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High-density genetic mapping based on whole-genome resequencing reveals a novel major locus for leaf-related traits in barley

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Abstract

Barley leaf morphology is a key determinant of photosynthetic efficiency and yield, but its genetic architecture requires further elucidation. In this study, a high-density genetic linkage map was constructed using a recombinant inbred line population of 172 lines from a cross between 'Yongjiabaidamai' (YJ) and 'Hua30' (H30). Whole-genome resequencing yielded 3,864,882 high-quality SNPs, which were condensed into 12,905 bin markers to generate a map spanning 1,086.46 cM with an average marker interval of 0.08 cM. This map showed high collinearity with the barley reference genome. Phenotypic data for nine leaf traits were evaluated across five environments. Quantitative trait locus analysis identified 74 QTLs. Among these, a major, stable cluster on chromosome 5H (527.49–528.08 Mbp) contained multiple trait-specific QTLs (*QFll.hzau-5H.2*, *QFlw.hzau-5H.2*, *QFla.hzau-5H.2*, *QSll.hzau-5H.2*, *QSlw.hzau-5H.2*, *QSla.hzau-5H.3*, *QTll.hzau-5H.2*, *QTlw.hzau-5H.2*, and *QTla.hzau-5H.2*), all of which exhibited significant and stable effects on the nine leaf traits. This interval harbored the vernalization gene *HvVRN1*. Sequence comparison between the two parents revealed a large length difference in the first intron of *HvVRN1*, which is a known regulatory region, suggesting that this structural variation may be associated with leaf size variation. Further analysis showed that this locus also affected plant height, spikelet number per spike, and heading date, but not spike length, spike density, thousand-grain weight, tiller number, or tiller angle, indicating pleiotropic effects of *HvVRN1* on both leaf morphology and plant architecture.

1. Introduction

Barley (*Hordeum vulgare* L.) is an important grain widely used in livestock feed, beer and various health foods (Newton et al., 2011; Collins et al., 2010). Leaf size is the main organ that affects plant photosynthesis, and it also significantly affects important agronomic traits such as yield (Vile et al., 2005; White et al., 2015). In cereals, the top three leaves absorb the most light energy and are the main source of carbohydrates during the accumulation process (Li et al., 1998). Leaves provide most of the photoassimilates for grain development and growth, mainly related to leaf size and shape (Foyer, 1987; Gladun and Karpov, 1993). Leaf size was estimated by length, width, and area, and was positively correlated with thousand-grain weight, panicle weight, and other yield-related traits in cereals (Cui et al., 2003; Wang et al., 2011, 2012). Optimizing plant leaf size and shape is a major goal in barley breeding (Richards et al., 2000).

Construction of high-density genetic linkage maps and quantitative trait loci (QTL) mapping have emerged as powerful tools for identifying genomic regions associated with key agronomic traits in barley (Romagosa et al., 1999; Ren et al., 2016; Zhang et al., 2017; Fang et al., 2020). The precision of QTL mapping was highly dependent on the density and quality of the genetic map used (Doerge and Rebai., 1996). Traditional molecular markers, including restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs), diversity array technology (DArT), and restriction site associated DNA (RAD), have been used to construct genetic linkage maps in barley (Wenzl et al., 2006; Hearnden et al., 2007; Marcel et al., 2007; Varshney et al., 2007; Szűcs et al., 2009; Ren et al., 2016). While these maps have provided valuable insights, their relatively low marker density has limited the precision of QTL mapping.

With the advancement of high-throughput sequencing technology, modern molecular markers derived from approaches such as whole-genome resequencing (WGR) of parental populations and single nucleotide polymorphism (SNP) discovery have been widely adopted for constructing high-density genetic maps across various species (Song et al., 2015; Cambiaso et al., 2019; Chen et al., 2023). In barley, several high-throughput sequencing-based mapping approaches, such as genotyping-by-sequencing (GBS), POPSEQ, whole-exome capture, and mapping-by-sequencing, have been developed and widely applied (Mascher et al., 2013a, b, c, 2014; Beier et al., 2017). These high-density maps served as crucial tools for investigating genome diversity and identifying key genes associated with agronomically important traits. The utilization of high-density, SNP-based genetic maps significantly enhanced the precision of gene fine-mapping (Huang et al., 2009; Xie et al., 2010). Furthermore, high-density mapping allowed for the resolution of closely linked QTLs into separate loci (Stange et al., 2013). Compared to low-density RFLP and SSR maps, high-density maps demonstrated

superior detection capacity and resolution for QTL mapping, particularly for QTLs with minor genetic effects (Yu et al., 2011). Consequently, the identification of a large number of SNP markers based on WGR proved conducive to the construction of whole-genome genetic maps, the mining of candidate genes for important traits, and the development of molecular markers closely linked to target genes.

Leaf development is regulated by multiple genes and is significantly influenced by the environment. Relevant studies were extensively conducted in barley (Yin et al., 1999; Elberse et al., 2004; Gyenis et al., 2007; Xue et al., 2008; Liu et al., 2015; Du et al., 2019; Ye et al., 2024). Early studies identified multiple QTLs associated with leaf size, including regions on chromosomes 5H and 7H that influenced leaf length and width (Xue et al., 2008). Two polygenic genomic regions influencing flag leaf morphology were identified on 2H and 7H (Liu et al., 2015). Du et al. (2019) measured the length, width, and area of the flag leaf and the second, third, and fourth leaves in barley, mapping a total of 57 QTLs to chromosomes 2H, 3H, 4H, and 7H. Furthermore, previous studies located QTLs related to flag leaf length on chromosomes 1H to 6H. Among these, a major QTL for leaf width was identified on chromosome 2H (Niu et al., 2022). Recently, Ye et al. (2024) identified stable QTLs for flag leaf traits on chromosomes 2H, 3H, and 5H. In addition, the *Blf1* gene on chromosome 5HL was cloned and shown to control leaf width by restricting lateral proliferation (Jöst et al., 2016). Collectively, these studies established a foundational genetic framework for barley leaf architecture. However, the resolution of many preceding analyses was constrained by the use of genetic maps with limited marker density, which might have hindered the precise identification of candidate genes.

High-density genetic mapping significantly enhances the resolution for dissecting complex agronomic traits. In this study, we performed whole-genome resequencing on a population of 172 recombinant inbred lines derived from a cross between ‘Yongjiabaidamai’ and ‘Hua30’ to construct a high-density genetic map. Using this map, we conducted QTL analysis for nine leaf-related traits across multiple environments to identify stable loci and candidate genes, thereby providing genomic resources for marker-assisted selection in barley breeding.

2. Methods

2.1 Plant materials and field trials

The QTL mapping population in this study consisted of a recombinant inbred line (RIL) population derived from a cross between ‘Yongjiabaidamai’ (YJ) and ‘Hua30’ (H30). The population included 172 lines at the F_{12} generation. The parental lines and the RIL population were cultivated at two locations: the Experimental Farm of Huazhong Agricultural University (30°48' N, 114°36' E) and the Xiangyang Experimental Farm (32°13' N, 112°36' E). Plantings were conducted during the 2021–2022, 2022–2023, and 2023–2024 growing seasons at the former location (2022WH, 2023WH, and 2024WH), and during the 2022–2023 and 2023–2024 seasons at the latter (2023XY, and 2024XY). A randomized complete block design was adopted, with each line planted in 2 rows (1.5 meters long, 20 cm row spacing) for three replicates, at a seeding density of 35 seeds per row, to identify agronomic traits between the parental lines and 172 lines. Crop management followed local farming practices. No severe damage from diseases, pests, or lodging was observed during the growing seasons.

2.2 Phenotype evaluation and statistical analysis

Ten days after the barley plants' heading, five main stem plants with consistent growth were randomly selected from each line of each repetition, and six morphological traits of the leaves on the main stems were measured, including flag leaf length (FLL, cm) and width (FLW, cm), second leaf length (SLL, cm) and width (SLW, cm), third leaf length (TLL, cm) and width (TLW, cm). The leaf areas of the flag leaf (FLA, cm²), the second leaf (SLA, cm²), and the third leaf (TLA, cm²) were calculated by FLL*FLW*0.75 (Rybiński and Garczyński 2004).

In this study, SPSS 25.0 (SPSS, Chicago, IL, USA; https://www.ibm.com/products/spss-statistics) was used to calculate the maximum, minimum, average, standard deviation, and coefficient of variation of the measured traits of each strain of the RIL population, and analysis of variance was conducted on the leaf-related trait data of the parents. The normality test (Shapiro-Wilk) of the trait distribution of the RIL population was conducted using OriginPro 2024 (https://www.originlab.com), and the frequency distribution histogram was plotted. The correlations among leaf traits of the barley RIL population were analyzed using R language, and the broad-sense heritability (*H2*) of each trait in various environments was calculated. The best linear unbiased prediction (BLUP) value was calculated in the R package "lme4".

2.3 DNA Extraction and genome sequencing

A total of 172 barley lines and their parental lines were selected at the three-leaf stage. From each line, five leaves were collected, pooled, and ground. DNA extraction was performed using the Plant Genome DNA Extraction Kit (DP305). Whole-genome resequencing of the 172 lines and the parental lines was performed based on next-generation sequencing technology. Sequencing libraries were first prepared following the protocol of the TruSeq Nano DNA HT Sample Preparation Kit (Illumina). Paired-end sequencing of the resulting libraries was conducted on an Illumina HiSeq 2500 platform with an insert size of 150 bp. The average sequencing depth was approximately 14.1× for YJ, 12.0× for H30, and 6.4× for the 172 RILs. The average raw data output per sample was approximately 71.80 Gb for YJ, 61.24 Gb for H30, and 32.50 Gb for each RIL.

