Genetic mapping and identification of Rht8-B1 that regulates plant height in wheat

Chunyun Zhou
Northwest A&F University
Hongchun Xiong
Chinese Academy of Agricultural Sciences, Institute of Crop Sciences
Meiyu Fu
Northwest A&F University
Huijun Guo
Chinese Academy of Agricultural Sciences, Institute of Crop Sciences
Linshu Zhao
Chinese Academy of Agricultural Sciences, Institute of Crop Sciences
Yongdun Xie
Chinese Academy of Agricultural Sciences, Institute of Crop Sciences
Jiayu Gu
Chinese Academy of Agricultural Sciences, Institute of Crop Sciences
Shirong Zhao
Chinese Academy of Agricultural Sciences, Institute of Crop Sciences
Yuping Ding
Chinese Academy of Agricultural Sciences, Institute of Crop Sciences
Yuting Li
Chinese Academy of Agricultural Sciences, Institute of Crop Sciences
Xuejun Li
Northwest A&F University
Luxiang Liu (liuluxiang@caas.cn)
Chinese Academy of Agricultural Sciences, Institute of Crop Sciences

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Abstract

Background

Plant Height (PH) and Spike Compactness (SC) are important agronomic traits that affect yield improvement in wheat crops. The identification of the *loci* or genes responsible for these traits is thus of great importance for marker-assisted selection in wheat breeding.

Results

In this study, we used a RIL population with 139 lines derived from crossing between the mutant *Rht8-2* and the local wheat variety NongDa5181 (ND5181) to construct a high-density genetic linkage map by applying the Wheat 40K Panel. We identified 7 stable QTLs for PH (3) and SC (4) under two environments using the RIL population, and found that *Rht8-B1* is the causal gene of *qPH2B.1* by further genetic mapping, gene cloning and gene editing analyses. Our results further showed that two natural variants from GC to TT in the coding region of *Rht8-B1* resulted in an amino acid change from G (ND5181) to V (*Rht8-2*) at the 175th position, reducing PH by 3.6%~6.2% in the RIL population. Moreover, gene editing analysis suggested that the height of T_2 generation in *Rht8-B1* edited plants was reduced by 5.6%, and that the impact of *Rht8-B1* on PH was significantly lower than *Rht8-D1*. Additionally, distribution analysis of *Rht8-B1* in various wheat resources suggested that *Rht8-B1b* have not been widely utilized in modern wheat breeding

Conclusions

The combination of *Rht8-B1b* with other favorable *Rht* genes might be an alternative approach for developing lodging-resistant crops. Our study brings important information for marker-assisted selection in wheat breeding.

Background

The global human population is predicted to continually expand and reach 10 billion by 2050, significantly increasing the need for the safe and reliable production of food. The improvement of crops using advanced technologies provides an effective strategy to meet the demand of food production in the future [1]. Wheat represents one of the most important staple crops worldwide, whereby the identification of quantitative trait loci (QTLs) important for agronomical traits, such as plant height (PH), and spikelet compactness (SC), offers critical information to ensure food security [2].

Plant height is tightly connected with lodging resistance and thus influences grain yield. To date, a total of 25 reduced height genes (*Rht1-Rht25*) have been documented in wheat [3] and classified into GA sensitive or insensitive dwarf genes based on response to GA treatment. The GA insensitive *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht2*) genes on chromosome 4B and 4D encoding truncated DELLA proteins significantly increased the harvest index and resulted in the well-known 'Green Revolution' [4, 5]; their allelic variations such as *Rht-B1c* (*Rht3*) [6], *Rht-B1e* (*Rht11*) [7], *Rht-B1p* (*Rht17*) [8], and *Rht-D1c* (*Rht10*) [9] have been identified. Moreover, several GA sensitive dwarf genes including *Rht8* [10, 11], *Rht12* [12], *Rht13* [13], *Rht18* [14], and *Rht24* [15], have been cloned or intensively studied in wheat. *Rht12* was located on chromosome 5A and mutations in *GA2oxA13* gene produced tall overgrowth phenotype in the *Rht12* background [12]. A missense mutation of *NB-LRR* gene in *Rht13* caused height reduction [13]. The dominant *Rht18* gene was identified by isolating and sequencing chromosome 6A of overgrowth mutants, and the increased

expression of *GA2oxA9* caused reduction of active GA content and thus resulted in dwarf phenotype [14]. Map-based cloning suggested that *GA2oxA9* was the causal gene of *Rht24*, which affected GA homeostasis and led to plant height reduction [15].

Since the *Rht8* gene does not influence coleoptile length, it well complements *Rht-B1b* and *Rht-D1b* weakness, and has been widely used in wheat breeding for several decades [16, 17]. *Rht8* was mapped on the short arm of chromosome 2D; and the SSR marker *Xgwm261* was regarded as a perfect diagnostic marker for *Rht8* previously [17, 18]. Using two wheat mutants *Rht8-2* and *Rht8-3* for construction of segregation populations, the *Rht8* gene was cloned recently, and it encodes an RNase H-like protein that affects bioactive GA content and changes plant height [10]. Via a wheat variety containing *Rht8* gene for map-based cloning, similar results were simultaneously obtained [11], and the results also showed that the dwarf allele of *Rht8* were positively selected during wheat breeding [10, 11].

Previous study suggested that *Rht8* not only reduced plant height but also significantly decreased spike length and thus increased spike compactness by analysis of near isogenic lines [19] and transgenic plants [11]. The modification of spike length or spikelet compactness plays an important role in the improvement of yield potential in wheat [20, 21]; and identification of its associated QTLs are critical for wheat improvement during the breeding process. In hexaploid wheat, spike morphology is regulated by three major genes, including *Q*, *C* (*Compactum*) and *S* (*Sphaerococcum*) located on chromosomes 5A, 2D and 3D [22–24]. These genes exert pleiotropic effects on spikelet compactness and length, plant height and grain shape. To date, a high number of QTLs associated with spike morphology have been identified on nearly all wheat chromosomes [25]. Additionally, the *VRN*, *Ppd* and *Eps* genes are also involved in spike length and development, and affect spikelet compactness in wheat [26–28].

In the present study, we dissected the genetic control of PH and SC by QTL mapping using a recombinant inbred line (RIL) population derived from the crossing between the wheat variety ND5181 and the mutant line *Rht8-2* and identified natural variation in the homoeologous gene *Rht8* that contributes to PH change for *qPH2B.1*. The linked markers and genes influencing PH and SC identified here can be applied to molecular breeding and promote wheat production.

