

RESOURCES

Chromosome-scale assembly of wheat cultivar Sumai 3, a major germplasm source for Fusarium head blight resistance

Rubylyn D. Mijan^{1,2}  | Bikash Poudel^{1,2}  | Sittal Thapa^{1,2} |
 Oluwatayo Ajayi-Moses^{2,3}  | Thomas Lux⁴ | Raz Avni⁵  | Steven Xu⁶  |
 Manuel Spannagl^{4,7} | Martin Mascher^{5,7}  | Jeff Maughan⁸  | Jason D. Fiedler² 

¹Department of Plant Pathology, North Dakota State University, Fargo, North Dakota, USA

²USDA-Agricultural Research Service, Cereal Crops Improvement Research Unit, Edward T. Schafer Agricultural Research Center, Fargo, North Dakota, USA

³Department of Plant Sciences, North Dakota State University, Fargo, North Dakota, USA

⁴Plant Genome and Systems Biology (PGSB), Helmholtz Center Munich, German Research Center for Environmental Health, Neuherberg, Germany

⁵Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Seeland, Germany

⁶USDA-ARS, Crop Improvement and Genetics Research Unit, Western Regional Research Center, Albany, California, USA

⁷Centre for Crop & Food Innovation, Food Futures Institute, Murdoch University, Perth, Western Australia, Australia

⁸Plant and Wildlife Sciences, Brigham Young University, Provo, Utah, USA

Correspondence

Jason D. Fiedler, USDA-ARS, Cereal Crops Improvement Research Unit, Edward T. Schafer Agricultural Research Center, Fargo, North Dakota, USA.
 Email: jason.fiedler@usda.gov

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Abstract

Fusarium head blight (FHB) is a devastating disease that severely impacts global wheat (*Triticum aestivum* L.) production. Sumai 3, a wheat cultivar widely used in breeding programs for its strong FHB resistance, has not been fully resolved at the chromosome level. Here, we present a high-quality chromosome-scale assembly of Sumai 3 using PacBio HiFi reads and chromosome conformation capture sequencing. The 14.6 Gb assembly consists of 832 contigs, with the longest contig being 245.2 Mb and a contig N50 of 41.90 Mb, which were scaffolded into 21 pseudomolecules. *De novo* annotation identified 104,620 high-confidence protein-coding genes and found 92.67% of the genome to consist of repetitive sequences. Synteny analysis showed strong collinearity between Sumai 3 and the wheat reference sequence Chinese Spring IWGSC RefSeq v2.1 (CS). Structural variant analysis identified chro-

Abbreviations: Ala-RNA, alanyl-tRNA synthetase; BLAST, basic local alignment search tool; BUSCO, benchmarking universal single-copy orthologs; CCS, circular consensus sequencing; Hyp, hypothetical protein; CS, Chinese Spring IWGSC RefSeq v2.1; FCS, Foreign Contamination Screen; FHB, Fusarium head blight; HC, high-confidence; Hi-C, chromosome conformation capture; His, histidine-rich calcium-binding protein; HMW, high-molecular weight; IWGSC, International Wheat Genome Sequencing Consortium; LTR, long terminal repeat; miRNA, microRNA; MT, tRNA methyltransferase; NAD, oxidoreductase NAD-binding; ncRNA, non-coding RNA; PAP, plastid-lipid associated proteins; PFT, pore-forming toxin-like protein; PG, polygalacturonase; QTL, quantitative trait locus; rRNA, ribosomal RNA; Sin, sina superfamily protein; snRNA, small nuclear RNA; SV, structural variant; TE, transposable elements; tRNA, transfer RNA; TS, terpene synthase.

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mosome 2A with the highest number of deletions (2773) and insertions (2645), while chromosome 3B had the most inversions (359). Duplications were most frequent on 2A (337), and contractions on 5B (122). The gene content of the major resistance quantitative trait loci on 3B, *Fhb1*, largely validates previous annotations for CS, although we discovered two new genes at approximately 12.4 Mb, including an additional copy of a terpene synthase, further suggesting homology with CS 3D over 3B. Differential expression analysis highlighted up-regulation of three genes coding pore-forming toxin-like protein, sina superfamily protein, and plastid-lipid-associated proteins potentially involved in FHB resistance. This assembly provides critical insights into FHB resistance and offers a valuable genomic resource for wheat breeding programs.

