 days. One obvious outlier in the
329 phenotypic scoring were the grain fertility measurements in Minnesota in 2019 where the
330 spikes got stuck in the flag leaf sheath due to high temperatures during the growing season
331 and did not emerge fully which led to a higher number of infertile florets. All phenotypic
332 information was combined into BLUPs except for 24 out of 165 phenotype datasets which
333 did not correlate with the rest (Supplemental Table 3 shows which phenotype values were
334 combined; Supplemental Figure 1 for distribution of BLUP values per phenotype). The
335 strongest positive correlation of the different phenotypes were the five different height
336 measurements to each other (Figure 3). Those were also strongly negative correlated with
337 year of registration. The earliness traits on the other hand showed a stronger positive
338 correlation with year of registration (awn tipping to heading; awn tipping to senescence). Of
339 the grain phenotypes, grain length and grain area were strongly correlated to each other and
340 to thousand kernel weight, but less so to grain width. Interestingly, grain length is positively
341 correlated with grain circularity, while grain width is negatively correlated with it.
342
343 Genotyping
344 To achieve the most extensive genotypic information for our population, variant calling from
345 RNA-seq data, whole genome shotgun data and previously established 50K SNP data was
346 combined (32,484,981 raw SNPs). For RNA-seq and WGS, the data was filtered to keep only
347 biallelic SNPs. We extracted the SNPs corresponding to the previous described BOPA markers
348 across all 1463 sequencing datasets (six tissue-specific RNA-seq datasets with 209 genotypes
349 each and one WGS dataset with 209 genotypes) for quality control. The Pearson correlation
350 coefficient for all genotypes between datasets was calculated. This identified mixed-up
351 samples where the genotype showed high correlation with a differently named sample and
352 therefore allowed for correction of the genotypic information. Samples with a high number
353 of heterogenous SNPs (above 10%) were removed as this pointed towards issues during
354 sample preparation. The filtering step reduced the number of genotypes per tissue. The final
355 numbers of genotypes per tissue varied between 191 to 199 (Supplemental Table 2 shows
356 which genotypes per tissue where retained). The merged SNP file was filtered to remove
357 highly heterozygous sites or those containing more than 20% missing data. The remaining
358 sites were imputed using haplotype imputation. SNPs were pruned by LD using Plink to
359 reduce the dataset size to the final 1,509,447 SNPs. SNP distribution along the 7
360 chromosomes showed overall low polymorphism in the centromeric region and higher
361 polymorphism towards the telomeric ends (Figure 2). For chromosome 1H, 2H and 7H almost

362 no polymorphism could be identified in the centromeric region, as previously observed by
363 Mascher *et al* [14].
364
365 Gene expression
366 RNA-seq data for six different tissues (crown, grain, inflorescence, peduncle, root, spikelet)
367 was mapped against the BaRTv2 transcriptome using Salmon[22]. The expression of all 39,434
368 genes in transcript per million (TPM) for each tissue were used as input to generate a
369 multidimensional scaling plot (MDS). The MDS shows all 209 genotypes cluster together by
370 tissue type (Figure 4). The tissue furthest separated by the first dimension from the rest was
371 the root tissue. The two tissues sampled from the spikelet at green anther stages (spikelet)
372 and developing grain at five days post anthesis (grain) show the highest overlap.
373
374 Data use-case scenarios
375 In the following three examples we show how the above datasets can be used.
376
377 In the first example the expression data has been used to filter for tissue-specific gene
378 expression. Tissue-specific genes showed that the root tissue was the most distinct with 776
379 genes identified as root specific (Figure 5). 572 genes were specifically expressed in grain,
380 437 in spikelet, 198 in inflorescence, 86 in peduncle and 64 in crown. Inflorescence and
381 peduncle shared the highest overlap of expressed genes with 927 genes and 13,215 genes
382 were expressed in all six tissues. While the MDS plot shows a high overlap of samples
383 between spikelet and grain in the first two dimensions, the third dimension divides those
384 tissues which fits with these two tissues showing the second and third highest tissue-specific
385 gene expression. Gene ontology for the peduncle resulted in no significant terms. The Gene
386 ontology results for all remaining five tissues are shown in Figure 6. Most terms were
387 identified for the root tissue showing terms one might expect in roots. Nitrate transport and
388 general transmembrane transport were identified in the Biological Process category;
389 apoplast, cell wall and extracellular region for the Cellular Compartment category. Different
390 transferase activities are highly significant in the Molecular Function category as well as
391 heme binding. The associated terms compare to those previously identified in maize[52].
392
393 In the second example, we studied the potential impact that genetic variation could have on
394 the gene activity or protein function by identifying premature stop codons or frameshift
395 mutations in a high confidence variant dataset. For the SNP dataset we started with 32
396 million SNPs, removed heterozygous SNPs and filtered for variants with less than 20%
397 missing data and a minor allele frequency of 2.5% which resulted in 4,012,229 SNPs[53]. Those
398 were used as input into SnpEff which identified 9,219,271 effects (as described by SnpEff:
399 http://pcingola.github.io/SnpEff/se_inputoutput/#eff-field-vcf-output-files) caused by those
400 4 million SNPs. 4% (368,650) of the effects were in exons, with 53.78% synonymous variants,
401 corresponding to 199,545 effects in 17,446 genes. 45.57% (169,105 effects) of the exon
402 effects were non-synonymous variants in 19,057 genes and 0.65% classified as nonsense.
403 The 0.65% corresponded to 2,425 transcripts and 1,105 genes with a premature stop codon
404 in the sequence. For the Indel identification only the RNA-seq variant files were considered
405 as those provided higher read depth for the genetic regions. They were also filtered by
406 removing heterozygous variants, keeping those with less than 20% missing data and a minor
407 allele frequency of 2.5%. A total of 50,865 variants remained which SnpEff predicted to cause
408 558,991 effects. 50.31% (281,228 effects) were upstream or downstream of the gene and
409 41.81% (233,706 effects) in the intronic region. After filtering for disruptive frame shifts
410 caused by insertions or deletions resulting in changes to the protein sequence, 1,912 genes
411 remained which we designated as potentially non-functional in some of the cultivars[53]. Such
412 structural variation can of course be explored in relation to gene expression. To illustrate,
413 within the tissue-specific gene expression data we highlighted two genes

(BaRT2v18chr5HG260690 and BaRT2v18chr2HG058650) with frameshift mutations in comparison to the Barke reference allele. Figure 7 shows the expression of those two genes. For BaRT2v18chr5HG260690 the deletion of four nucleotides is associated with lower gene expression. For BaRT2v18chr2HG058650 an A insertion is sociated with higher gene expression. The consequence of such variation still needs to be explored.