2.4 Reads mapping and variants calling

The raw sequencing data were subjected to quality control using Trimmomatic (version 0.39) software (Bolger et al., 2014). The raw reads were filtered to remove low-quality reads, reads with an N-content ratio exceeding 10%, and reads containing adapters, thereby obtaining clean, effective, and high-quality reads. The clean reads were aligned with the barley reference genome MorexV3 (https://plants.ensembl.org/Hordeum_vulgare/Info/Index) using BWA software (Bailey et al., 2009), and SAM files were generated. The resulting SAM files were compressed into BAM files using Samtools (version 1.9) (Li et al., 2009), and multiple BAM files were merged. The MarkDuplicates module in GATK (version 4.1.9.0) (McKenna et al., 2010) was used to remove duplicate sequences, construct the index file of BAM, and call SNPs and InDels. Using VCFTools (version 0.1.16) (Danecek et al., 2011), the parameters "--remove-indels --max-missing 0.90 --minQ 30 --minDP 3" were applied to eliminate insertions, deletions, and low-quality single nucleotide polymorphisms for subsequent analysis.

2.5 SNP screening and Bin marker division

The obtained SNP markers were filtered according to the following criteria: (a) markers exhibiting inconsistent homozygosity in the parents were discarded; (b) markers with a read depth of less than 5× in the parents or less than 3× in the 172 lines were removed. The 172 lines were genotyped based on the parental genotypes, and only markers located on the chromosomes were retained.

For bin marker division and screening, a sliding window approach was employed. A window size of 15 SNPs and a step size of 1 SNP were used to scan each chromosome. Within each window, the genotype was classified as ‘aa’ if the number of ‘aa’ SNP genotypes exceeded 11 compared to ‘bb’; as ‘bb’ if the number was less than 11; and as ‘ab’ in all other cases. Genotype imputation and correction were subsequently performed. The resulting bin markers were assigned based on the recombination patterns observed across the 172 lines, and the data for each sample were organized according to the physical position along the chromosomes.

2.6 Genetic map construction and collinearity analysis

The Bin markers were used as markers for the genetic linkage map, and the Bin markers were grouped and sorted using the MAP function of IciMapping4.2 (Meng et al., 2015) to construct the genetic linkage map. The R package "LinkageMapView" (https://github.com/louellette/LinkageMapView) was used to visualize the genetic map.

The genetic locations of all Bin markers after constructing the genetic map and the corresponding barley reference genome locations were analyzed to evaluate their genetic linkage collinearity with the reference genome. Collinearity analysis and visualization processing were conducted using the R packages "ggplot2" and "dplyr".

2.7 QTL analysis

QTL analysis of agronomic traits was performed using the inclusive composite interval mapping (ICIM) function in QTL IciMapping 4.2. The ICIM-ADD method within the “BIP (QTL mapping in biparental populations)” module was selected for whole-genome QTL scanning. The scan parameters were set as follows: probability in stepwise regression (PIN) = 0.001, and a logarithm of odds (LOD) threshold of ≥ 3.0, with a walk speed of 1 cM. QTLs detected in two or more environments or for two or more traits were considered important. Those identified in three or more environments were further defined as environment-stable QTLs. QTLs associated with the same trait across different environments and with overlapping confidence intervals were considered to represent the same locus. QTL nomenclature followed standard genetic conventions (McIntosh et al., 2017). The abbreviation “HZAU” refers to Huazhong Agricultural University. The positions of the identified QTLs on the genetic map were visualized using the R package “LinkageMapView.”

2.8 Identification and Cloning of Candidate Genes

Candidate genes were predicted based on the annotation of all genes within the stable QTL intervals. These intervals were delineated using the flanking bin markers and their corresponding physical positions on the barley reference genome MorexV3 (https://plants.ensembl.org/Hordeum_vulgare/Info/Index). Specific primers for the selected candidate genes were designed using the Primer Server online tool (http://202.194.139.32/PrimerServer/) in conjunction with Oligo 7 software to genotype the parental lines YJ and H30. PCR amplification was performed using 1× Fast Taq PCR Master Mix. The amplified products were ligated into the pTOPO-BLUNT Simple Vector and verified by Sanger sequencing. Sequences were aligned using MEGA or ClustalW to identify polymorphisms between the parents.

3. Results

3.1 Whole-genome Resequencing and SNP Marker Development

This study conducted whole-genome resequencing of two parental lines, ‘YJ’ and ‘H30’, along with their 172 progeny lines. The average sequencing data output per sample was 71.80 Gb for YJ, 61.24 Gb for H30, and 32.50 Gb for each RIL. Using the MorexV3 barley reference genome for alignment, the mapping rate of sequencing reads to the reference genome was 99.81% for all three sample groups. Quality assessment yielded the following results: Q20 scores were 97.51%, 97.16%, and 97.36%; Q30 scores were 92.86%, 91.82%, and 92.38%; GC content was 44.40%, 44.38%, and 44.11%; and the average sequencing depths were 14.1×, 12.0×, and 6.4×, respectively (Table S1).

Based on the aforementioned high-quality alignment data, 202,887,646 variant loci were obtained. By removing InDel markers, filtering low-depth markers (parental depth < 5×, progeny depth < 3×), and genotyping progeny according to the parental "aa×bb" genotype pattern, only chromosomal markers were retained, ultimately yielding 3,864,882 high-quality SNP markers. These SNP markers were utilized for the subsequent construction of high-density genetic linkage maps.

3.2 Construction of High-density Genetic Linkage Map

In this study, a sliding window strategy was employed to partition 3,864,882 SNP markers into bin markers, resulting in a total of 12,905 bin markers (Table 1). The genetic distance between bin markers was calculated by QTL IciMapping 4.2 software, and high-density linkage maps were constructed. The total length of the map is 1,086.46 cM, with the genetic lengths of each chromosome ranging from 124.16 cM to 176.44 cM. Chromosome 2H has the longest genetic distance (176.44 cM), while chromosome 4H has the shortest (124.16 cM). The average genetic distance between adjacent

markers on the linkage map was 0.08 cM, with the 7H chromosome showing the largest average genetic distance of 0.15 cM and the 6H chromosome the smallest average genetic distance of 0.06 cM (Table 1).

The constructed linkage map comprised 12,905 Bin markers, with an average of 1,843.57 markers distributed per chromosome. Among these, chromosome 3H contained the highest number of markers (2,714), while chromosome 7H had the fewest (1,058) (Table 1). Overall, the bin markers on each chromosome exhibited a relatively uniform distribution (Fig. 1).

Table 1
Basic information of high-density genetic linkage map

Chromosome	Chromosome length (cM)	Bin Markers	Average distance (cM)
1H	144.26	1,175	0.12
2H	176.44	2,110	0.08
3H	165.85	2,714	0.06
4H	124.16	1,485	0.08
5H	173.05	1,938	0.09
6H	144.85	2,425	0.06
7H	157.85	1,058	0.15
Total	1,086.46	12,905	0.08

The seven linkage groups (1H to 7H) are shown vertically. Black bars on the left depict the genetic linkage maps with bin markers. The corresponding genetic distances (cM) are indicated on the left scale.

3.3 Comparison of genetic and physical maps

To evaluate the accuracy of the constructed genetic map, we performed collinearity analysis with the Morex V3 reference genome physical map. Overall, the distribution of markers along the diagonal indicated a high consistency between genetic and physical orders, confirming the overall reliability of the map (Fig. 2A). However, in regions adjacent to the centromeres, distinct map distortion was observed, characterized by a marked reduction in the rate of genetic distance increase within specific physical intervals (Fig. 2B). The rapid increase in genetic distance toward chromosome ends suggested the presence of recombination hotspots, whereas the slowed genetic progression in the central region surrounding the centromere corresponded to a recombination cold zone. This pattern was attributed to the suppressive effect of centromeres on recombination. From a genetic mapping perspective, this confirmed that chromosomal termini are recombination-active regions, while interstitial regions are recombination-suppressed. This synteny analysis thus validated the map's accuracy at the genomic structural level and provided critical information on recombination suppression for subsequent QTL fine-mapping.

1. Collinearity analysis of barley genetic and physical maps. The scatter plot displays the normalized genetic positions of all Bin markers on chromosomes versus their physical positions. B. Map distortion in centromeric regions. An enlarged view of the area within the box in Figure A reveals recombination suppression near the centromere.

3.4 Phenotypic variation and correlation analysis of leaf traits

This study evaluated the phenotypic variation of nine leaf-related traits (FLL, FLW, FLA, SLL, SLW, SLA, TLL, TLW, and TLA) in the RIL population and its parental lines across five environments (Fig. 3). The phenotypic mean of parent YJ was consistently higher than that of parent H30 across all environments. For flag leaf traits (FLL, FLW, FLA), significant

differences ($P < 0.01$) were observed in all five environments. For the second leaf, SLL, SLW, and SLA showed highly significant differences ($P < 0.01$) in most environments, except for SLW in the 2024WH environment, which showed a significant difference ($P < 0.05$). For the third leaf, TLL, TLW, and TLA exhibited highly significant differences ($P < 0.01$) in three environments, while TLW showed significant differences ($P < 0.05$) in the 2023WH and 2024WH environments (Fig. 3). Broad-sense heritability (H^2) estimates for the nine traits, calculated across five environments and using best linear unbiased prediction (BLUP), ranged from 0.80 to 0.92 (Table S2), indicating that variation in these leaf traits was primarily controlled by genetic factors. Normality tests indicated that all leaf-related traits in the RIL population followed a normal distribution across the five environments and BLUP estimates (Figure S1). These results demonstrate that the leaf-related traits in this population are quantitative traits, likely controlled by multiple genes.