Plain Language Summary

Fusarium head blight is a destructive disease of small grains that decreases yield and grain quality, leading to reduced revenue for producers. The most efficient method of limiting disease in the field is cultivation of genetically resistant crop varieties, which are developed through deployment of resistance gene regions. Several of these gene regions arise from the remarkably resistant variety Sumai 3, which has been the most important source for small grains breeders throughout the world. Here we present a high-quality genome sequence of this variety along with a characterization of all its genes. This information will provide an accurate framework to accelerate research into the molecular mechanism of how this variety combats disease and make it easier to utilize these gene regions in the future.

1 | INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is one of the most widely grown and important staple crops, providing a significant source of calories and nutrients to the global population (Shiferaw et al., 2020). However, wheat production faces numerous challenges, including the threat of Fusarium head blight (FHB), also known as scab, caused by the fungal pathogen *Fusarium graminearum* Schwabe (Dweba et al., 2017; Lilleboe & Roth, 2011; McMullen et al., 2012; Salgado et al., 2015). FHB is a disease of wheat spikes that severely reduces grain quality and leads to substantial yield losses (Rojas et al., 2020). Spring grain crops are especially susceptible to FHB since their flowering usually occurs during warm and humid environmental conditions that favor the growth, infection, and spread of the fungus (Birr et al., 2020; Doohan et al., 2003; Parry et al., 1995; X. M. Xu et al., 2008). A major FHB epidemic in 1993 severely impacted wheat production in the northern United States hard red spring wheat growing areas of Minnesota, North Dakota, and South Dakota, resulting in a yield loss of 4.8 million metric tons and causing an economic loss of approximately \$704 million (Beard & Hamlin, 1995). This epidemic underscored the urgent need for the

development of wheat varieties with enhanced resistance to FHB to ensure the sustainability and profitability of wheat production in the region.

Resistance to FHB in wheat is a complex trait that is inherited quantitatively and involves multiple genetic loci and environmental interactions (Mesterhazy, 2020). The resistance is classified into four major types: type I, which confers resistance to initial infection, type II, which limits the spread of the fungus within the spike, type III, which is resistance to kernel infection, and type IV, which is resistance to mycotoxin accumulation (Mesterhazy, 1995; Miller & Amison, 1986; Schroeder & Christensen, 1963). Genetic studies have identified numerous quantitative trait loci (QTLs) and genes associated with disease resistance, with *Fhb1* (Cuthbert et al., 2006; S. Liu et al., 2006 and *Fhb7* (Guo et al., 2015; Wang et al., 2020) being among the most significant loci controlling type II resistance. The *Fhb1* gene, located on the short arm of chromosome 3B, has been “cloned” by three different groups identifying different genes as the causative agent. Rawat et al. (2016) described the *Fhb1* gene as a gene coding pore-forming toxin-like (PFT) protein, suggesting a direct antimicrobial role in FHB resistance. In contrast, Su et al. (2019) later identified *Fhb1* as the nearby gene

encoding a putative nuclear-localized, histidine-rich calcium-binding protein, working through a susceptibility mode of action where resistance results from a loss-of-function mutation. Building on this, G. Li et al. (2019) fine-mapped *Fhb1* to a 23.8-kb interval and pinpointed TaHRC as the sole candidate gene within that region. However, unlike Su et al., G. Li et al. interpreted the resistance-conferring allele as a functional variant rather than a knockout, emphasizing the complexity of *Fhb1*'s molecular nature. Over the years, *Fhb1* has been extensively incorporated into more than 20 modern cultivars used for wheat production (Z. Zhu et al., 2019). However, this locus has shown inconsistent effectiveness when introgressed into many different genetic backgrounds (Brar et al., 2019; G. Li et al., 2023; Neupane et al., 2024; Yan et al., 2025). Durum wheat is especially vulnerable to this variable efficacy, and it has been postulated that accessory genes on other chromosomes interact with *Fhb1* to promote or limit its function (X. Zhu et al., 2022; 2016).