Materials And Methods

Plant materials and phenotypic evaluation

We established an F_7 RIL population comprising 139 lines derived from a cross between wheat cultivar NongDa5181 (ND5181) and the mutant *Rht8-2* using a single seed descend method. The RIL population and their parents were planted in Zhongpuchang and Changping experimental fields in the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences (ICS-CAAS), Beijing, China, during 2021–2022 crop season. PH and SC were evaluated in this study. PH and SC were measured at maturity with 8 replicates for each line [29].

Genotyping and QTL mapping

Genomic DNA of the RIL population and parent lines were extracted and assessed as previously described [29]. The DNA samples were hybridized to the GenoBaits Wheat 40K Panel containing 202971 markers. Genotyping was performed at the MOLBREEDING (Shijiazhuang) Biotech Co., Ltd. (http://www.molbreeding.com). A total of 15258 homozygote SNPs were selected from ND5181 and *Rht8-2* for follow-up analyses. The BIN and MAP functions of IciMapping 4.1 were used to remove redundant markers and construct the genetic map, respectively. The genetic linkage map included 1847 Bin markers across 21 chromosomes. The threshold of the logarithm of odds (LOD) score was set to 2.5, and the Kosambi map function was used to calculate the map distance from recombination

frequencies. Composite interval mapping (ICIM) on IciMapping 4.1 was selected to identify QTLs for PH and SC. The mean values of the phenotypic traits in each line were used for QTL analysis. QTLs detected in two environments were regard as stable QTL.

KASP marker development and QTL validation

We developed the KASP assay based on the SNPs identified within or around ND5181 and *Rht8-2* from the genotyping 40K SNP array. The online primer design pipeline PolyMarker (http://polymarker.tgac.ac.uk/) was used to design specific primers. The KASP assays were performed as previously described [29]. End-point fluorescence data were screened using the microplate reader FLUOstar Omega SNP (BMG LABTECH, Germany) and analyzed by the Klustering Caller Software. The KASP markers were tested with the two parents and then the developed polymorphic KASP markers were used for the identification of genotypes in the F_7 RIL population. QTL analysis was conducted using IciMapping 4.1.

Cloning and sequencing of Rht8-B1

Genome specific primers were designed to amplify the full length of *Rht8-B1*. The genomic DNA of ND5181 and *Rht8-2* were extracted and amplified by PCR as follows: total volume 20 μL, including buffer 10 μL, dNTP 4 μL, genomic DNA 1 μL, forward primer 0.8 μL, reverse primer 0.8 μL, KOD FX 0.4 μL and ddH_2O 3 μL. The reaction conditions were 94 ℃ for 2 min, followed by 32 cycles of 98 ℃ for 10 s, annealing at 65 ℃ for 20 s, and 68 ℃ for 2 min, with a final extension of 68 ℃ for 5 min. The PCR products were sequenced at the Shanghai Sangon Biotech Co., Ltd. (https://www.sangon.com/).

Generating Rht8-B1 and Rht8-D1 mutants by gene editing

For CRISPR/Cas9-based gene editing, sgRNA target sequences and plant transformations were performed as previously described [10]. We sequenced T_2 plants and validated the mutations produced by CRISPR/Cas9-based gene editing. Single mutants of *Rht8-B1* and *Rht8-D1* were successfully selected, and the height of edited and WT plants recorded and compared.

Quantitative RT-PCR

Quantitative RT-PCR was performed according to our previous report [30]. Briefly, total RNA was isolated from the first internode below spike by using TRNzol-A^+ Reagent (Tiangen Biotech), and was then purified using the RNA purification kit (Tiangen). The first-strand cDNA was synthesized using iScript cDNA synthesis kit (Bio-Rad), and the SsoFast EvaGreen Supermix Kit (Bio-Rad) was used for quantitative RT-PCR. This experiment was conducted on a CFX 96 Real-Time System (Bio-Rad) following the manufacturer's instruction. The actin gene was used as an internal control and primers used for quantitative RT-PCR are listed in Supplementary table 5.

Distribution analysis of Rht8-B1b in wheat accessions

A total of 305 worldwide accessions with genotypic information obtained from the Wheat Union Database (http://wheat.cau.edu.cn/WheatUnion/) were used for analysis of allelic variation in *Rht8-B1* [31–34]. The frequency of *Rht8-B1b* in wheat accessions from different geographical regions was calculated according to the number of accessions carrying *Rht8-B1b* alleles.

Accession numbers

Sequence data of *Rht8-B1a* and *Rht8-B1b* are deposited in the GenBank data libraries with the following accession numbers, OQ512875 and OQ512876, respectively.

Results

Phenotypic variation of two parent lines and the RIL population

We have previously identified a semi-dwarf wheat mutant line *Rht8-2* with high yield potential [10]. To explore QTLs associated with important agronomic traits, we constructed a RIL population including 139 lines derived from the crossing between the wheat variety ND5181and the mutant line *Rht8-2*. The two parent lines showed significant differences in Plant Height (PH) and Spike Compactness (SC) under two environmental conditions (Fig. 1A). Specifically, compared to ND5181, *Rht8-2* decreased PH by 11 cm, and increased SC by 0.45 (Fig. 1; Table S1). In the RIL population, the PH and SC of RILs displayed an obviously transgressive segregation. These three traits showed normal distributions under two environmental conditions, suggesting they are controlled by multiple genes (Fig. 2). Pairwise correlation analysis between PH and SC indicated a significantly negative correlation between PH and SC in the RIL population under the two environmental conditions (Table S2).

QTL mapping analysis

A total of 23 QTLs associated with PH and SC were detected under the two environmental conditions on chromosomes 1A, 1B, 2B, 2D, 3A, 3B, 4B, 5A, 5B, 6B, 6D, 7A, 7B, and 7D, respectively. Among these QTLs, *qPH2B.1*, *qPH2D*, *qPH4B*, *qSC1B*, *qSC2B.1*, *qSC2D.1*, *qSC7D*, could be stably detected in the two environments (Fig. 3; Table 1).

Table 1
QTLs associated with plant height (PH), heading date (HD), and spikelet compactness (SC) in the Zhongpuchang and Changping environments identified with IciMapping 4.1.