A. Phenotype of parents 'YJ' and 'H30'. Bar 10 cm; B-D Leaf morphology of parents and partial RILs. Bar 1 cm. F-G. Differences in flag leaf length (FLL), flag leaf width (FLW), flag leaf area (FLA) of parental flag leaves. H-J. Differences in second leaf length (SLL), second leaf width (SLW), second leaf area (SLA) of the second leaf of parents. K-M. Differences in third leaf length (TLL), third leaf width (TLW), and third leaf area (TLA) of the third leaf of parents. Designations * and ** represent $P < 0.05$ and $P < 0.01$, respectively.

Correlation analysis of barley leaf-related traits was performed based on Best Linear Unbiased Prediction (BLUP) values (Fig. 4). The results showed that every pairwise comparison among the nine traits exhibited a highly significant positive correlation ($p < 0.001$), with correlation coefficients ranging from 0.59 to 0.97. Among all 36 trait pairs, 24 pairs (66.7%) showed correlation coefficients exceeding 0.80, indicating strong positive correlations among these leaf size traits.

3.5 QTL mapping for leaf-related traits

A total of 74 QTLs associated with leaf traits were identified, including 7 for FLL, 7 for FLW, 5 for FLA, 11 for SLL, 7 for SLW, 13 for SLA, 9 for TLL, 12 for TLW, and 3 for TLA (Table 2, Table S3). The increasing allele of 51 QTLs originated from 'YJ', and those of 23 QTLs originated from 'H30'. The phenotypic variation explained by these different environmental QTLs ranged from 0.61% to 54.15% (Table 2, Table S3).

QTL mapping for flag leaf-related traits identified seven QTLs for FLL distributed on chromosomes 1H, 2H, 3H, 5H, 6H, and 7H, explaining 3.14% to 26.78% of the phenotypic variation. *QFll.hzau-5H.2*, located at 128.5–129.5 cM on chromosome 5H, was repeatedly detected across four environments and the BLUP dataset, representing the most stable major QTL for this trait. For FLW, seven QTLs were identified on chromosomes 2H, 3H, 5H, and 6H, accounting for 4.95% to 36.08% of the phenotypic variation. Among these, *QFlw.hzau-5H.1* and *QFlw.hzau-5H.2* were detected across multiple environments and the BLUP dataset, constituting the primary stable QTLs for FLW. Five QTLs were mapped for FLA on chromosomes 2H, 5H, and 6H, with phenotypic contributions ranging from 6.03% to 30.38%. The major locus *QFla.hzau-5H.2* was stably expressed across all environments and the BLUP dataset. *QFla.hzau-2H.1* was detected only in the 23WH environment, with a contribution of 25.22%, and its increasing allele originated from YJ. In contrast, the adjacent locus *QFla.hzau-2H.2* was detected in the 24XY and 22WH environments, with its increasing allele derived from H30 (Table 2, Table S3).

QTL analysis of the second leaf traits showed that twelve QTLs were identified for SLL across chromosomes 1H to 7H, contributing 3.23% to 34.90% of the phenotypic variation. The key QTL *QSll.hzau-5H.2* was stably detected in three environments and the BLUP dataset, and showed a contribution rate of up to 34.90% in the BLUP analysis. Seven QTLs were detected for SLW on chromosomes 2H, 4H, 5H, and 6H. Among them, *QSlw.hzau-5H.2* was consistently expressed across all tested environments and the BLUP dataset, with a contribution rate ranging from 25.78% to 54.15%. Twelve QTLs were mapped for SLA on chromosomes 1H to 6H, with a contribution rate ranging from 0.61% to 42.17%. The major QTL *QSla.hzau-5H.3* was stably expressed in four environments and the BLUP dataset, with a maximum contribution rate of 42.17% (Table 2, Table S3).

For the third leaf traits, nine QTLs were identified for TLL on chromosomes 1H, 2H, 3H, 4H, 5H, and 7H, accounting for 2.96% to 28.47% of the phenotypic variation. The major QTL *QTll.hzau-5H.2* was stably detected in three environments and the BLUP dataset. Twelve QTLs were detected for TLW across chromosomes 1H to 6H, with contribution rates ranging from 2.93% to 51.93%. Among these, *QTlw.hzau-5H.2* was detected in all environments and the BLUP dataset, with a maximum contribution rate of 51.93%, representing a highly effective and stable major QTL. For TLA, three QTLs were mapped on chromosomes 4H and 5H, contributing 3.65% to 43.13% of the phenotypic variation. The major QTL *QTla.hzau-5H.2* was stably expressed in all five individual environments and the BLUP dataset, with a maximum contribution rate of 43.13% (Table 2, Table S3).

Comprehensive analysis showed that the genomic interval 128.5–129.5 cM on chromosome 5H was the key QTL locus for leaf morphology. The quantitative trait loci within this region (including *QFll.hzau-5H.2*, *QFlw.hzau-5H.2*, *QFla.hzau-5H.2*, *QSll.hzau-5H.2*, *QSlw.hzau-5H.2*, *QSla.hzau-5H.3*, *QTll.hzau-5H.2*, *QTlw.hzau-5H.2* and *QTla.hzau-5H.2*) exhibited significant and stable genetic effects on all nine leaf traits. These QTLs showed high reproducibility across most tested environments and the BLUP analysis, along with substantial phenotypic contributions, and their increasing allele was consistently derived from the parent YJ (Table 2).

In addition to the stable QTLs identified in multi-environment and BLUP analyses, this study also identified two QTL clusters located on chromosomes 2H and 5H, respectively. In the marker region BM1212-BM1294 of chromosome 2H, we identified a cluster comprising six QTLs (*QFla.hzau-2H.2*, *QFll.hzau-2H*, *QFlw.hzau-2H.1*, *QSla.hzau-2H.2*, *QSll.hzau-2H*, and *QTll.hzau-2H.1*), with phenotypic variation explained rates ranging from 3.03% to 15.58%. However, the QTL cluster on chromosome 2H was not a stable major effect locus and was only detected in individual environments. Additionally, another cluster comprising eight QTLs (*QFlw.hzau-5H.1*, *QFla.hzau-5H.1*, *QFll.hzau-5H.1*, *QSla.hzau-5H.1*, *QSll.hzau-5H.1*, *QSlw.hzau-5H.1*, *QTla.hzau-5H.*1, and *QTlw.hzau-5H.1*) was identified within the labeled region BM0–BM9 of chromosome 5H, with a phenotypic contribution ranging from 3.30% to 12.42%. This QTL cluster on chromosome 5H was classified as a minor effect locus; all QTLs except *QTlw.hzau-5H.1* were replicated in two or more environments (Table 2, Table S3).

Table 2
Environmental stability QTLs for leaf-related traits in YJ and H30 RIL populations.

Trait	QTL	Env	Chr	Interval	Position	LeftMarker	RightMarker	LOD	PVE(%)	Add
FLA	*QFla.hzau-5H.2*	22WH	5	128.5–129.5	129	EM1742	EM1746	15.49	24.76	-4.26
		23WH	5	128.5–129.5	129	EM1742	EM1746	12.67	0.80	-4.91
		23XY	5	128.5–129.5	129	EM1742	EM1746	14.45	26.35	-5.25
		24WH	5	128.5–129.5	129	EM1742	EM1746	13.00	20.72	-3.55
		24XY	5	128.5–129.5	129	EM1742	EM1746	7.89	13.37	-3.43
		FLA-BLUP	5	128.5–129.5	129	EM1742	EM1746	20.70	30.38	-3.54
FLL	*QFll.hzau-5H.2*	23WH	5	128.5–129.5	129	EM1742	EM1746	13.40	21.31	-2.84
		23XY	5	128.5–129.5	129	EM1742	EM1746	18.48	23.67	-2.80
		24WH	5	128.5–129.5	129	EM1742	EM1746	11.61	16.39	-1.62
		24XY	5	128.5–129.5	129	EM1742	EM1746	7.64	12.48	-1.66
		FLL-BLUP	5	128.5–129.5	129	EM1742	EM1746	25.72	26.78	-1.85
FLW	*QFlw.hzau-5H.1*	22WH	5	0-0.5	0	EM1	EM2	20.13	16.37	-0.13
		23XY	5	0-1.5	0	EM1	EM2	4.41	6.42	-0.07
		24XY	5	0-1.5	1	EM8	EM9	6.25	10.04	-0.08
		FLW-BLUP	5	0-1.5	1	EM8	EM9	5.88	8.31	-0.04
FLW	*QFlw.hzau-5H.2*	22WH	5	128.5–129.5	129	EM1742	EM1746	28.05	25.89	-0.21
		23WH	5	128.5–129.5	129	EM1742	EM1746	17.41	34.06	-0.21
		23XY	5	128.5–129.5	129	EM1742	EM1746	16.54	28.93	-0.20
		24WH	5	128.5–129.5	129	EM1742	EM1746	20.13	27.79	-0.17
		24XY	5	128.5–129.5	129	EM1742	EM1746	11.31	19.37	-0.14
		FLW-BLUP	5	128.5–129.5	129	EM1742	EM1746	20.59	36.08	-0.11
SLA	*QSla.hzau-5H.3*	23WH	5	128.5–129.5	129	EM1742	EM1746	30.60	3.94	-10.14