As a final example, we show a genome wide association study (GWAS) using the 1,509,447 SNP markers and the awn length phenotype. We used the Mixed Linear Model (MLM) in Gapit[47] to identify associations in the genome. Using a -log10(p) cut-off of 5 resulted in 6 significant peaks (Figure 8). The most significant SNP was found on chromosome 5H at position 441Mb. This peak had a very rare MAF of 3% and was located within 1kb of a previously identified gene involved in awn length, *HvDep1* (BaRT2v18chr5HG247460). The here identified allele corresponded to the previously described ari-eGP allele on chromosome 5H which is a one base pair "A" insertion in the *HvDep1* gene[54]. The insertion was confirmed for four cultivars in the population: Golden Promise, Tyne, Midas and Chad. Gene expression of *HvDep1* (Figure 8b) showed potentially lower expression in crown and inflorescence tissue but similar levels for the peduncle. This simple example highlights the fact that variants causing a loss of function do not necessarily induce strong changes in gene expression.

Usage Notes

To perform the analysis using the Snakemake[55] pipeline (see code availability) a high-performance computing (HPC) cluster is needed. For example, the Salmon indexing step in this setup needed 56Gb of memory using 16 cores, mapping of each individual sample needed 31Gb of memory using 8 cores. Downstream analyses like the genome wide association studies can be performed by downloading the BLUPs of the phenotypes and the marker file from Germinate.

Code Availability

The code for analysing the RNA-sequencing data from mapping to genome and transcriptome to variant calling was combined into a Snakemake[55] pipeline and is available on GitHub: https://github.com/SchreiberM/BARN.

Acknowledgements

The authors would like to acknowledge Richard Keith and Chris Warden for maintaining the JHI field trials, Nicola McCallum and Ruth Hamilton for the help with phenotypic scoring and John Fuller for the RNA-seq library preparation. We thank Micha Bayer, Runxuan Zhang, John Brown and Wenbin Guo for advice on the project design and bioinformatics. We would like to acknowledge Shane Heinen, Yadong Huang, Ismaél Pfeifer, and Letícia Pasqualino for the help in the lab and field work at the University of Minnesota. We acknowledge current and former members of the Genomics of Genetic Resources research group at IPK Gatersleben: Mary Ziems for planning and maintaining field trials, phenotypic scoring and tissue sampling; Mark Timothy Rabanus-Wallace, Hélène Pidon, Sudharsan Padmarasu, Mingjiu Li, Jayavardhan Reddy Kunam, Mohammed Rafaqat, Mohammad Awais, Jaqueline Pohl, Susanne König, Ines Walde, Manuela Knauft, Manuela Kretschmann and Beate Kamm for phenotypic scoring and tissue sampling. We thank Ines Walde and Susanne König at IPK for preparing sequencing libraries and generating sequencing data.

Thanks are also given to the Research/Scientific Computing teams at The James Hutton Institute and NIAB for providing computational resources and technical support for the "UK's

Crop Diversity Bioinformatics HPC" (BBSRC grant BB/S019669/1), use of which has contributed to the results reported. The work was supported by funding from the Rural and Environment Science and Analytical Services Division of the Scottish Government. The authors acknowledge the Minnesota Supercomputing Institute at the University of Minnesota.

The project received funding in frame of the ERA-CAPS Research Programme with funding (i) of the German partners through German Research Foundation (DFG) to NS under the project references MU 3589/1-1 | STE 1102/15-1 | WA 3336/4-1, (ii) the Scottish partner to RW through BBSRC (award number BB/S004610/1), (iii) the US partner to GJM through NSF (award number 1844331)

Author contributions

MS wrote the manuscript, conducted data collection and analysis. RWo conducted RNA-seq and WGS, data collection and analysis. AHa conducted RNA-seq data collection and analysis. MC did RNA-seq and data collection. JR provided seed material and did field trials. AHi conducted RNA sequencing, whole genome shotgun sequencing and primary data analysis. AF did data management. GJM, NS and RWa designed the experiment and supervised the work.

Competing interests

There is no conflict of interest.

Figure Legends

Figure 1: Selection of a two-row spring population. a) Multidimensional scaling plot of 647 European two-row spring cultivars. Genotype information came from 2,336 previously published BOPA markers. The 209 selected cultivars forming the population in this paper are shown as triangles. The year range represents the year each individual cultivar was registered. b) Distribution of the selected population of 209 cultivars (orange) as part of the total 647 European two-row spring cultivars (green) by year of registration.

Figure 2: SNP distribution and density of the final 1,509,447 SNPs in the genotypic marker file along the seven barley chromosomes in 1 Mb bins. The SNPs were identified from the 50k SNP array, RNA-sequencing and whole genome shotgun sequencing datasets and filtered to remove missing values and heterozygosity.

Figure 3: Pearson correlation coefficient between the 29 scored phenotypes and as 30th variable the year of registration. Phenotypic values were provided as best linear unbiased predictions for each phenotype for each of the 209 cultivars.

Figure 4: Gene expression of 209 cultivars across six tissues. Multidimensional scaling plot of all genes expressed in any of the six studied tissues: root, crown, peduncle, inflorescence, spikelet and grain.

Figure 5: An UpSet plot showing the overlap of the expressed genes for each of the tissues and tissue combinations.

Figure 6: Gene ontology enrichment for the tissue-specific genes a) root, b) grain, c) inflorescence, d) spikelet and e) crown. X-axis shows the percentage of genes associated with the GO term out of all genes in BaRTv2 associated with this term. Y-axis shows the significance as FDR adjusted -log(p-value) of the GO term. The area of the circle corresponds to the number of genes associated with the GO term.

Figure 7: Gene expression in TPM (transcripts per million) for two genes identified with changes in the protein sequence. a) BaRT2v18chr5HG260690 and b) BaRT2v18chr2HG058650 split by haplotype on the x-axis. The first haplotype always represents the reference allele from the genotype Barke, and the second allele represents the alternative.

Figure 8: Genome wide association of awn length. a) GWAS result of awn length showing a high significant association on chromosome 5H. b) The gene expression in TPM of the candidate gene *HvDep1* (BaRT2v18chr5HG247460). The first haplotype on the x-axis represents the reference allele from the genotype Barke, and the second allele represents the alternative.

Supplemental Figure 1: Violin and boxplots of BLUP values for the phenotypic variation in all 29 scored traits.