Environment	Trait Name	QTL	Chromosome	Left Marker	Right Marker	LOD	PVE (%)	Add
Zhongpuchang	PH	qPH2B.1	2B	A44806	A44812	11.4684	7.994	2.5902
		qPH2B.2	2B	A48303	A48463	8.2893	5.5607	2.1144
		qPH2D	2D	A61464	A61529	22.651	19.2436	4.0568
		qPH3A	3A	A69302	A69519	5.8247	3.943	-1.7645
		qPH4B	4B	A112983	A113076	30.1221	29.7012	-4.901
		qPH7B.1	7B	A193948	A194156	10.2915	6.9504	2.414
		qPH7B.2	7B	A194789	A194814	3.9033	2.449	-1.4358
	SC	qSC1B	1B	A18513	A19081	5.0487	3.7798	0.0687
		qSC2B.1	2B	A44792	A44806	24.5623	24.9755	-0.1801
		qSC2B.2	2B	A45030	A45063	11.673	9.482	0.1119
		qSC2D.1	2D	A61140	A61372	13.6018	13.006	-0.1288
		qSC2D.2	2D	A64193	A64287	5.4426	3.9538	-0.0703
		qSC7B	7B	A193641	A193948	3.9214	2.7592	-0.058
		qSC7D	7D	A202015	A202077	2.7953	1.9425	0.0493
Changping	PH	qPH2B.1	2B	A44792	A44806	4.0065	2.1155	1.6056
		qPH2B.3	2B	A56299	A56338	5.2987	2.8879	1.9184
		qPH2D	2D	A61464	A61529	23.5792	17.8166	4.7225
		qPH3B	3B	A94610	A94625	4.2256	2.2634	-1.6462
		qPH4B	4B	A112816	A112833	40.1532	41.7687	-7.0218
		qPH5A	5A	A133195	A133216	7.5183	4.2383	2.6582
		qPH5B	5B	A134432	A141070	5.1174	3.5318	-2.0132
		qPH7A	7A	A179460	A179521	6.4762	3.6198	2.132
	SC	*qSC1A*	1A	A7344	A7362	4.4992	4.6783	-0.0555
		qSC1B	1B	A18513	A19081	6.2316	6.9554	0.0686
		qSC2B.1	2B	A44757	A44782	12.7453	14.9828	-0.1018
		qSC2D.1	2D	A61578	A61731	19.362	27.6476	-0.1415
		qSC6B	6B	A161877	A161905	3.8673	3.9563	0.0521
		qSC6D	6D	A174738	A174762	3.3927	3.504	0.0514
		qSC7B	7B	A192944	A193088	3.8252	3.8496	-0.0502

Environment	Trait Name	QTL	Chromosome	Left Marker	Right Marker	LOD	PVE (%)	Add
		qSC7D	7D	A202015	A202077	5.3974	5.6152	0.0617

Three stable QTLs associated with PH were identified on chromosomes 2B, 2D, and 4B in the two environments. The major QTL *qPH4B* showed higher LOD scores (30.1 and 40.2) that explained 29.7% and 41.8% of the phenotypic variation, respectively. In addition, *qPH2D* were identified with LOD scores 22.7 and 23.6 under the two environments and accounted for 19.2% and 17.8% of the phenotypic variation, respectively; while *qPH2B.1* had LOD scores 11.5 and 4.0 explained 7.9% and 2.1% of the phenotypic variation, respectively, being detected on the two environments. Finally, the *qPH4B* allele from ND5181 decreased PH, while the *Rht8-2* allele in *qPH2D* and *qPH2B.1* reduced PH (Table 1).

And, we identified four stable QTLs associated with SC on chromosomes 1B, 2B, 2D and 7D in the two environments. *qSC2B.1* was detected in both environments with LOD scores 24.6 and 12.7, explaining 25.0% and 15.0% of the phenotypic variation, respectively. *qSC2D.1* with LOD scores 13.6 and 19.4 was also identified in two environments and explained 13.0% and 27.6% of the phenotypic variation, respectively. *qSC1B*, with LOD scores 5.0 and 6.2, explained 3.8% and 7.0% of the phenotypic variation, respectively and was detected in the two environments. Finally, *qSC7D* with LOD scores 2.8 and 5.4 explained 1.9% and 5.6% of the phenotypic variation, respectively. Among these QTLs in ND5181, the *qSC1B* and *qSC7D* alleles increased SC and the *qSC2B.1* and *qSC2D.1* alleles decreased SC (Table 1).

Rht8-B1 **is the candidate gene of** qPH2B.1

To validate the mapped region of *qPH2B.1*, we successfully developed 9 KASP makers around this region based on 660K SNP array analysis between the ND5181 and *Rht8-2* varieties, and delimited it to a physical interval of 3.5 Mb between markers *2B-4* and *2B-5*. This QTL showed a LOD score of 3.4 and explained 11.8% of the observed phenotypic variation (Fig. 4). Given that this region included *Rht8-B1* (TraesCS2B02G073600), an homoeologous gene to *Rht8*, we sequenced the region and uncover two genetic variants (39418567–39418568, GC to TT) in the coding region of the gene that resulted in an amino acid change from G (ND5181) to V (*Rht8-2*) at the 175th position. We then analyzed the effects of *Rht8-B1* on PH and SC in the RIL population, and found that TT alleles reduced PH by 6.2% and 3.6%, shortened spike length by 6.6% and 4.7%, and increased SC by 5.8% and 6.3% in the Zhongpuchang and Changping field experiments, respectively (Fig. 4).

Rht8-B1 **exhibited a lower impact on PH reduction than** Rht8-D1

We compared the effects of *Rht8-B1* and previously reported *Rht8-D1* on PH reduction by knocking out both genes in a Fielder background and evaluating the PH of the T_2 lines. The results showed that the PH of edited plants was significantly lower than control plants. Specifically, edited *Rht8-B1* and *Rht8-D1* plants showed a PH reduction of 5.6% and 17.5%, respectively, suggesting a lower impact of *Rht8-B1* than *Rht8-D1* (Fig. 5A, B).

Expression comparison of Rht8-B1 **and** Rht8-D *1*

In order to examine the difference of expression pattern between *Rht8-B1* and *Rht8-D1*, we analyzed their transcript level using Hexaploid Wheat Expression Dataset [35], and found that both genes were highly expressed in stem at the jointing stage, while the expression of *Rht8-D1* was significantly higher than that of *Rht8-B1* (Fig. 6A). Our previous study suggested that the frameshift mutation in *Rht8-D1* caused dwarfism phenotype in *Rht8-2* and its expression

was significantly decreased in the mutant compared to that of WT [10]. To investigate the effects of mutation of *Rht8-D1* on the B subgenome of *Rht8*, we analyzed the expression of *Rht8-B1* in the first internode below spike of the mutant *Rht8-2* and WT. The results showed that the expression of *Rht8-B1* was remarkably increased in *Rht8-2* (Fig. 6B), indicating that the mutation of *Rht8-D1* affected the transcript expression of *Rht8-B1*.