Trait	QTL	Env	Chr	Interval	Position	LeftMarker	RightMarker	LOD	PVE(%)	Add
		23XY	5	128.5–129.5	129	EM1742	EM1746	15.21	29.63	-7.79
		24WH	5	128.5–129.5	129	EM1742	EM1746	26.74	23.83	-6.02
		24XY	5	128.5–129.5	129	EM1742	EM1746	16.02	26.26	-6.53
		SLA-BLUP	5	128.5–129.5	129	EM1742	EM1746	32.55	42.17	-5.46
SLL	*QSll.hzau-5H.2*	23WH	5	128.5–129.5	129	EM1742	EM1746	10.52	21.00	-3.00
		23XY	5	128.5–129.5	129	EM1742	EM1746	11.55	22.25	-3.20
		24WH	5	128.5–129.5	129	EM1742	EM1746	18.38	20.22	-2.12
		SLL-BLUP	5	128.5–129.5	129	EM1742	EM1746	26.94	34.90	-2.13
SLW	*QSlw.hzau-5H.2*	22WH	5	128.5–129.5	129	EM1742	EM1746	23.19	42.50	-0.19
		23WH	5	128.5–129.5	129	EM1742	EM1746	34.50	51.91	-0.25
		23XY	5	128.5–129.5	129	EM1742	EM1746	20.48	38.69	-0.19
		24XY	5	128.5–129.5	129	EM1742	EM1746	12.19	25.78	-0.14
		SLW-BLUP	5	128.5–129.5	129	EM1742	EM1746	39.06	54.15	-0.16
		24WH	5	129.5-130.5	130	EM1757	EM1758	28.13	39.87	-0.17
TLA	*QTla.hzau-5H.2*	22WH	5	128.5–129.5	129	EM1742	EM1746	12.53	28.98	-5.43
		23WH	5	128.5–129.5	129	EM1742	EM1746	11.68	27.39	-6.22
		23XY	5	128.5–129.5	129	EM1742	EM1746	8.10	17.44	-7.52
		24WH	5	128.5–129.5	129	EM1742	EM1746	27.00	35.93	-6.60
		24XY	5	128.5–129.5	129	EM1742	EM1746	15.70	25.49	-6.69
		TLA-BLUP	5	128.5–129.5	129	EM1742	EM1746	35.33	43.13	-5.23
TLL	*QTll.hzau-5H.2*	23WH	5	128.5–129.5	129	EM1742	EM1746	8.51	18.17	-2.53
		24WH	5	128.5–129.5	129	EM1742	EM1746	20.30	21.52	-2.27

Trait	QTL	Env	Chr	Interval	Position	LeftMarker	RightMarker	LOD	PVE(%)	Add
		24XY	5	128.5–129.5	129	EM1742	EM1746	15.52	18.83	-2.21
		TLL-BLUP	5	128.5–129.5	129	EM1742	EM1746	27.44	28.47	-1.56
TLW	*QTlw.hzau-5H.2*	22WH	5	128.5–129.5	129	EM1742	EM1746	27.66	43.84	-0.19
		23WH	5	128.5–129.5	129	EM1742	EM1746	28.33	41.81	-0.21
		23XY	5	128.5–129.5	129	EM1742	EM1746	21.65	19.32	-0.21
		24WH	5	128.5–129.5	129	EM1742	EM1746	31.76	40.64	-0.19
		24XY	5	128.5–129.5	129	EM1742	EM1746	12.54	17.40	-0.13
		TLW-BLUP	5	128.5–129.5	129	EM1742	EM1746	45.90	51.93	-0.17

Env: the environment of QTL mapping; Chr: chromosome; LOD: logarithm of odds; PVE(%): the percentage of phenotypic variation explained by a QTL; Add: additive effect of a QTL, where positive and negative values represent alleles increasing the phenotypic values from H30 and YJ, respectively.

3.6 Candidate genes at major loci of chromosome 5H

In this study, QTL analysis was conducted on nine traits, including the length, width, and area of the flag leaf, second leaf, and third leaf under all five environments and BLUP conditions. A stable major QTL was identified in the genomic region 128.5–129.5 cM on chromosome 5H (Fig. 5A). By integrating the genetic map with the barley reference genome Morex V3, 28 genes were annotated within this interval, of which 16 were classified as high-confidence genes (Table S3). Based on functional annotations, *HORVU.MOREX.r3.5HG0511210* was selected as a candidate gene. This gene encodes a MADS-box transcription factor known as the vernalization gene *HvVRN1* (Trevaskis et al., 2006); however, its role in regulating leaf size has not been previously reported in barley.

To investigate the genetic basis of this locus, we amplified and sequenced the entire *HvVRN1* gene in both parents. The first intron of the large-leaf parent YJ was approximately 4,500 bp, whereas that of the small-leaf parent H30 was approximately 11,000 bp (Fig. 5B). This sequence difference was located in a known regulatory region of *HvVRN1*. To evaluate the association between this variant and leaf traits, we classified the 172 RILs into YJ-type and H30-type genotypes based on polymorphisms in the first intron, and compared leaf size under five environments and BLUP conditions (Figure S2). Nine leaf traits showed significant differences between the two genotype classes, suggesting that cis-regulatory variation at *HvVRN1* may contribute to leaf size variation. However, functional validation is required to confirm the causal role of this sequence variant.

- A. QTL mapping on chromosome 5H. LOD distribution of nine leaf traits (FLL, FLW, FLA, SLL, SLW, SLA, TLL, TLW, TLA) across five environments and BLUP conditions on chromosome 5H. The dashed horizontal line indicates the significance threshold (LOD = 3.0). B. Gene structure and annotation within the candidate interval. The vertical axis represents physical location (Mb). A model of the key high-confidence candidate gene is displayed, with the first intron length polymorphism between the parents annotated.

3.7 Effects of the major QTL on other agronomic traits

To evaluate whether the major QTL on chromosome 5H affects other agronomic traits, we classified the 172 RILs into two groups based on the parental allele at the QTL interval (YJ-type and H30-type) and compared eight traits under field conditions: plant height (PH), spike length (SL), spikelet number per spike (SNS), spike density (SD), thousand-grain weight (TGW), heading date (HD), effective tiller number (ETN), and tiller angle (TA). Lines carrying the YJ allele showed significantly greater plant height (5.08 cm increase, $P < 0.05$), greater SNS (1.94 more spikelets, $P < 0.01$), and delayed heading date (4.5 days later, $P < 0.01$) compared with lines carrying the H30 allele (Fig. 6). In contrast, no significant differences were detected for SL, SD, TGW, ETN, or TA between the two genotype groups ($P > 0.05$).

These results indicate that the 5H QTL has pleiotropic effects on plant height, spikelet number, and heading date, but does not significantly affect spike length, spike density, grain weight, tillering, or tiller angle. The co-localization of leaf size, plant height, and heading date at this locus is consistent with the known pleiotropic nature of *HvVRN1*, which regulates vernalization and flowering time and can indirectly influence plant architecture.

- RILs were grouped based on the parental allele at the QTL interval: "+" indicates lines carrying the YJ allele, and "–" indicates lines carrying the H30 allele. Box plots show the median (central line), interquartile range (box), and range (whiskers). Significant differences are indicated by $*P < 0.05$ and $**P < 0.01$; NS, not significant.

4. Discussion

Genetic maps served as the core analytical platform in plant genetics research and played a pivotal role in crop genetic improvement. They provide critical support for gene mapping of important agronomic traits and marker-assisted selection (MAS) breeding (Aghnoum et al., 2010; Bankole et al., 2017; Xu et al., 2018). Traditional molecular markers, such as RFLP (Wenzl et al., 2006), AFLP (Marcel et al., 2007), SSR (Varshney et al., 2007), DArT (Hearnden et al., 2007) and RAD (Ren et al., 2016), were widely used to construct genetic linkage maps in barley. However, these markers often suffered from limitations such as limited quantity, low genotyping throughput, and high costs, resulting in insufficient map density and consequently restricting the precision of quantitative trait locus (QTL) mapping (Hyten et al., 2010; Sun et al., 2013). With the advancement of high-throughput sequencing technologies, strategies based on simplified genomes or whole-genome sequencing could efficiently develop large-scale single nucleotide polymorphism (SNP) markers, thereby enabling the construction of denser and more evenly covered genetic maps. This strategy was successfully applied to the construction of high-density genetic maps in various plants (Xia et al., 2015; Liu et al., 2016; Wen et al., 2020; Fang et al., 2020; Chen et al., 2023).