References

1 FAO.FAOstat. *License: CC BY-NC-SA 3.0 IGO. Extracted from: https://www.fao.org/faostat/. Data of Access: 16-12-2022*, 2013).

2 O'Connor, A. Brewing and distilling in Scotland–economic facts and figures. *Scottish Parliament Information Centre* (2018).

3 Dawson, I. K. *et al.* Barley: a translational model for adaptation to climate change. *New Phytol* **206**, 913-931, doi:10.1111/nph.13266 (2015).

4 Fischbeck, G. in *Developments in Plant Genetics and Breeding* Vol. 7 (eds Roland von Bothmer, Theo van Hintum, Helmut Knüpffer, & Kazuhiro Sato) 29-52 (Elsevier, 2003).

5 Ortiz, R., Nurminiemi, M., Madsen, S., Rognli, O. A. & Bjørnstad, Å. Genetic gains in Nordic spring barley breeding over sixty years. *Euphytica* **126**, 283–289, doi:10.1023/A:1016302626527 (2002).

6 Schuster, W. H. Welchen Beitrag leistet die Pflanzenzüchtung zur Leistungssteigerung von Kulturpflanzenarten? *Pflanzenbauwissenschaften* **1**, 9-18 (1997).

7 Gustafsson, A., Hagberg, A., Persson, G. & Wiklund, K. Induced mutations and barley improvement. *Theor Appl Genet* **41**, 239-248, doi:10.1007/BF00277792 (1971).

8 Dockter, C. *et al.* Induced Variations in Brassinosteroid Genes Define Barley Height and Sturdiness, and Expand the Green Revolution Genetic Toolkit. *Plant Physiology* **166**, 1912-1927, doi:10.1104/pp.114.250738 (2014).

9 Mammadov, J., Aggarwal, R., Buyyarapu, R. & Kumpatla, S. SNP Markers and Their Impact on Plant Breeding. *International Journal of Plant Genomics* **2012**, 728398, doi:10.1155/2012/728398 (2012).

10 Cockram, J. *et al.* Genome-wide association mapping to candidate polymorphism resolution in the unsequenced barley genome. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 21611-21616 (2010).

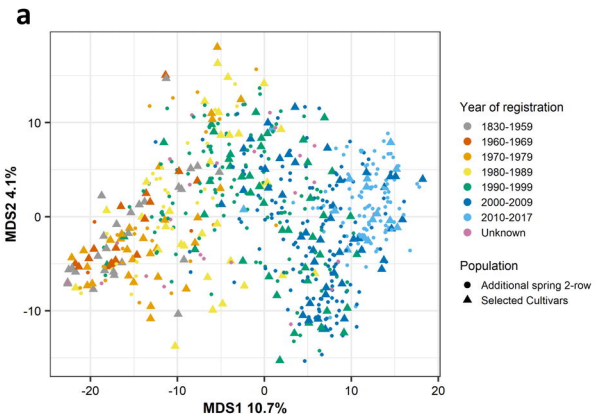
11 Comadran, J. *et al.* Natural variation in a homolog of *Antirrhinum CENTRORADIALIS* contributed to spring growth habit and environmental adaptation in cultivated barley. *Nature Genetics* **44**, 1388-1392, doi:10.1038/ng.2447 (2012).

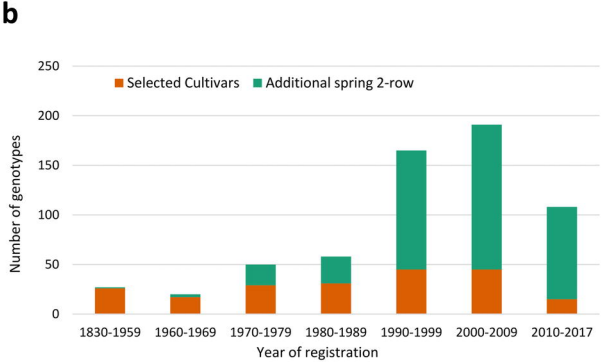
12 Matros, A. *et al.* Genome-wide association study reveals the genetic complexity of fructan accumulation patterns in barley grain. *J Exp Bot* **72**, 2383-2402, doi:10.1093/jxb/erab002 (2021).