Distribution of Rht8-B1 in wheat varieties worldwide

We used a total of 305 worldwide accessions from the Wheat Union Database (http://wheat.cau.edu.cn/WheatUnion/), including 193 accessions from China and 112 accessions from other countries, for the analysis of *Rht8-B1* allelic variation. Among these, 68 Chinese accessions (35.2%) contained the *Rht8-B1b* TT alleles, compared to only 6 accessions (5.4%) in other countries (Fig. 7). In China, we analyzed 118 modern varieties and 75 landrace accessions, with 20.3% and 58.7% with *Rht8-B1b* alleles, respectively (Table S3). These results suggest *Rht8-B1b* alleles were not widely used historically for wheat breeding.

Discussion

Semi-dwarf wheat usually possesses high lodging resistance with high yield stability [4]. The identification of novel genes regulating PH is pivotal for improving lodging resistance in wheat breeding. In this study, we identified *Rht8-B1* as a novel regulator of plant height by genetic mapping and gene editing analyses. By combining mapping with the Wheat40K array and developing molecular markers, *qPH2B.1* was mapped between the markers *2B-4* and *2B-5* within a physical position of 36.5–40.0 Mb on chromosome 2B of the Chinese Spring reference genome v1.0 (Fig. 4A). The *Rht8-B1* gene was located to position ~ 39.4 Mb on chromosome 2B, suggesting it is the candidate gene for *qPH2B.1*. Genotyping analysis of *Rht8-B1* in the RIL population showed genetic variants associated with PH (Fig. 4B). Further gene editing of *Rht8-B1* validated its function on the regulation of PH (Fig. 5). These results suggest *Rht8-B1* is the causal gene of *qPH2B.1*.

We recently identified *Rht8* on chromosome 2D (*Rht8-D1*) that encodes a Ribonuclease H-like protein and modifies PH by regulating the bioactive GA content using map-based cloning with two dwarfing mutants *Rht8-2* and *Rht8-3* [10]. Here, we used *Rht8-2* for the construction of the RIL population and found a major QTL – *qPH2D* – on chromosome 2D with a high LOD score value that contributes to phenotypic variation, probably representing *Rht8-D1*. In contrast to the *Rht8-D1* (*qPH2D*) locus, QTL mapping revealed the *qPH2B.1* had a minor impact on PH (Fig. 3, 4A), which is consistent with gene editing results (Fig. 5) and reports by Chai et al. [11]. The higher impact of *Rht8-D1* on height reduction is also accordance with the higher expression of *Rht8-D1* in the stem (Fig. 6A). Previous studies extensively investigated the function of the *Rht8* orthologous gene *TAC4/ sg2* in rice [36, 37]. A stop-gain mutation in *TAC4* led to a greater tiller angle and a dwarf phenotype that may result from changes in IAA content and distribution. In addition, an 8 bp-deletion in *sg2* resulted in a smaller grain phenotype due to repressed cell expansion in spikelet hulls and a semi-dwarf phenotype. These observations indicate that orthologous genes to *Rht8* affect multiple agronomic traits and play different roles and functions, highlighting the need for further exploration of the effects of *Rht8-B1* and *Rht8-D1* on PH reduction in the future.

The semidwarf *Rht8-B1b* allele has high potential for utilization in wheat breeding. By analyzing the genetic sequence of *Rht8-B1* in the mutant *Rht8-2* and wildtype (WT) variety Jing411, we found the *Rht8-B1b* allele was derived from the WT, indicating that it is a natural variation. Importantly, the distribution of the *Rht8-B1b* allele in global wheat varieties suggests it has not been widely utilized historically in wheat breeding outside of China (Fig. 7). Similar to *Rht8-D1b*, the *Rht8-B1b* allele had no effects on thousand grain weight. Several studies suggested dwarf or semidwarf genes are associated with a decrease in thousand grain weight and ultimately affect wheat yield [38–40].

The combination of *Rht8-B1b* and other dwarf genes in wheat breeding can thus be an alternative approach for developing high lodging resistant wheat.

We found consistency between the stable QTLs associated with PH and SC detected in this study and previous reports. For example, *Rht-B1b* was located at ~ 30.8 Mb on chromosome 4B within the chromosomal region corresponding to *qPH4B* [5]; *qSC2D.1* was identified between molecular markers A61578 and A61731 in positions 20.8–30.3 Mb, closely linked to the *Rht8-D1* reported to significantly reduce spike length by Chai et al. [11]; *qSC7D* was located between molecular markers A202015 and A202077 in positions 584.5-588.2 Mb, closely linked to the *WAPO1-7D* that regulates spikelet number per spike [41]. We also identified several putatively novel QTLs for PH and SC, including *qPH7B.1* on chromosome 7BL with LOD score 10.3 that explained 7.0% of the phenotypic variation in PH; and *qSC1B* located on the long arm of chromosome 1B between molecular markers A18513 and A19081, which is associated with SC.

Conclusion

We identified 7 stable QTLs for PH and SC under two environments using a RIL population in this study. We used mapping and gene editing analyses and found that *Rht8-B1* is the causal gene of *qPH2B.1* and affects PH variation. *Rht8-B1* showed lower effects on PH than *Rht8-D1* and has not been widely utilized in wheat breeding. This implies that combining *Rht8-B1b* with other favorable *Rht* genes has great potential for breeding lodging resistant wheat varieties. Our study revealed novel linked markers and QTLs that provide important information for marker-assisted selection in wheat breeding.

Declarations

Ethics approval and consent to participate

Experimental research and field studies on plants including the collection of plant material are comply with relevant guidelines and regulation.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in the main text article and its supplementary files. The variant data for this study have been deposited in the European Variation Archive (EVA) at EMBL-EBI under accession number PRJEB60409 (http:// www.ebi.ac.uk/eva/?eva-study=PRJEB60409).

Competing interests

The authors declare that they have no conflict of interest.

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Author contributions

L. L. and X. L. conceived the project and revised the manuscript. C. Z., H. X, and M. F. conducted most of the experiments and analyzed the data. H. G., L. Z., Y. X., J. G., S. Z., Y. D. Y. L. helped in RIL population planting and data analysis. C. Z., and H. X. wrote the first draft of the manuscript. All authors have read and approved the final manuscript.