Despite extensive investment in research, the complete set of genes conferring resistance or tolerance to FHB has eluded complete characterization for decades. This challenge has largely been due to the complex genetic architecture of wheat, combined with the lack of comprehensive sequence information in the reference genome (The International Wheat Genome Sequencing Consortium [IWGSC], 2018; Walkowiak et al., 2020). The lack of detailed genetic maps has confounded efforts to pinpoint the specific interacting loci and resistance haplotypes associated with FHB resistance (Bai & Shaner, 2004). Traditional mapping techniques have often fallen short in resolving the intricate network of QTLs that govern this resistance, leading to a significant gap in our understanding of the genetic basis of FHB resistance (Buerstmayr et al., 2009). The multi-genic nature of FHB resistance, coupled with environmental interactions, further complicates the identification and functional validation of resistance genes (Mesterhazy, 1995). Recent advancements in genomic technologies have begun to overcome these hurdles. The advent of high-throughput sequencing methods, including short-read sequencing (The International Wheat Genome Sequencing Consortium [IWGSC], 2018), third generation long-read sequencing (Pucker et al., 2022), and high-resolution genotyping platforms (Geethanjali et al., 2024), have revolutionized our ability to dissect the wheat genome. These technologies provide the resolution necessary to identify small polymorphisms, structural variants, and presence-absence variations that determine FHB resistance. For instance, the development of long-read sequencing platforms such as PacBio and Nanopore has enabled the rapid assembly of contiguous genome sequences, revealing previously hidden genetic elements that contribute to disease resistance (Murigneux et al., 2020). Moreover, the integration of chromosome conformation capture (Hi-C) sequencing data has facilitated the scaffolding of these contigs into chro-

Core Ideas

- Chromosome-scale genome of Sumai 3, a key source of fusarium head blight resistance in wheat.
- High-quality assembly improves resolution of key loci like *Fhb1*, a major contributor to Fusarium head blight resistance.

mosomal pseudomolecules, allowing for the production of many new wheat genomes (Akpınar et al., 2022; Athiyanan et al., 2022; Aury et al., 2022; Kale et al., 2022; Z. Liu et al., 2024; S. Liu et al., 2025; Sato et al., 2021; T. Zhu et al., 2021) and more accurate mapping of resistance loci (Ghurye et al., 2019). Here, we report the utilization of these technologies to generate a high-quality de novo assembly of the Sumai 3 cultivar for use as a robust foundation to build genomics research upon and better understand this disease.

2 | MATERIALS AND METHODS

2.1 | Plant materials, library preparation, and sequencing

T. aestivum L. cv Sumai 3, used to generate a chromosome-level genome assembly, was developed in China by crossing two moderately susceptible parents, Funo and Taiwanxiao-mai, and was found to exhibit high-level FHB resistance and other important agronomic traits (Bai & Shaner, 1994). Seedlings of Sumai 3 were grown in a controlled hydroponic growth chamber adjusted with 12-h photoperiod and day/night temperature set to 20°C/18°C. The growth solution was made using MaxiBloom Hydroponics Plant Food (General Hydroponics) at a concentration of 1.7 g/L. In preparation for Pacific Biosciences High-Fidelity (PacBio HiFi) sequencing, high-molecular weight (HMW) DNA was extracted from 2-week-old young leaf tissues after 72-h dark treatment using a cetyltrimethylammonium bromide-Qiagen Genomic-tip protocol as described previously (Vaillancourt & Buell, 2019). DNA quantity and quality were checked using the Qubit double-stranded DNA high sensitivity assay and Nanodrop spectrophotometer, respectively. HMW genomic DNA was sheared to 17 kb on a Diagenode Megaruptor and then processed into single molecule real-time (SMRT) bell-adapted libraries using the SMRTbell Express Template Prep Kit 2.0. Size selection was performed using a Sage BluePip-pin to select fragments greater than 15 kb and then sequenced at the Brigham Young University DNA Sequencing Center (Provo, UT) using Sequel II Sequencing Kit 2.0 with Sequenc-