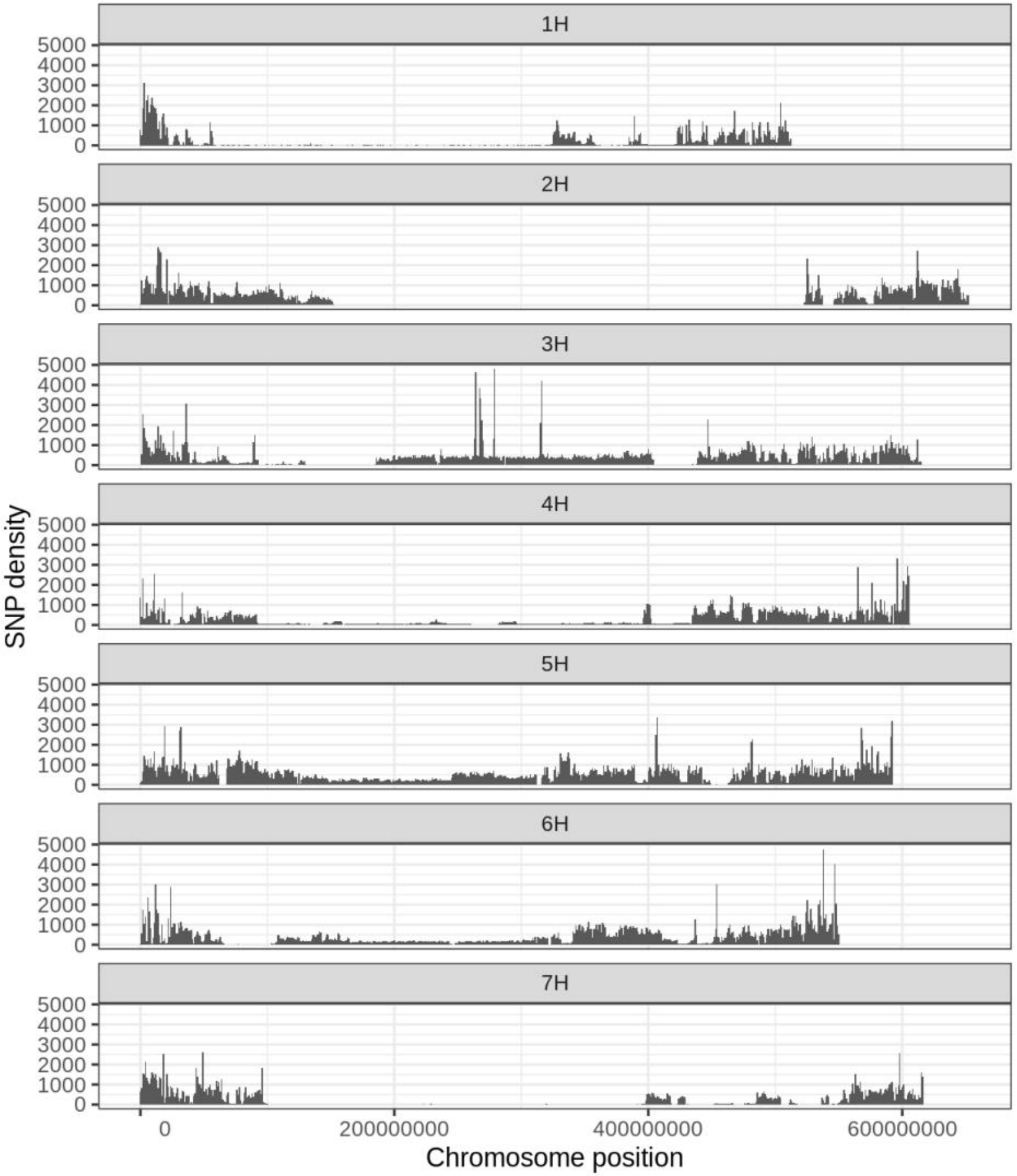
13 Ramsay, L. *et al.* INTERMEDIUM-C, a modifier of lateral spikelet fertility in barley, is an ortholog of the maize domestication gene TEOSINTE BRANCHED 1. *Nature Genetics* **43**, 169, doi:10.1038/ng.745 (2011).
14 Mascher, M. *et al.* A chromosome conformation capture ordered sequence of the barley genome. *Nature* **544**, 427-433, doi:10.1038/nature22043 (2017).
15 Jayakodi, M. *et al.* The barley pan-genome reveals the hidden legacy of mutation breeding. *Nature* **588**, 284-289, doi:10.1038/s41586-020-2947-8 (2020).
16 Bayer, M. M. *et al.* Development and Evaluation of a Barley 50k iSelect SNP Array. *Front Plant Sci* **8**, 1792, doi:10.3389/fpls.2017.01792 (2017).
17 Tondelli, A. *et al.* Structural and Temporal Variation in Genetic Diversity of European Spring Two-Row Barley Cultivars and Association Mapping of Quantitative Traits. *The Plant Genome* **6**, plantgenome2013.2003.0007, doi:10.3835/plantgenome2013.03.0007 (2013).
18 Looseley, M. E. *et al.* Association mapping of malting quality traits in UK spring and winter barley cultivar collections. *Theor Appl Genet* **133**, 2567-2582, doi:10.1007/s00122-020-03618-9 (2020).
19 Thomas, W. *et al.* HGCA Project Report 528: Association genetics of UK elite barley (AGOUEB). (2014).
20 Russell, J. R. *et al.* A retrospective analysis of spring barley germplasm development from `foundation genotypes' to currently successful cultivars. *Molecular Breeding* **6**, 553-568, doi:10.1023/A:1011372312962 (2000).
21 Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114-2120, doi:10.1093/bioinformatics/btu170 (2014).
22 Patro, R., Duggal, G., Love, M. I., Irizarry, R. A. & Kingsford, C. Salmon provides fast and bias-aware quantification of transcript expression. *Nature methods* **14**, 417-419, doi:10.1038/nmeth.4197 (2017).
23 Coulter, M. *et al.* BaRTv2: a highly resolved barley reference transcriptome for accurate transcript-specific RNA-seq quantification. *Plant J*, doi:10.1111/tpj.15871 (2022).
24 Srivastava, A. *et al.* Alignment and mapping methodology influence transcript abundance estimation. *Genome Biol* **21**, 239, doi:10.1186/s13059-020-02151-8 (2020).
25 R Core Team. (R Foundation for Statistical Computing, 2021).
26 Soneson, C., Love, M. & Robinson, M. Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences [version 2; referees: 2 approved]. *F1000Research* **4**, doi:10.12688/f1000research.7563.2 (2016).
27 Robinson, M. D., McCarthy, D. J. & Smyth, G. K. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26**, 139-140, doi:10.1093/bioinformatics/btp616 (2010).
28 Conway, J. R., Lex, A. & Gehlenborg, N. UpSetR: an R package for the visualization of intersecting sets and their properties. *Bioinformatics* **33**, 2938-2940, doi:10.1093/bioinformatics/btx364 (2017).
29 Gu, Z. Complex heatmap visualization. *iMeta* **1**, e43, doi:https://doi.org/10.1002/imt2.43 (2022).
30 Schreiber, M., Orr, J., Barakate, A. & Waugh, R. Barley (Hordeum Vulgare) Anther and Meiocyte RNA Sequencing: Mapping Sequencing Reads and Downstream Data Analyses. *Methods Mol Biol* **2484**, 291-311, doi:10.1007/978-1-0716-2253-7_20 (2022).
31 Dobin, A. *et al.* STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **29**, 15-21, doi:10.1093/bioinformatics/bts635 (2013).