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Figures

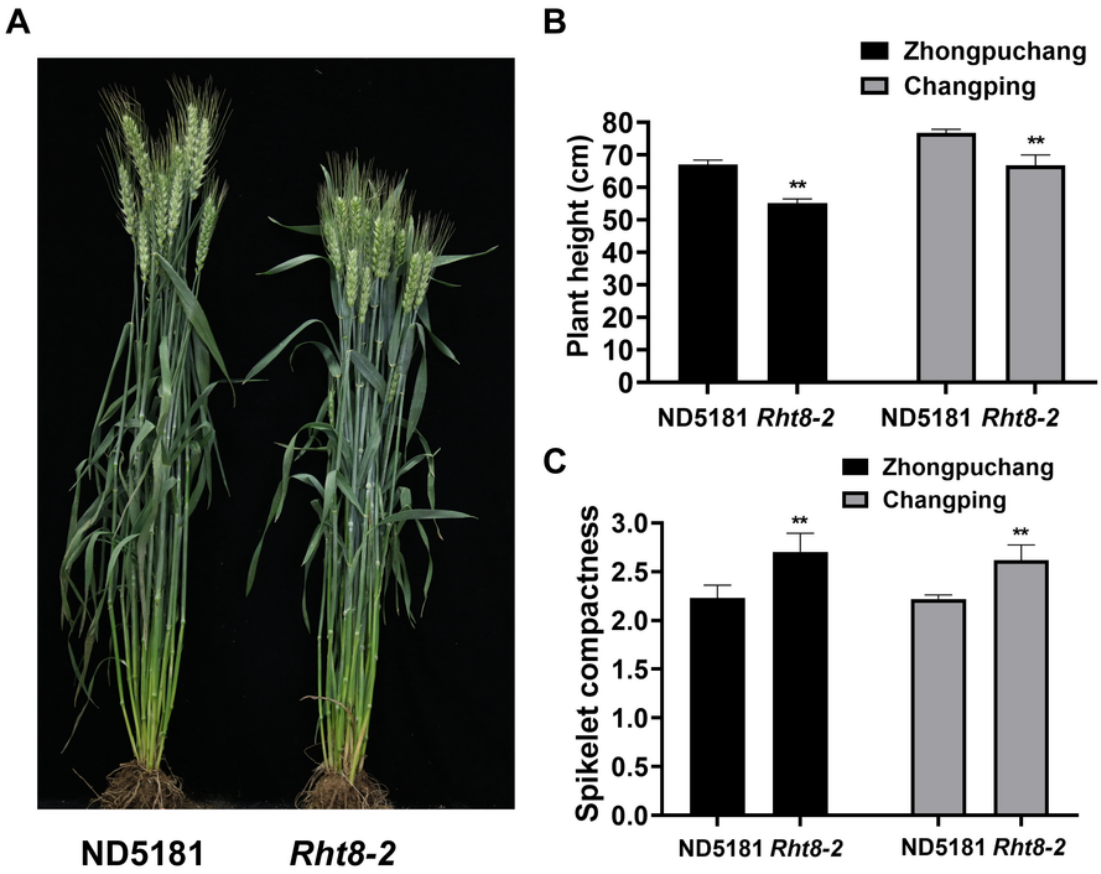

Figure 1

Phenotype comparisons between ND5181 and *Rht8-2*. A. Phenotype of ND5181 and *Rht8-2*. B. Plant height of ND5181 and *Rht8-2*. C. Spikelet compactness of ND5181 and *Rht8-2*.

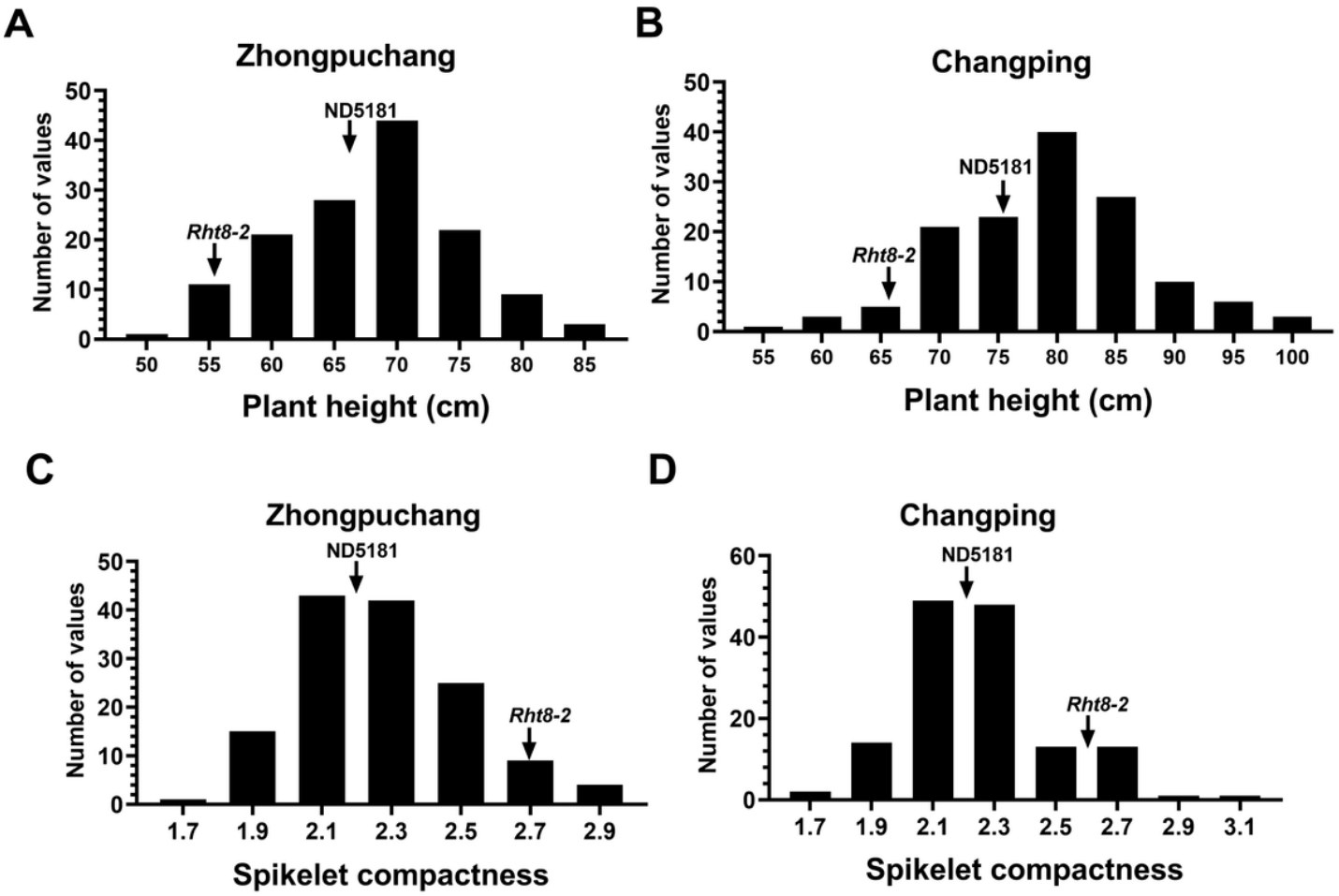

Figure 2

The frequency distribution of plant height, heading date and spikelet compactness in the RIL population. A-B. Frequency distribution of plant height in the Zhongpuchang (A) and Changping (B) environments. C-D. Frequency distribution of spikelet compactness in in the Zhongpuchang (C) and Changping (D) environments. Phenotypic values of the two parental lines are marked by vertical arrows.