ing Primer v5 and Sequel Binding Kit 2.2 for 30 h with adaptive loading using PacBio SMRT Link recommendations. In total 21 cells were run resulting in 397 Gb of circular consensus sequencing (HiFi CCS) data, which is approximately 25.6x genome coverage. For Hi-C sequencing, two independent libraries were prepared by Phase Genomic, Inc. using the four-base restriction endonuclease DPNII and sequenced on the Illumina NovaSeq platform (Illumina) with a total output of 45x coverage. Total RNA was extracted from stem, leaf, spikelet, and rachis tissues with a Qiagen RNA Easy Plant Kit, pooled at equimolar concentrations, and converted into a single IsoSeq library. This library was sequenced with one PacBio HiFi cell, resulting in 7.3 Gb of data. Total RNA was also extracted from spikelet tissues collected 5 days post-inoculation with water (treated as mock) and *F. graminearum* conidia spores under three replications. High-quality RNA (RNA integrity number [RIN] ≥ 8) was sent out to Novogene where libraries were prepared and sequenced, yielding ~10 Gb raw reads per individual samples. Raw reads were trimmed using Trimmomatic (Bolger et al., 2014), and quality was checked using FASTQC (fast quality control; Andrews, 2010). Clean reads ($Q \geq 30$) were mapped to a de novo high-quality Sumai 3 genome using HISAT2, and differentially expressed genes were identified using the Hisat2-featurecounts-DESeq2 pipeline. Differential gene expression analysis was performed using DESeq2 (Love et al., 2014), applying a stringent false discovery rate (FDR) cutoff of < 0.05 and $\log_2$ fold change ($\log_2\text{FCI}$) > 1 to identify significantly regulated genes.

2.2 | Genome assembly and scaffolding

Primary assembly contigs were generated by assembling PacBio HiFi reads with Hifiasm v0.16.1 using default parameters and option `-l0` (disable duplicate purging). The TRITEX pipeline (Monat et al., 2019) was used to inspect and curate the contig sequences and scaffold primary contigs into pseudomolecules with Hi-C proximity. Briefly, Hi-C reads were aligned to the primary contigs and only uniquely aligned paired-end reads were retained for downstream analyses. The resulting files were plotted as part of the TRITEX software pipeline (Monat et al., 2019; Figure S1b) and to facilitate manual curation in cases where the Hi-C map did not show a contiguous sequence. The final assembly consisted of 21 pseudomolecules and 4362 unanchored contigs that were concatenated into an unknown chromosome pseudomolecule. NCBI Foreign Contamination Screen (FCS) (Astashyn et al., 2024) was employed to detect and eliminate contaminant sequences. The FCS identified 67 contaminant fragments, including 66 adaptor fragments and one mitochondrial fragment across 21 pseudomolecules, totaling 3898 (0.03%) contaminant nucleotides that were subsequently hard-masked.

2.3 | Genome assembly quality and annotation of repetitive and non-coding elements

Genome completeness of Sumai 3 was assessed relative to conserved orthologous genes with the poales_odb10 database using benchmarking universal single-copy orthologs (BUSCO) v5.7.1 (Manni et al., 2021). Transposable elements (TEs) were annotated using a homology-based approach implemented in RepeatMasker v4.2.0 (Smit et al., 2015). A custom repeat library was constructed by combining wheat TE sequences from ClariTeReP (<https://github.com/jdaron/CLARI-TE>) and 801 *T. aestivum* TE elements (https://trep-db.uzh.ch/?db=TREP_main). Long terminal repeats (LTRs) were initially identified using LTR_FINDER v1.06 (Z. Xu & Wang, 2007). To enhance detection efficiency and accuracy for estimating LTR assembly index (LAI), LTR_FINDER_parallel v1.2 (Ou & Jiang, 2019) was employed. Non-coding RNAs (ncRNAs), including microRNAs (miRNAs), small nuclear RNAs (snRNAs), ribosomal RNAs (rRNAs), and other regulatory elements, were annotated using Infernal v1.1.2 (Nawrocki et al., 2009) against the Rfam database v14.8 (where Rfam is RNA families) (Griffiths-Jones et al., 2005). Further identification of ribosomal and transfer RNAs (tRNAs), RNAmmer v1.2 (Lagesen et al., 2007) and tRNAscan-SE v1.3.1 (Lowe & Eddy, 1995) were used, respectively, identifying functional RNA elements across the genome.