The barley genome is extensive, and achieving uniform coverage was challenging with traditional polymorphic markers. For instance, the integrated map by Wenzl et al. (2006) contained 2,935 loci with an average genetic distance of 0.7 ± 1.0 cM, and the map constructed by Hearnden et al. (2007) using 1,000 SSR and DArT markers had an average map distance of 0.91 cM; both maps exhibited limited marker density, which could constrain trait mapping. Although subsequent studies adopted high-throughput sequencing strategies to improve density, Zhou et al. (2015) developed 12,998 SNP markers through RAD-seq and constructed a map with an average distance of 0.07 cM and a total length of 967.6 cM; Fang et al. (2020) obtained 12,635 SLAF markers and 26,693 SNPs using SLAF-seq technology and constructed a map with a span of 896.74 cM and an average distance of 0.07 cM. However, when analyzed based on a single SNP, potential genotyping errors may still introduce false positive mapping results. In this study, whole-genome resequencing was performed on an RIL population of 172 lines derived from 'YJ' and 'H30', yielding 3,864,882 high-quality SNPs. These were consolidated into 12,905 highly reliable bin markers to produce a high-density genetic map with a total length of 1,086.46 cM and an average marker interval of 0.08 cM.This map provides an ultra-dense genetic linkage map of barley constructed using whole-genome resequencing, with both SNP and bin marker numbers surpassing previous reports. Bin markers are determined by multiple linked SNPs, which effectively reduces genotyping errors associated with individual SNP loci, thereby enhancing marker accuracy and genetic map precision (Chen et al., 2023).

Leaves serve as the primary “source” organ, converting atmospheric CO_2 into crop grain yield through photosynthesis, making leaf traits critically important for yield formation. Previous studies have localized QTLs associated with leaf size on barley chromosomes 1H, 2H, 3H, 4H, 5H, 6H, and 7H (Xue et al., 2008; Liu et al., 2015; Du et al., 2019; Jabbari et al., 2018; Zheng et al., 2022; Niu et al., 2022). This study identified 74 QTLs across 9 leaf-related traits (FLL, FLW, FLA, SLL, SLW, SLA, TLL, TLW, TLA) distributed on chromosomes 1H to 7H (Table 2, Table S4). Among these, three QTL clusters were identified on chromosomes 2H (72.5–76.5 cM) and 5H (0–1.5 cM and 128.5–129.5 cM) (Fig. 7).

In this study, a cluster containing six QTLs (*QFla.hzau-2H.2*, *QFll.hzau-2H*, *QFlw.hzau-2H.1*, *QSla.hzau-2H.2*, *QSll.hzau-2H*, and *QTll.hzau-2H.1*) was identified within the chromosome 2H marker region BM1212–BM1294 (Fig. 7). Previous studies have reported QTL clusters controlling leaf size on chromosome 2H (Liu et al., 2015; Du et al., 2019; Zheng et al., 2022; Niu et al., 2022). Among these, the QTL clusters localized by Liu et al. (2015) and Zheng et al. (2022) were near the *Vrs1* gene, whereas the QTL cluster identified in this study was close to the regions *qFLL2-2* and *qFLA2-2* reported by Du et al. (2019), as well as the loci *Qfll-2H*, *Qflt-2H*, *Qflw-2H.1*, and *Qfla-2H* reported by Niu et al. (2022). However, the QTL cluster located on chromosome 2H was not a stable major effect locus in this study and was only detected in individual environments.

This study identified a cluster of eight QTLs (*QFlw.hzau-5H.1*, *QFla.hzau-5H.1*, *QFll.hzau-5H.1*, *QSla.hzau-5H.1*, *QSll.hzau-5H.1*, *QSlw.hzau-5H.1*, *QTla.hzau-5H.1*, and *QTlw.hzau-5H.1*) within the BM0–BM9 marker region of chromosome 5H. This cluster constituted a minor locus, with all QTLs except QTlw.hzau-5H.1 detected in two or more environments (Fig. 7). Previously, Niu et al. (2022) also identified a QTL (*Qflt-5H*) that controlled flag leaf thickness (FLT) in the same region of chromosome 5H, which further supported that this locus was an important region in regulating leaf traits. In this study, a major and stable QTL cluster covering all nine leaf-related traits was identified within the 128.5–129.5 cM interval on chromosome 5H.

The seven linkage groups (chromosomes 1H to 7H) are displayed vertically. The black lines on the left represent the genetic linkage maps with Bin markers, with genetic distances (cM) indicated on the left scale. QTLs identified for nine leaf traits (FLL, FLW, FLA, SLL, SLW, SLA, TLL, TLW, TLA) were identified and marked in box form at their corresponding peak locus positions. The specific QTL names were labeled on the right. Green indicates the stable QTLs detected in multiple environments, while the orange and sky blue colors represent the QTL clusters of 2H and 5H, respectively.

In earlier barley QTL mapping, the FLA-related QTL *Qfla-5H* (Niu et al., 2022) and the FLW-related QTL *qFW5.1* (Xue et al., 2008) were mapped to similar regions, although both differed in physical position from the interval identified here (527.49–528.08 Mbp). In wheat, multiple QTLs for leaf traits have been reported in adjacent regions of chromosome 5BL (approximately 493.7–534.3 Mbp), including *QFll.sicau-5B.3* and *QFlr.sicau-5B* (Ma et al., 2020), *Qbafl.acs–5B.2* (Yang et al., 2016), and the major QTLs *QFll.cau-5B* and *QFlr.cau-5B* (Wu et al., 2016). These loci are all located near the wheat vernalization gene *Vrn-B1* (at 573.8 Mbp on 5BL) (Yan et al., 2004; Chu et al., 2011). Further analysis revealed that the 5HL interval (527.49–528.08 Mbp) identified in this study harbors the barley vernalization gene *HvVRN1* (Trevaskis et al., 2006). This interval is distinct from the previously cloned Blf1 locus (Jöst et al., 2016), which also resides on 5HL but at a different physical position. While *Blf1* limits leaf width by restricting lateral proliferation, our locus harbors *HvVRN1* and is associated with length, width, and area of multiple leaves, suggesting a different regulatory mechanism.

The first intron of *HvVRN1* is a known regulatory region. Fu et al. (2005) reported that spring alleles carry large deletions in this region, overlapping a ~ 4 kb segment, and Hemming et al. (2009) further delimited a ~ 1 kb core repressive region. In our study, the first intron of YJ was approximately 4,500 bp, whereas that of H30 was approximately 11,000 bp. This large difference suggests that YJ may carry a large deletion in the first intron similar to the known spring alleles, thereby removing cis-regulatory elements that maintain the repressed state of *HvVRN1* (Fu et al., 2005; Hemming et al., 2009). Consistent with this, prior work has shown that allelic variation in intron 1 is closely associated with increased *VRN1*

expression (Zhang et al., 2015). This sequence difference makes *HvVRN1* a compelling candidate for leaf size regulation, although functional validation is still required.

HvVRN1 is a core regulatory factor in the barley vernalization response and the transition from vegetative to reproductive growth (Trevaskis et al., 2006). It is expressed at a low level before vernalization to inhibit flowering, while prolonged cold induces its transcription and promotes rapid flowering (Murai et al., 2003). In addition to flowering time, *HvVRN1* is associated with cold resistance and spike development (Koemel et al., 2004; Deng et al., 2015), but its role in regulating barley leaf size remains unclear. Notably, MADS-box transcription factors can influence leaf development; overexpression of the tomato MADS-box gene *SlMBP22* leads to dwarfism and reduced leaf size (Li et al., 2020). Therefore, HvVRN1 may possess a previously unrecognized function in regulating leaf morphology in barley. Consistent with this hypothesis, the major QTL on chromosome 5H not only controlled leaf size but also affected plant height, spikelet number, and heading date, which is in line with the pleiotropic role of *HvVRN1* in regulating the transition from vegetative to reproductive growth (Trevaskis et al., 2006; Deng et al., 2015). The delayed heading date and increased plant height associated with the YJ allele may reflect an extended vegetative growth phase, which could also contribute to larger leaves. However, the lack of significant effects on spike length, spike density, thousand-grain weight, tiller number, and tiller angle suggests that the leaf size effect is not simply a consequence of overall plant size. These findings provide valuable information for breeding programs aiming to optimize leaf architecture while maintaining desirable agronomic traits.

5. Conclusions

In summary, this study conducted whole-genome resequencing of 172 progeny lines from the ‘YJ’ and ‘H30’ cross, constructing a high-density genetic linkage map containing 12,905 bin markers. A total of 74 QTLs were identified, among which a stable locus on chromosome 5H (128.5–129.5 cM) was found to be associated with all nine leaf traits examined. This region harbored the vernalization gene *HvVRN1*. Sequence comparison between the parents revealed a large length difference in the first intron of *HvVRN1*, suggesting that cis-regulatory variation at this gene may contribute to leaf size variation.The locus also affected plant height, spikelet number per spike, and heading date, but had no significant effect on spike length, spike density, thousand-grain weight, tiller number, or tiller angle, indicating pleiotropic effects on leaf morphology and plant architecture. The results demonstrate the utility of high-density bin maps in precise QTL mapping and identify *HvVRN1* as a candidate gene for leaf morphology. These findings provide a basis for marker-assisted selection and a candidate gene for future breeding programs aimed at optimizing leaf architecture and improving yield in barley.

Declarations

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Conflict of interest

The authors declare no competing financial interest.

Author contributions

X.F. Wang: Experimental design, data collection, analysis, genetic map construction, and manuscript drafting; J.P. Chen, M.W. Wang, C. Zhang, W.J. Ge, and C.Y. Wang: assisted in data collection for the RIL population; Y.X. Tong and Y. Zhou: conducted bioinformatics analysis and data validation; X.F. Wang and J.P. Chen: conducted statistical analysis and visualization; J.H. Zhu: collected the parental materials and developed the RIL population; X.F. Ren (corresponding author): conceptualized the study, supervised the research, acquired funding, provided methodology guidance, and revised the manuscript. All authors contributed to the study and approved the final version for submission.

Ethics declarations

Conflicts of interest

The authors declare no conflicts of interest.

Ethical standards

I would like to declare on behalf of my co-authors that the work described is original, previously unpublished research. All the authors listed have approved the enclosed manuscript.