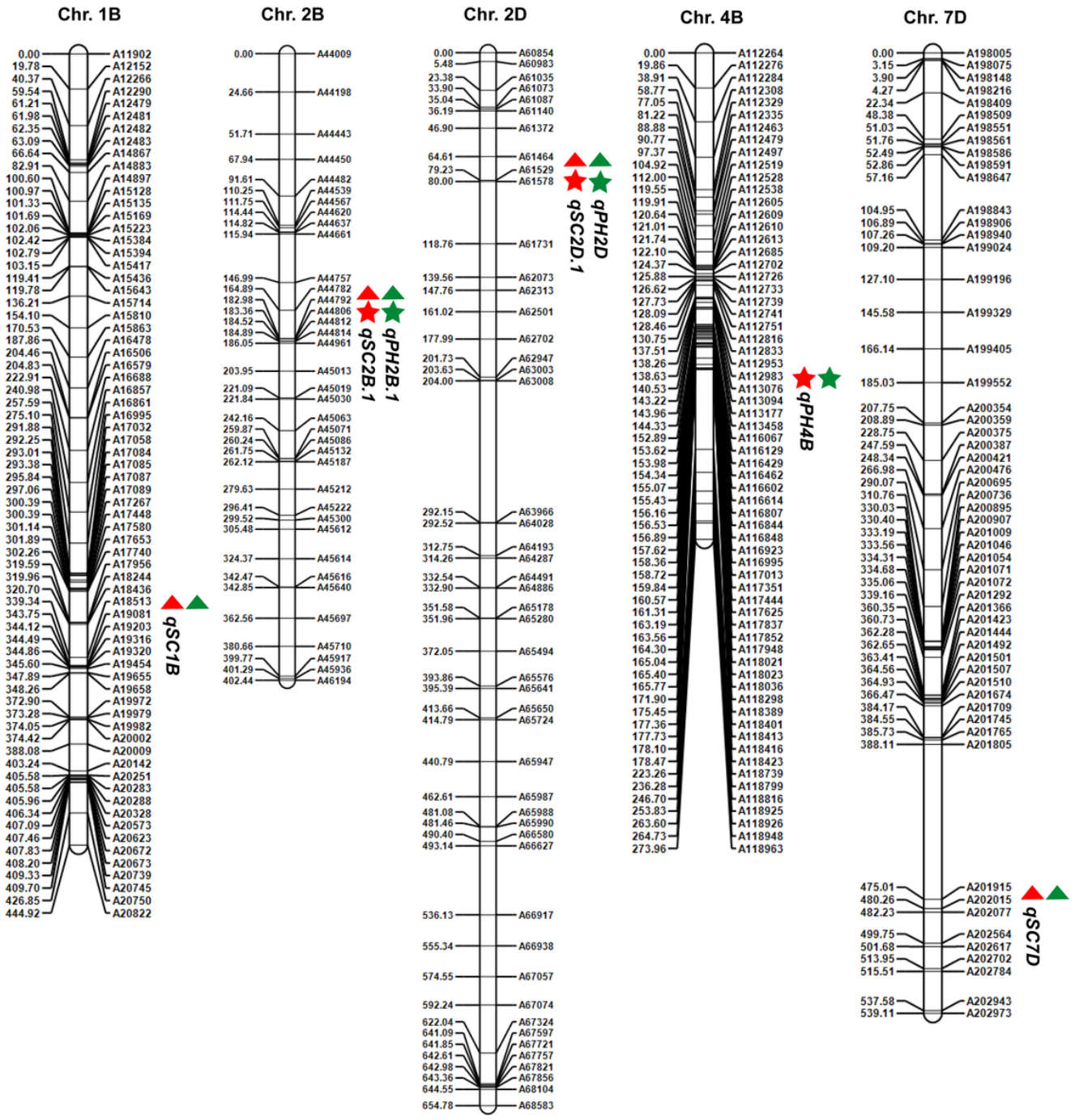

Figure 3

Chromosomal locations of the identified stable QTLs associated with PH and SC. The stars and triangles represent PH and SC, respectively. The red and green colors indicate data from the Zhongpuchang and Changping environments, respectively.