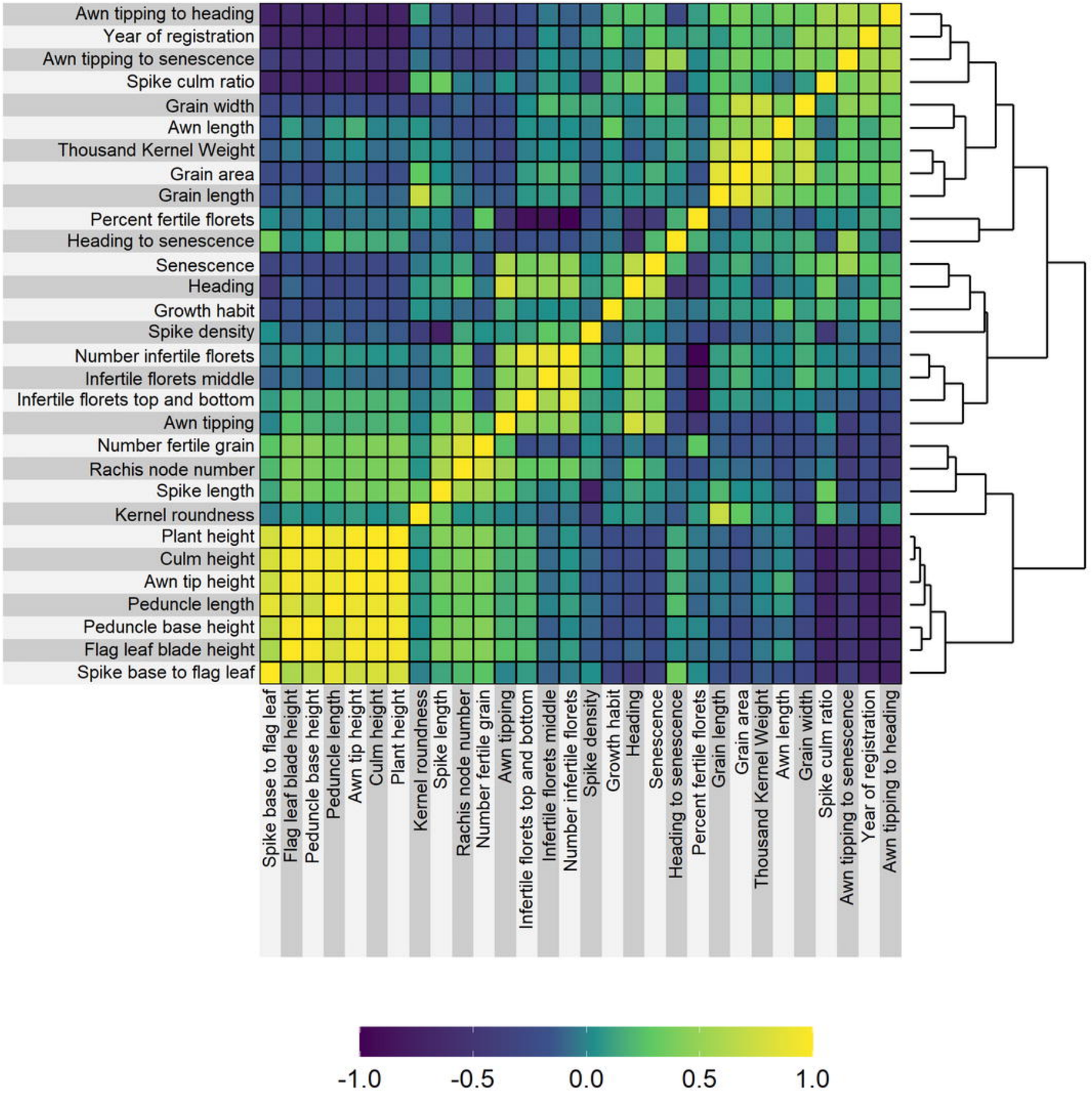
617 32 Broad Institute. Picard tools. *Broad Institute, GitHub repository* **Version 2.18.4**,
618 http://broadinstitute.github.io/picard/ (2018).
619 33 Barnett, D. W., Garrison, E. K., Quinlan, A. R., Stromberg, M. P. & Marth, G. T.
620 BamTools: a C++ API and toolkit for analyzing and managing BAM files.
621 *Bioinformatics* **27**, 1691-1692, doi:10.1093/bioinformatics/btr174 (2011).
622 34 Garrison, E. M. G. Haplotype-based variant detection from short-read sequencing.
623 *ArXiv e-prints*, 9 (2012).
624 35 Milner, S. G. *et al.* Genebank genomics highlights the diversity of a global barley
625 collection. *Nat Genet* **51**, 319-326, doi:10.1038/s41588-018-0266-x (2019).
626 36 Martin, M. Cutadapt removes adapter sequences from high-throughput sequencing
627 reads. *2011* **17**, 3, doi:10.14806/ej.17.1.200 (2011).
628 37 Li, H. Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics* **34**,
629 3094-3100, doi:10.1093/bioinformatics/bty191 (2018).
630 38 Novocraft Technologies Sdn Bhd. Novosort. **Version 3.06.05**,
631 https://www.novocraft.com/products/novosort/.
632 39 Li, H. *et al.* The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**,
633 2078-2079, doi:10.1093/bioinformatics/btp352 (2009).
634 40 Danecek, P. *et al.* Twelve years of SAMtools and BCFtools. *Gigascience* **10**,
635 doi:10.1093/gigascience/giab008 (2021).
636 41 Bradbury, P. J. *et al.* TASSEL: software for association mapping of complex traits in
637 diverse samples. *Bioinformatics* **23**, 2633-2635, doi:DOI
638 10.1093/bioinformatics/btm308 (2007).
639 42 Swarts, K. *et al.* Novel Methods to Optimize Genotypic Imputation for Low-Coverage,
640 Next-Generation Sequence Data in Crop Plants. *The Plant Genome* **7**,
641 plantgenome2014.2005.0023, doi:10.3835/plantgenome2014.05.0023 (2014).
642 43 Purcell, S. *et al.* PLINK: a tool set for whole-genome association and population-
643 based linkage analyses. *Am J Hum Genet* **81**, 559-575, doi:10.1086/519795 (2007).
644 44 Cingolani, P. *et al.* A program for annotating and predicting the effects of single
645 nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster
646 strain w1118; iso-2; iso-3. *Fly* **6**, 80-92, doi:10.4161/fly.19695 (2012).
647 45 Alvarado, G. *et al.* META-R: A software to analyze data from multi-environment plant
648 breeding trials. *The Crop Journal* **8**, 745-756, doi:10.1016/j.cj.2020.03.010 (2020).
649 46 Yu, J. *et al.* A unified mixed-model method for association mapping that accounts for
650 multiple levels of relatedness. *Nature Genetics* **38**, 203-208, doi:10.1038/ng1702
651 (2006).
652 47 Lipka, A. E. *et al.* GAPIT: genome association and prediction integrated tool.
653 *Bioinformatics* **28**, 2397-2399, doi:10.1093/bioinformatics/bts444 (2012).
654 48 Raubach, S. *et al.* From bits to bites: Advancement of the Germinate platform to
655 support prebreeding informatics for crop wild relatives. *Crop Science* **61**, 1538-1566,
656 doi:https://doi.org/10.1002/csc2.20248 (2021).
657 49 Arend, D. *et al.* e!DAL--a framework to store, share and publish research data. *BMC*
658 *Bioinformatics* **15**, 214, doi:10.1186/1471-2105-15-214 (2014).
659 50 Close, T. J. *et al.* Development and implementation of high-throughput SNP
660 genotyping in barley. *BMC Genomics* **10**, 582, doi:10.1186/1471-2164-10-582 (2009).
661 51 Shaw, P. D., Graham, M., Kennedy, J., Milne, I. & Marshall, D. F. Helium: visualization
662 of large scale plant pedigrees. *BMC Bioinformatics* **15**, 259, doi:10.1186/1471-2105-
663 15-259 (2014).
664 52 Li, Y. *et al.* Genome-scale mining of root-preferential genes from maize and
665 characterization of their promoter activity. *BMC Plant Biol* **19**, 584,
666 doi:10.1186/s12870-019-2198-8 (2019).
667 53 Schreiber, M. *et al.* Data record for the genomic resources of cultivated European
668 two-rowed spring barley genotypes. doi:https://doi.ipk-