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Data availability

The WGS data that support the findings of this study have been deposited in NCBI under the BioProject PRJNA1545627. All data generated or analyzed during this study are included in the main text article and its supplementary files.

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Figures

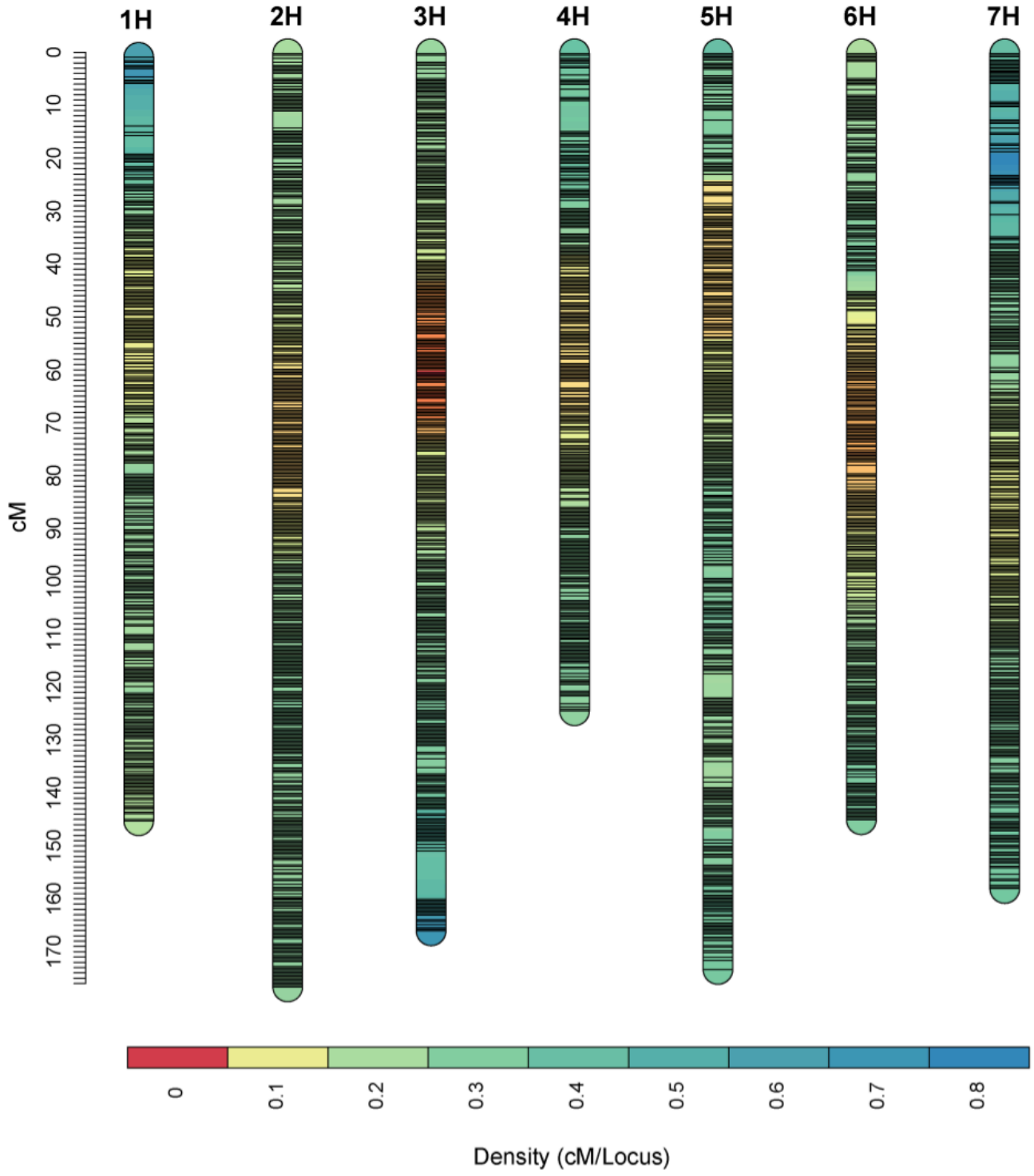


Figure 1

High-density genetic linkage map of the RIL population derived from a cross between YJ and H30.

The seven linkage groups (1H to 7H) are shown vertically. Black bars on the left depict the genetic linkage maps with bin markers. The corresponding genetic distances (cM) are indicated on the left scale.

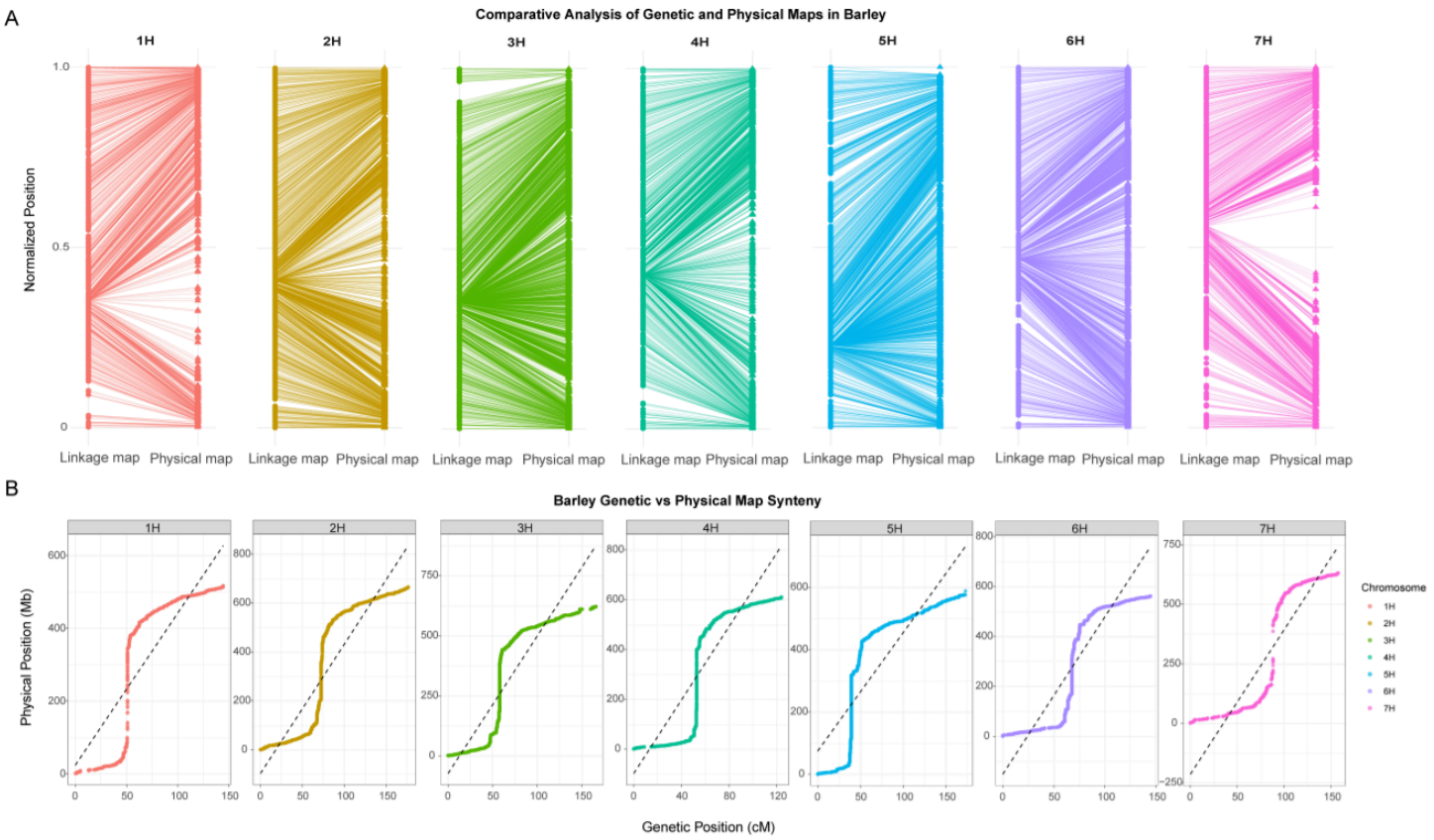


Figure 2

Correlation between the genetic and physical maps of barley.

A. Collinearity analysis of barley genetic and physical maps. The scatter plot displays the normalized genetic positions of all Bin markers on chromosomes versus their physical positions. B. Map distortion in centromeric regions. An enlarged view of the area within the box in Figure A reveals recombination suppression near the centromere.