2.4 | Gene prediction, functional annotation, and comparative gene analysis

We performed de novo structural gene prediction, confidence classification, and functional annotation, following the protocol described by Mascher et al. (2021). The strategy applied in this study only differs in the use of Helixer (Stiehler et al., 2021), run with standard parameters, as an additional ab initio input source for Evidence Modeller (weight set to 10). Further refinement, untranslated region (UTR) as well as isoform calling was performed using Mikado, improving the confidence and completeness of the final gene set (Venturini et al., 2018). To assess gene conservation and classification, a comparative analysis of high-confidence (HC) and low-confidence genes was conducted using Chinese Spring IWGSC RefSeq v2.1 (CS) as reference and Sumai 3 as the query.

2.5 | Whole-genome alignment and structural variant analysis

Whole-genome alignments between CS and Sumai 3 genomes were performed using Minimap2 v2.24 (H. Li,

2018) to generate pairwise alignments and assess large-scale genome structure and conservation. Dot plots visualizing the alignments were generated with a custom R script (<https://github.com/rubylyn-mijan/whole-genome-dotplot-from-PAF-alignment.git>) with the ggplot2 package to illustrate overall syntenic relationships. Structural variants, including insertion, deletion, duplication, translocation, inversion, and contraction, were detected between the telomere-to-telomere (T2T) reference genome CS-IAAS (Chinese Spring-Institute of Advanced Agricultural Sciences; S. Liu et al., 2025) and the query Sumai 3 with MUM&Co (v 3.8) using default parameters, including a minimum alignment length of 50 bp (O'Donnell & Fisher, 2020), enabling identification of genome-wide differences contributing to structural divergence.

2.6 | *Fhb1* region syntenic analysis

For the syntenic analysis of the *Fhb1* region, coding sequences of annotated genes within the known *Fhb1* locus were aligned to Sumai 3 chromosome 3BS bacterial artificial chromosome (BAC) sequence (GenBank Accession KX907434.1) and the Chinese Spring International Wheat Genome Sequencing Consortium (IWGSC) RefSeq v2.1 3BS and 3DS chromosomes using basic local alignment search tool (BLAST) v2.16.0 (Altschul et al., 1990) to assess sequence similarity and identify homoeologous regions. This alignment-based comparison enabled quantification of coding sequence conservation and identification of potential homologs across wheat subgenomes. Gene collinearity and syntenic relationships within *Fhb1* region were visualized using Jbrowse2 (Diesh et al., 2023) and the interactive web-based platform SynVisio (Bandi & Gutwin, 2020) (<http://synvisio.github.io/>), facilitating detailed examination of gene orientation, and structural conservation (Figure S2). To complement the structural comparison, RNA-seq data from Sumai 3 and control plants inoculated with *F. graminearum* spores were analyzed to evaluate expression patterns of genes annotated within *Fhb1* region, enabling integration of transcriptomics evidence with structural comparisons.

3 | RESULTS AND DISCUSSION

3.1 | Chromosome-scale genome assembly of Sumai 3

Using PacBio HiFi CCS and Hi-C reads, the final assembly of Sumai 3 genome (Figure 1; Figure S1b) was compared against five other wheat varieties, including Fielder (Sato et al., 2021), Kariaga (Athiyannan et al., 2022), Attraktion (Kale et al., 2022), Renan (Aury et al., 2022), and CS (T. Zhu et al., 2021).