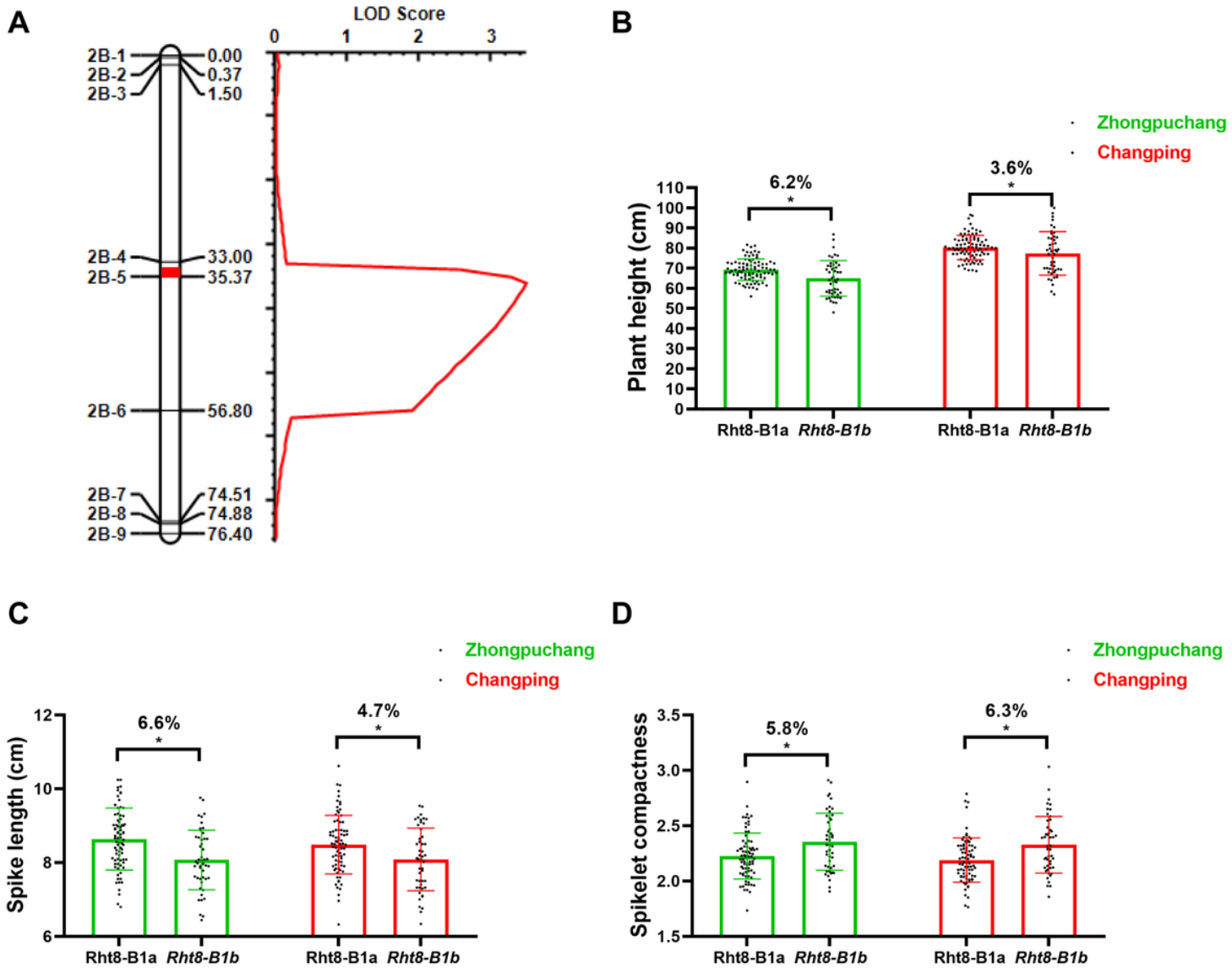

Figure 4

QTL validation of *qPH2B.1*and the predicted effects of *Rht8-B1b* in the RIL population. A. QTL validation of *qPH2B.1*. B-D. The effects of *Rht8-B1b* on plant height (B), spike length (C) and spikelet compactness (D).

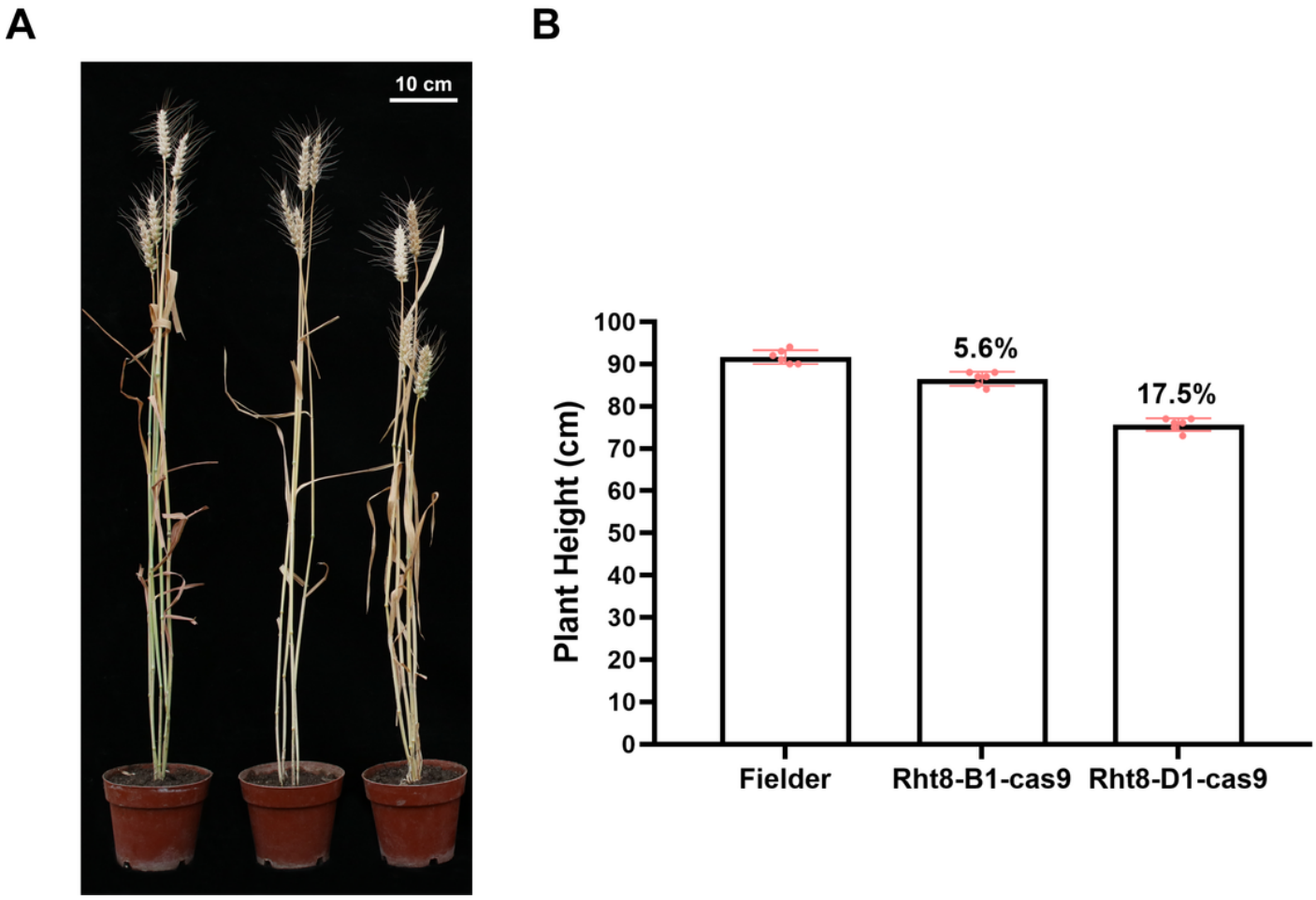

Figure 5

The effect of *Rht8-B1*and *Rht8-D1* on plant height of edited plants. A. Phenotype of Fielder and edited lines (*Rht8-B1* and *Rht8-D1*). B. Plant height of Fielder and edited lines (*Rht8-B1* and *Rht8-D1*).