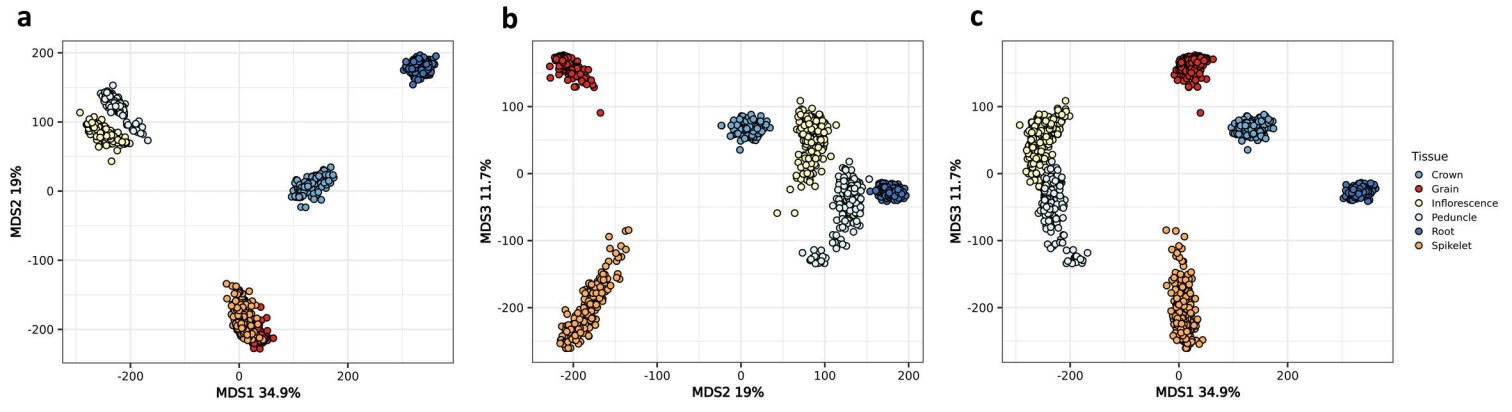
669 gatersleben.de/DOI/815787d8-4036-408b-999e-725f7645eacf/6e532074-b4d7-
670 4393-9221-8fe643e100f2/2/1847940088 (2023).
671 54 Wendt, T. *et al.* HvDep1 Is a Positive Regulator of Culm Elongation and Grain Size in
672 Barley and Impacts Yield in an Environment-Dependent Manner. *PLoS One* **11**,
673 e0168924, doi:10.1371/journal.pone.0168924 (2016).
674 55 Molder, F. *et al.* Sustainable data analysis with Snakemake. *F1000Res* **10**, 33,
675 doi:10.12688/f1000research.29032.2 (2021).
676

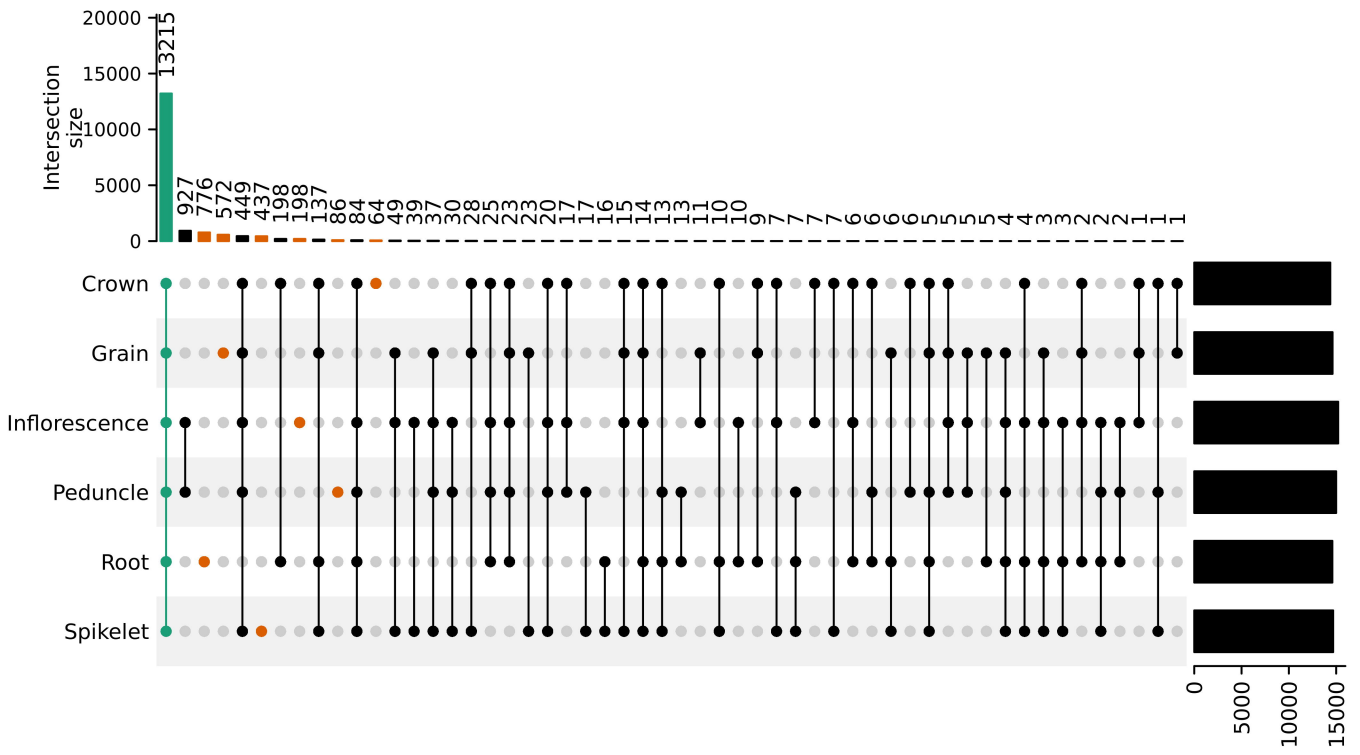

a
MDS2 4.1%
MDS1 10.7%
10
0
-10
-20
-10
0
10
20
Year of registration
1830-1959
1960-1969
1970-1979
1980-1989
1990-1999
2000-2009
2010-2017
Unknown
Population
Additional spring 2-row
Selected Cultivars