The total genome sizes across all varieties remain comparable, ranging from 14.26 Gb (Renan) to 14.7 Gb (Fielder). Sumai 3's genome spans 14.6 Gb, slightly larger than the reference genome CS (14.57 Gb) and similar to the other cultivars. The total chromosome lengths for Sumai 3 are closely aligned with CS. The D-genome chromosomes (Chr1D, Chr4D, and Chr6D) remain the smallest across assemblies supporting their relatively compact and conserved nature (Athiyannan et al., 2022; Aury et al., 2022; Kale et al., 2022; Sato et al., 2021; T. Zhu et al., 2021). In contrast, the largest chromosomes are consistently found in the A and B subgenomes, particularly Chr3B (858.38 Mb), which shows only minor size variation among cultivars, further indicating strong structural conservation (Table S1). The longest contig length in Sumai 3 (245.2 Mb; Table S2) is markedly larger than any of the other genomes, with the next highest being 171.22 Mb in Fielder and 158.43 Mb in Kariaga. CS, being assembled from short read sequences, exhibits the most fragmented assembly, with its longest contig being only 3.53 Mb. Similarly, contig N50, which represents the contig length at which 50% of the genome is contained in contigs of at least that size, is 41.9 Mb in Sumai 3, double that of Fielder (20.70 Mb) and higher than Kariaga (30.22 Mb), and significantly higher than CS (0.34 Mb). The contig count further highlights the fragmentation of CS (306,274 contigs) compared to Sumai 3 (832 contigs), suggesting a more contiguous and high-quality assembly in Sumai 3. Across all assemblies, BUSCO completeness remains high, with Sumai 3 at 99.7% (Table 1; Figure S1a), slightly surpassing CS (99.3%) and other cultivars (99.2%–99.4%). In terms of HC genes, Sumai 3 contains 104,620 genes, which is fewer than CS (106,913 genes) and other varieties such as Fielder (116,480 genes) and Kariaga (116,838 genes), likely due to the limited number of tissues that were used in the IsoSeq library preparation. While Sumai 3 has a slightly lower number of annotated genes (Table 1), its high contiguity and assembly quality suggest improved structural integrity and accuracy in gene localization (Michael & VanBuren, 2020).

Despite this overall conservation, differences in assembly quality were observed across subgenomes. The LAI values indicate a high-quality assembly, with A subgenome showing slightly higher values, ranging from 23.93 (Chr4A) to 25.61 (Chr5A) (Table S3), suggesting more complete representation of repetitive elements consistent with previous findings associating higher LAI scores with extensive retention and continuity of LTR elements (Ou et al., 2018). The D subgenome exhibited comparatively lower LAI values, with Chr3D (22.42) and Chr7D (22.44), but also showed reduced fragmentation, with fewer contigs and higher contiguity. In contrast, the B subgenome displayed higher contig counts (92 contigs), making it the most fragmented chromosome in the Sumai 3 assembly. ncRNA distribution varied across chromosomes, with enrichment patterns differing by subgenome.