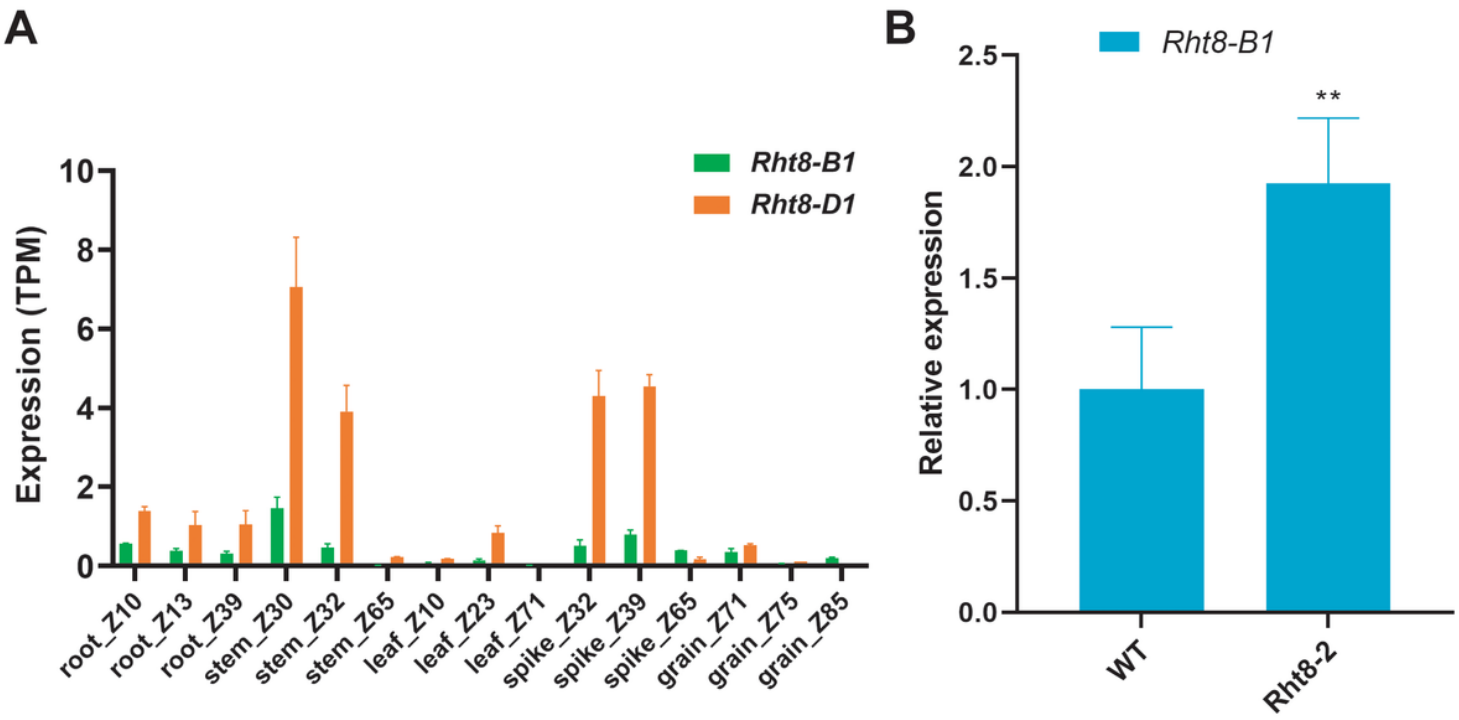

Figure 6

A. The expression data of *Rht8-B1* and *Rht8-D1* from Hexaploid Wheat Expression Database. B. The expression analysis of *Rht8-B1* in the first internode below spike between WT and *Rht8-2*.

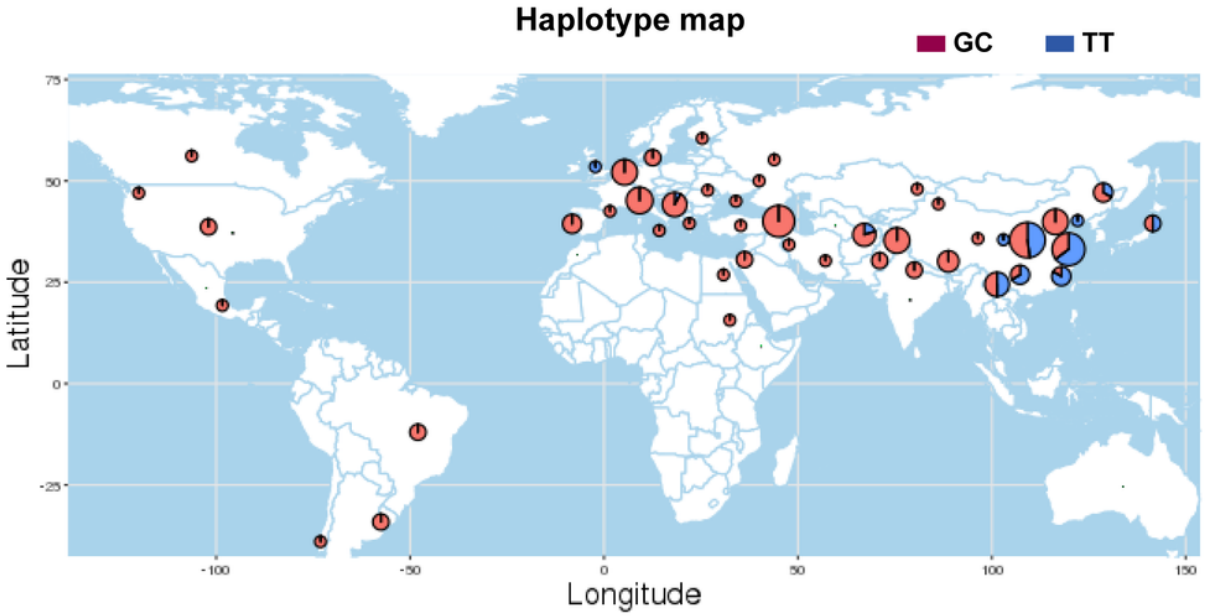

Figure 7

Distribution analysis of *Rht8-B1b*in wheat accessions worldwide. GC and TT represent *Rht8-B1a* and *Rht8-B1b*, respectively.

Supplementary Files

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