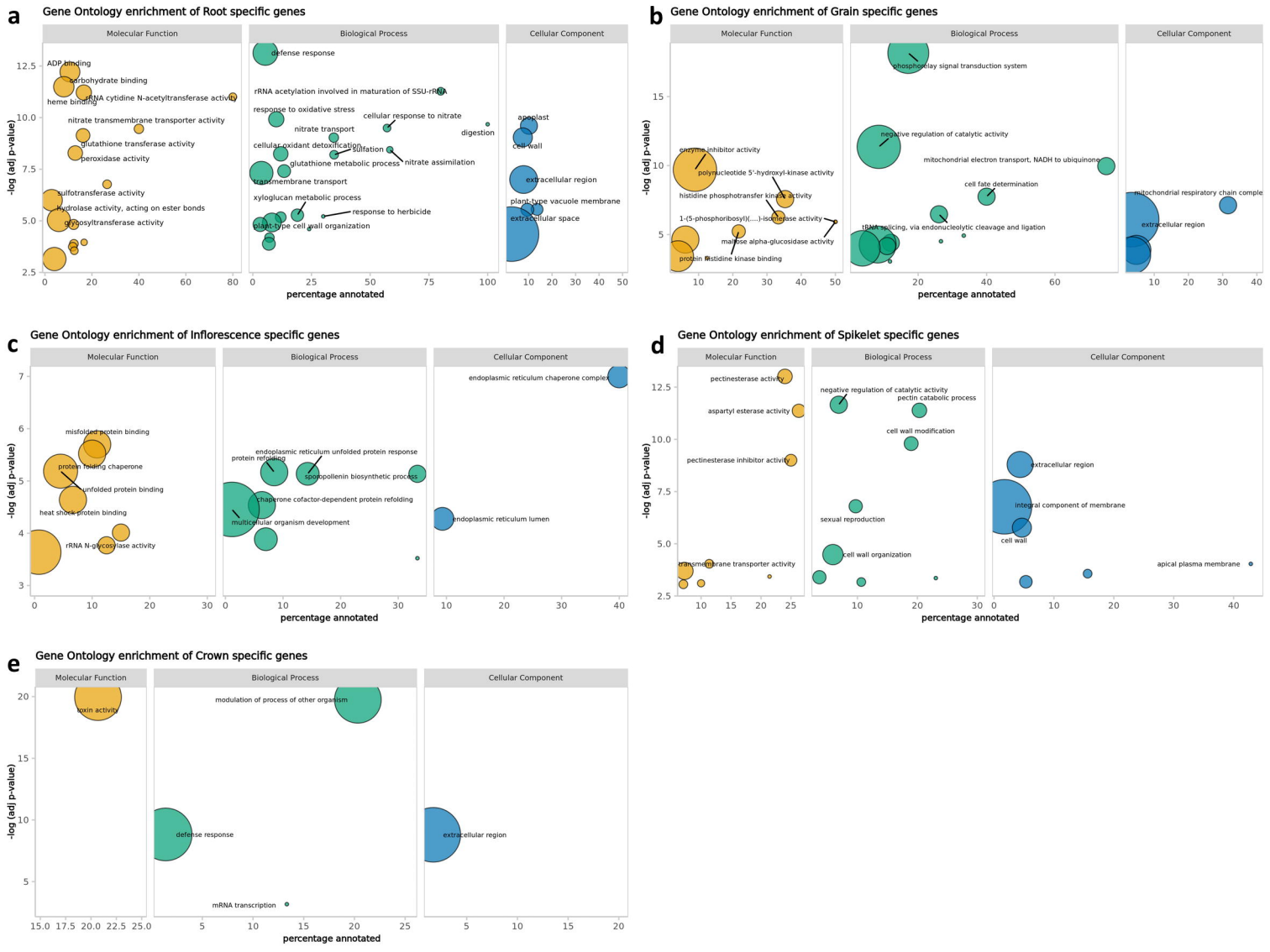

b
Selected Cultivars
Additional spring 2-row
Number of genotypes
250
200
150
100
50
0
1830-1959
1960-1969
1970-1979
1980-1989
1990-1999
2000-2009
2010-2017
Year of registration

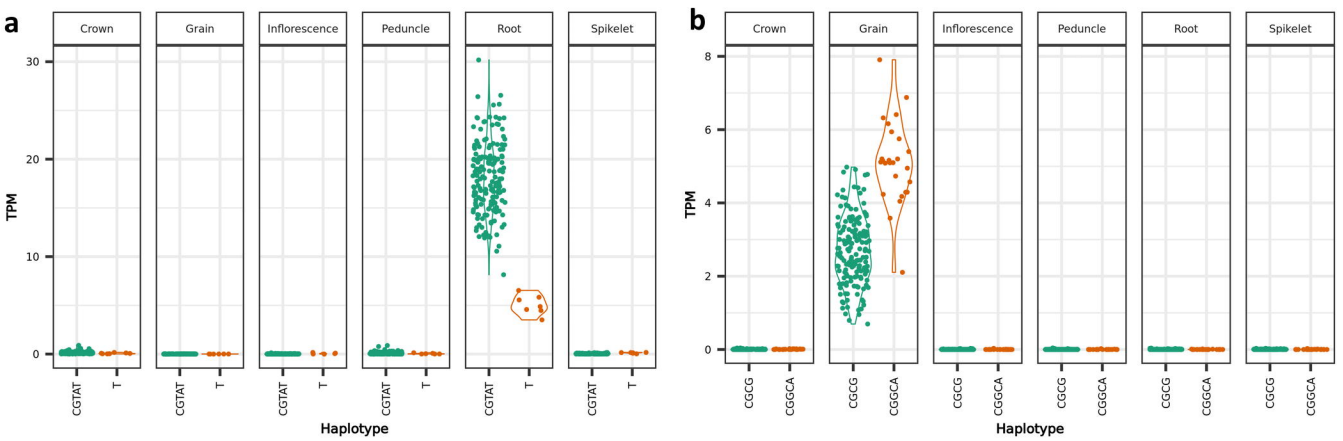

1H
2H
3H
4H
5H
6H
7H
5000
4000
3000
2000
1000
0
SNP density
0
200000000
400000000
600000000
Chromosome position