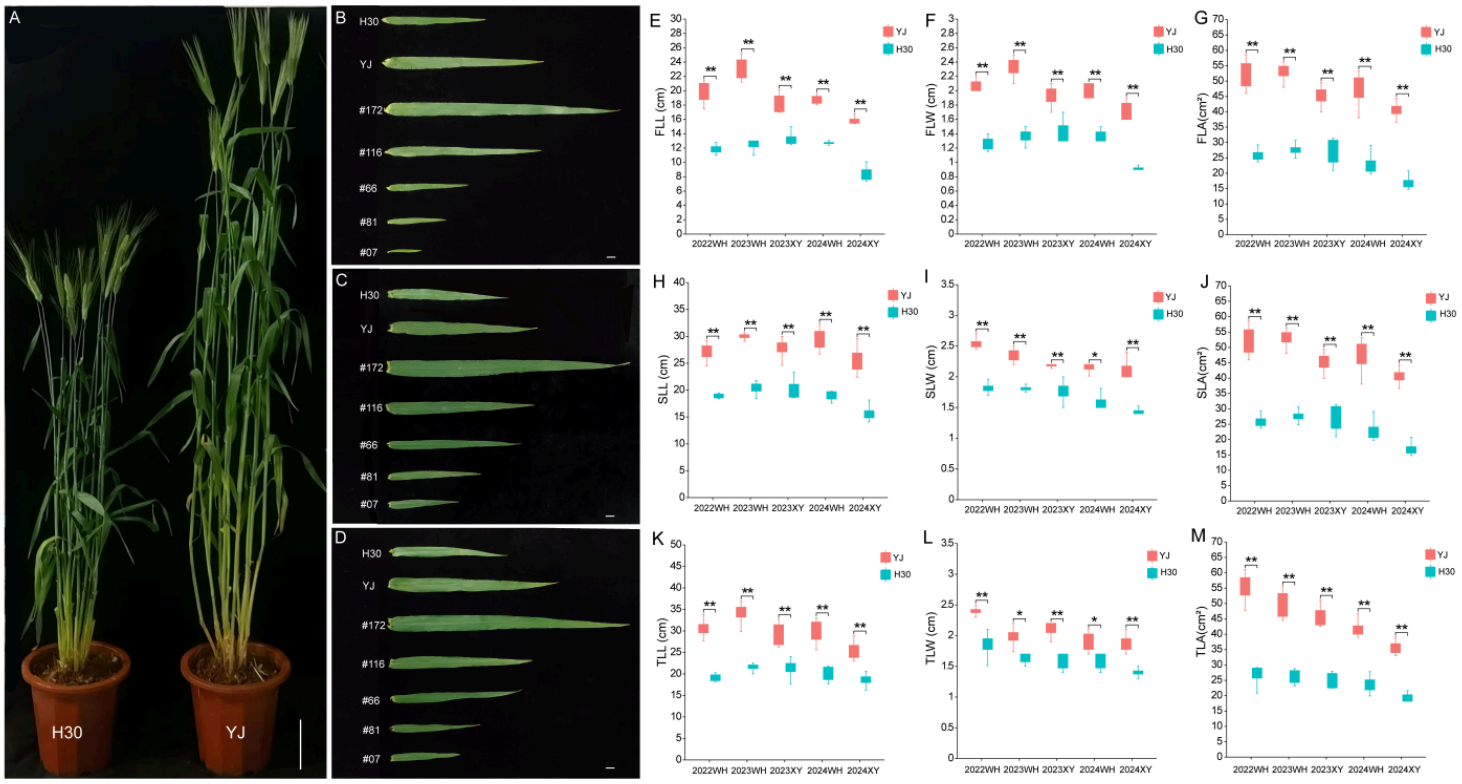


Figure 3

The correlation analysis of leaf traits between parents.

A. Phenotype of parents ‘YJ’ and ‘H30’. Bar 10 cm; B-D Leaf morphology of parents and partial RILs. Bar 1 cm. F-G. Differences in flag leaf length (FLL), flag leaf width (FLW), flag leaf area (FLA) of parental flag leaves. H-J. Differences in second leaf length (SLL), second leaf width (SLW), second leaf area (SLA) of the second leaf of parents. K-M. Differences in third leaf length (TLL), third leaf width (TLW), and third leaf area (TLA) of the third leaf of parents. Designations * and ** represent $P< 0.05$ and $P < 0.01$, respectively.

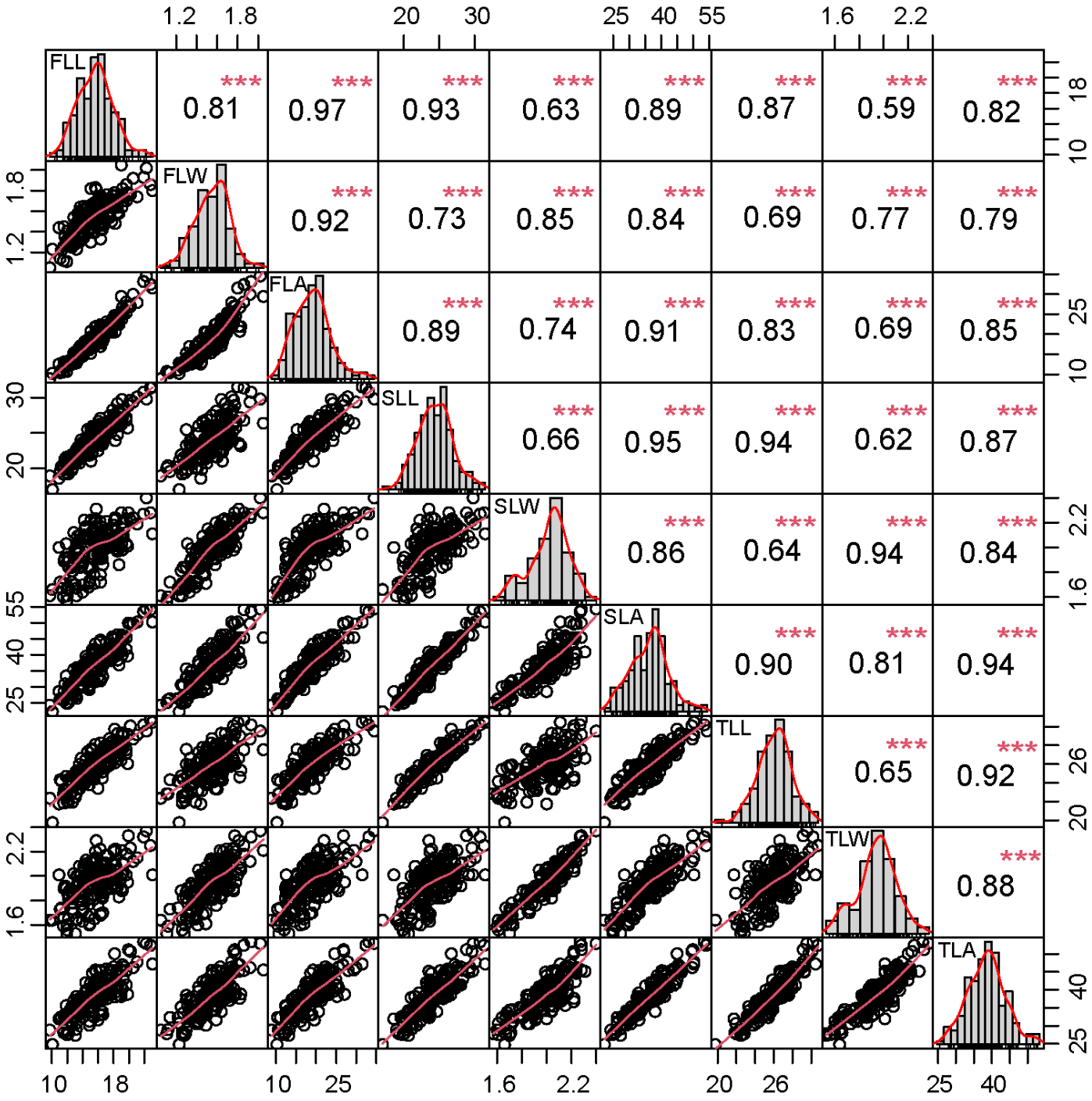


Figure 4

Pearson correlation coefficients among nine leaf-related traits in the RIL population. Abbreviations: FLL, flag leaf length; FLW, flag leaf width; FLA, flag leaf area; SLL, second leaf length; SLW, second leaf width; SLA, second leaf area; TLL, third leaf length; TLW, third leaf width; TLA, third leaf area. Asterisks indicate significance levels: *** $P < 0.001$.