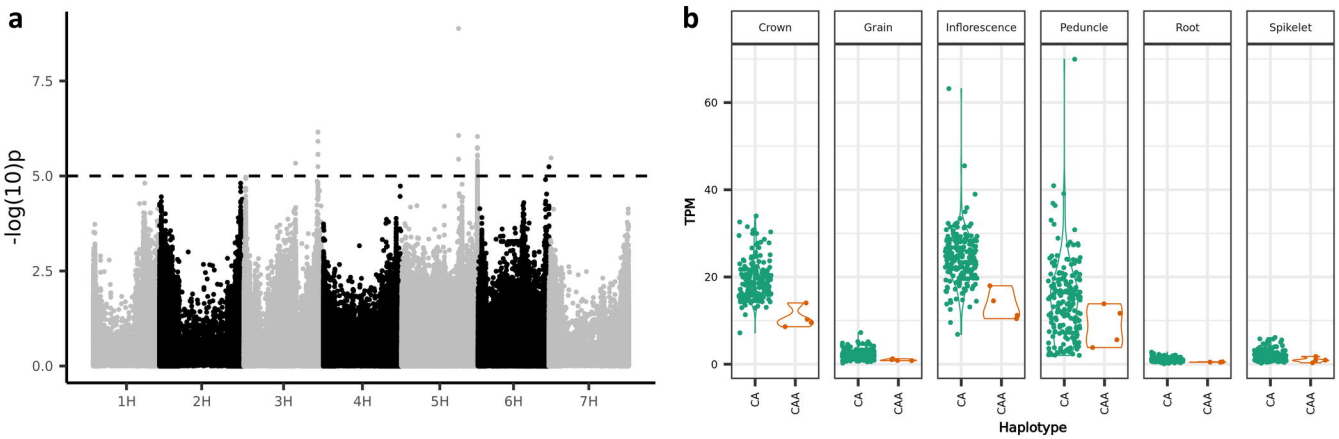

Awn tipping to heading
Year of registration
Awn tipping to senescence
Spike culm ratio
Grain width
Awn length
Thousand Kernel Weight
Grain area
Grain length
Percent fertile florets
Heading to senescence
Senescence
Heading
Growth habit
Spike density
Number infertile florets
Infertile florets middle
Infertile florets top and bottom
Awn tipping
Number fertile grain
Rachis node number
Spike length
Kernel roundness
Plant height
Culm height
Awn tip height
Peduncle length
Peduncle base height
Flag leaf blade height
Spike base to flag leaf
-1.0
-0.5
0.0
0.5
1.0


a
b
c
MDS2 19%
MDS1 34.9%
MDS3 11.7%
MDS2 19%
MDS3 11.7%
MDS1 34.9%
Tissue
Crown
Grain
Inflorescence
Peduncle
Root
Spikelet


Intersection size
20000
15000
10000
5000
0
13215
927
776
572
449
437
198
198
137
86
84
64
49
39
37
37
30
28
25
23
23
20
17
17
16
15
14
13
13
11
10
10
9
7
7
7
7
6
6
6
6
5
5
5
5
4
4
3
3
2
2
1
1
1
Crown
Grain
Inflorescence
Peduncle
Root
Spikelet
0
5000
10000
15000


a
Gene Ontology enrichment of Root specific genes
Molecular Function
Biological Process
Cellular Component
ADP binding
carbohydrate binding
heme binding
tRNA cytidine N-acetyltransferase activity
nitrate transmembrane transporter activity
glutathione transferase activity
peroxidase activity
sulfotransferase activity
hydrolase activity, acting on ester bonds
glycosyltransferase activity
defense response
rRNA acetylation involved in maturation of SSU-rRNA
response to oxidative stress
cellular response to nitrate
nitrate transport
digestion
cellular oxidant detoxification
sulfation
glutathione metabolic process
nitrate assimilation
transmembrane transport
xyloglucan metabolic process
response to herbicide
plant-type cell wall organization
apoplast
cell wall
extracellular region
plant-type vacuole membrane
extracellular space
-log (adj p-value)
percentage annotated
b
Gene Ontology enrichment of Grain specific genes
Molecular Function
Biological Process
Cellular Component
phosphorelay signal transduction system
enzyme inhibitor activity
negative regulation of catalytic activity
mitochondrial electron transport, NADH to ubiquinone
polynucleotide 5'-hydroxyl-kinase activity
cell fate determination
histidine phosphotransfer kinase activity
1-(5-phosphoribosyl)(...)-isomerase activity
tRNA splicing, via endonucleolytic cleavage and ligation
maltose alpha-glucosidase activity
protein histidine kinase binding
mitochondrial respiratory chain comple
extracellular region
-log (adj p-value)
percentage annotated
c
Gene Ontology enrichment of Inflorescence specific genes
Molecular Function
Biological Process
Cellular Component
endoplasmic reticulum chaperone complex
misfolded protein binding
endoplasmic reticulum unfolded protein response
protein refolding
protein folding chaperone
sporopollenin biosynthetic process
unfolded protein binding
chaperone cofactor-dependent protein refolding
heat shock protein binding
multicellular organism development
endoplasmic reticulum lumen
rRNA N-glycosylase activity
-log (adj p-value)
percentage annotated
d
Gene Ontology enrichment of Spikelet specific genes
Molecular Function
Biological Process
Cellular Component
pectinesterase activity
negative regulation of catalytic activity
pectin catabolic process
aspartyl esterase activity
cell wall modification
pectinesterase inhibitor activity
extracellular region
integral component of membrane
sexual reproduction
cell wall
cell wall organization
transmembrane transporter activity
apical plasma membrane
-log (adj p-value)
percentage annotated
e
Gene Ontology enrichment of Crown specific genes
Molecular Function
Biological Process
Cellular Component
toxin activity
modulation of process of other organism
defense response
extracellular region
mRNA transcription
-log (adj p-value)
percentage annotated


a
Crown
Grain
Inflorescence
Peduncle
Root
Spikelet
TPM
30
20
10
0
CGTAT
T
Haplotype
b
Crown
Grain
Inflorescence
Peduncle
Root
Spikelet
TPM
8
6
4
2
0
CGCG
CGGCA
Haplotype


a
-log(10)p
7.5
5.0
2.5
0.0
1H
2H
3H
4H
5H
6H
7H
b
Crown
Grain
Inflorescence
Peduncle
Root
Spikelet
TPM
60
40
20
0
CA
CAA
Haplotype