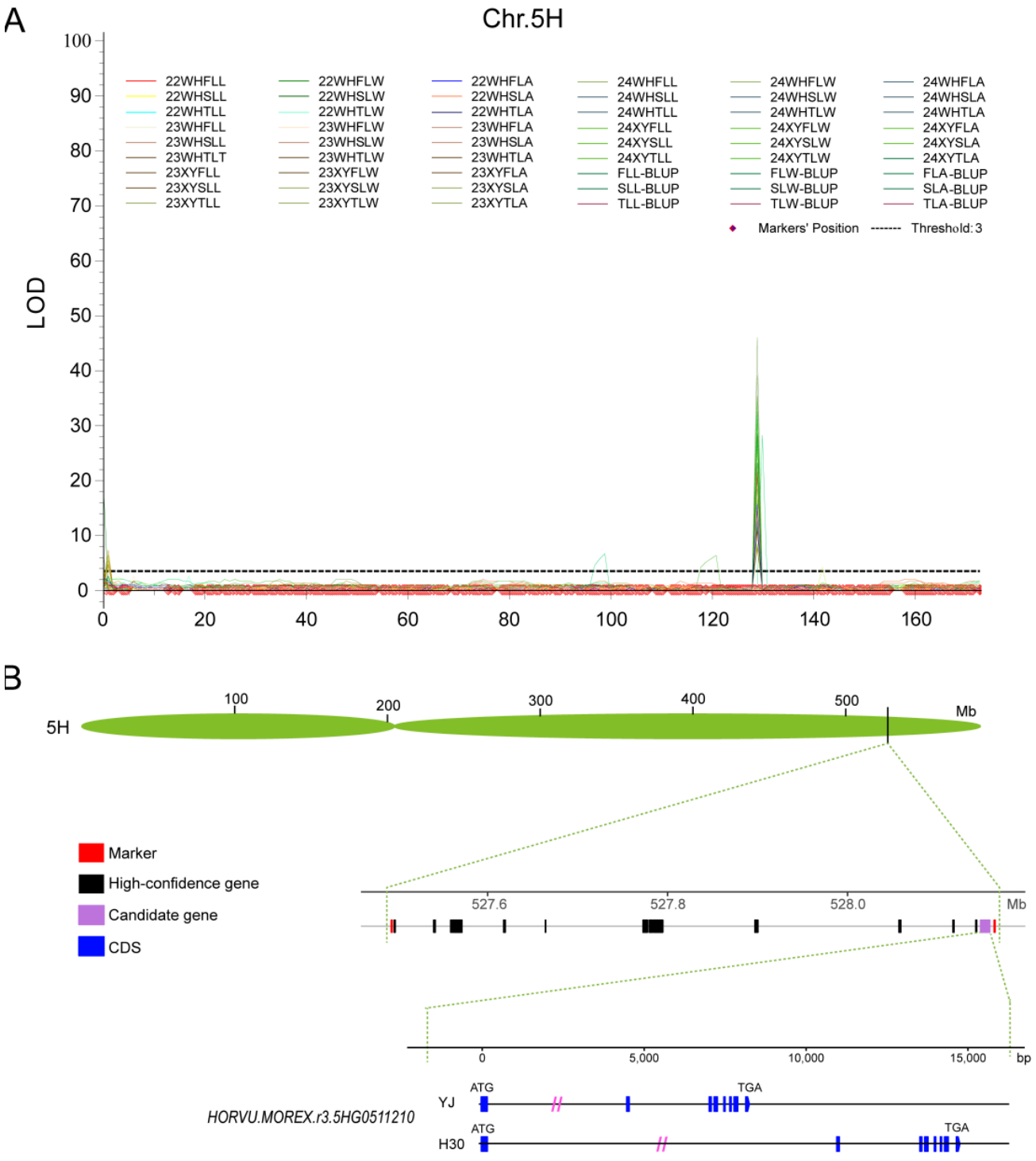


Figure 5

Localization of major effect QTLs and candidate gene structure on chromosome 5H.

A. QTL mapping on chromosome 5H. LOD distribution of nine leaf traits (FLL, FLW, FLA, SLL, SLW, SLA, TLL, TLW, TLA) across five environments and BLUP conditions on chromosome 5H. The dashed horizontal line indicates the significance threshold (LOD = 3.0). B. Gene structure and annotation within the candidate interval. The vertical axis represents physical

location (Mb). A model of the key high-confidence candidate gene is displayed, with the first intron length polymorphism between the parents annotated.

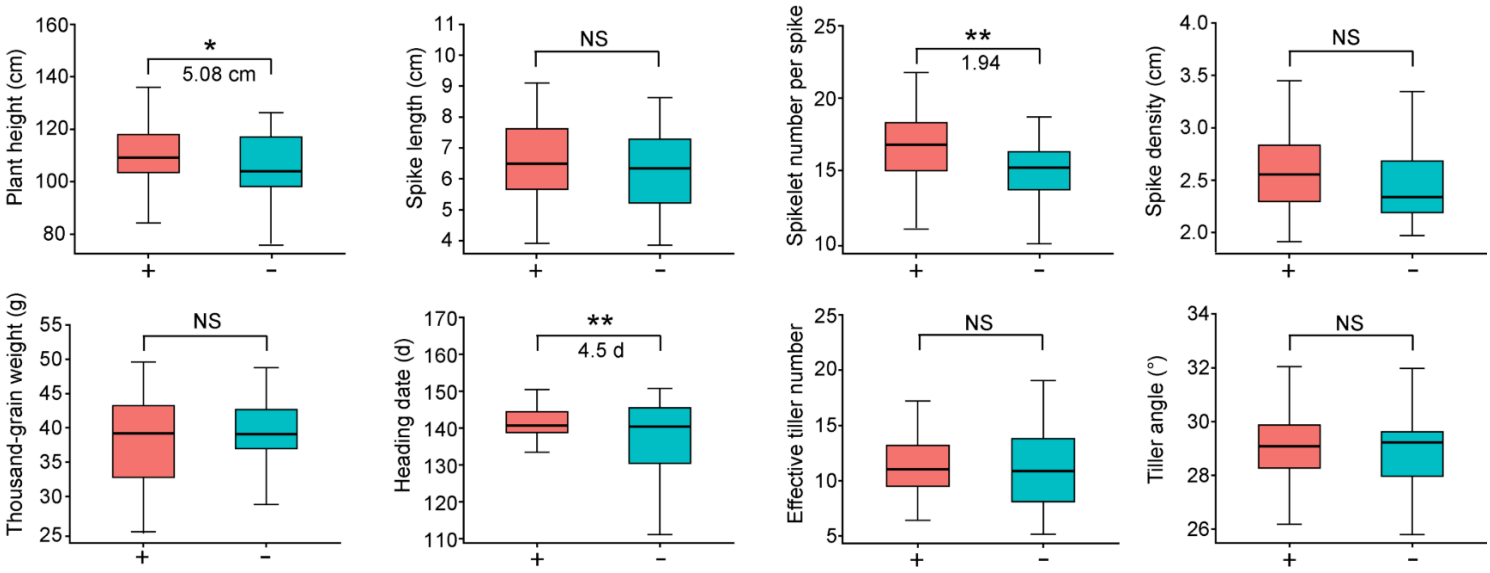


Figure 6

Effects of the major QTL on chromosome 5H on eight agronomic traits.

RILs were grouped based on the parental allele at the QTL interval: "+" indicates lines carrying the YJ allele, and "–" indicates lines carrying the H30 allele. Box plots show the median (central line), interquartile range (box), and range (whiskers). Significant differences are indicated by *P< 0.05 and **P < 0.01; NS, not significant.

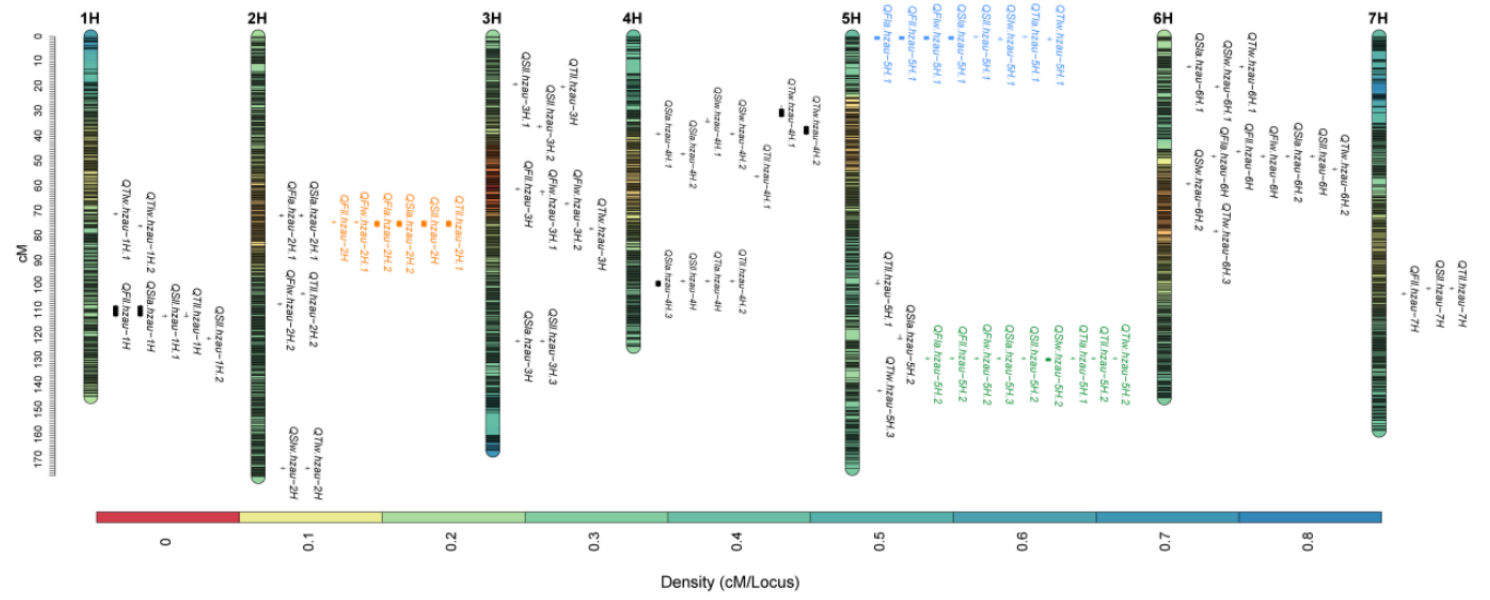


Figure 7

Distribution of barley leaf trait-associated QTLs in the genetic linkage map.

The seven linkage groups (chromosomes 1H to 7H) are displayed vertically. The black lines on the left represent the genetic linkage maps with Bin markers, with genetic distances (cM) indicated on the left scale. QTLs identified for nine

leaf traits (FLL, FLW, FLA, SLL, SLW, SLA, TLL, TLW, TLA) were identified and marked in box form at their corresponding peak locus positions. The specific QTL names were labeled on the right. Green indicates the stable QTLs detected in multiple environments, while the orange and sky blue colors represent the QTL clusters of 2H and 5H, respectively.

Supplementary Files

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