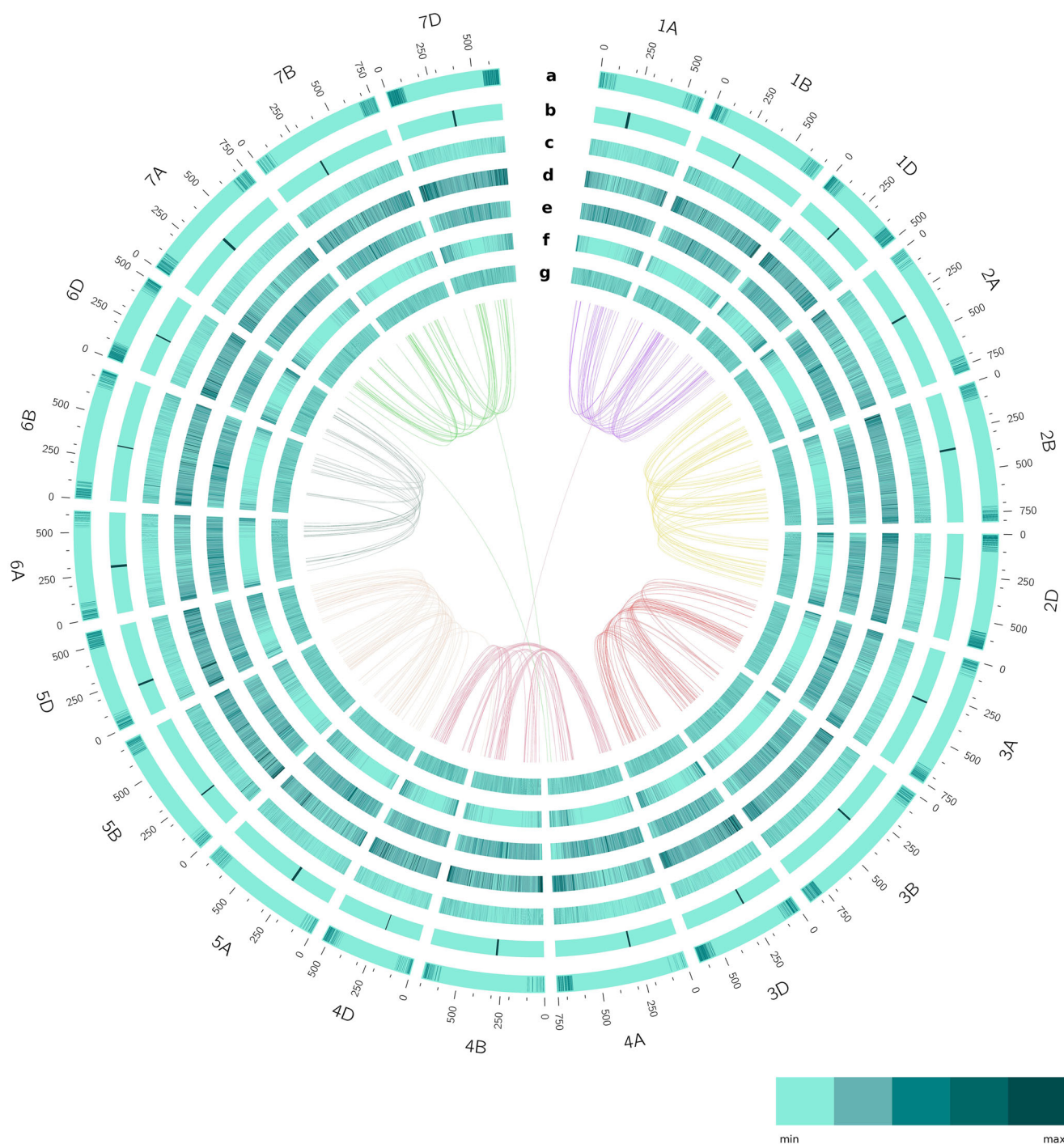


FIGURE 1 Overview of Sumai 3 chromosome-scale assembly. (a) Distribution of the *Aegilops tauschii* Coss. clone A6-10 subtelomeric tandem repeat sequence (GenBank Accession AY249980.1). (b) Distribution of the *A. tauschii* clone 6C6-3 (GenBank Accession AY249981.1) and 6C6-4 (GenBank Accession AY249982.1) and *Triticum monococcum* ssp. *aegilopoides* (Link) Thell. clone BAC TbBAC5 (GenBank Accession DQ904440.1) and TbBAC30 (GenBank Accession EF624064.1) centromere-specific tandem repeat sequences. (c) Distribution of the noncoding gene density. (d) Distribution of tandem repeat density (e) Distribution of the long terminal repeat density. (f) the high-confidence protein-coding gene density. (g) Distribution percentage of GC content. Links between chromosomes are collinearity blocks, which are colored according to the homeologous group.

TABLE 1 The summary results of genome assemblies of wheat cultivars Sumai 3, Fielder, Karioga, Attraktion, Renan, and Chinese Spring IWGSC RefSeq v2.1 (CS).

Genome features	Sumai 3	Fielder	Karioga	Attraktion	Renan	CS
Total size (Gb)	14.60	14.70	14.68	14.68	14.26	14.57
Longest contig (Mb)	245.2	171.22	158.43	113.94	15.12	3.53
# Contigs	832	1428	717	1553	12,982	306,348
Contig N50 (Mb)	41.90	20.70	30.22	17.3 Mb	2.20	0.34
BUSCO completeness (%)	99.70	99.30	99.30	99.40	99.20	99.30
# High confidence genes	104,620	116,480	116,838	NA	109,543	106,913

Abbreviations: BUSCO, benchmarking universal single-copy orthologs; IWGSC, International Wheat Genome Sequencing Consortium; NA, not available.

miRNAs were most abundant in the B subgenome, with Chr2B (5948) and Chr3B (5753) showing the highest count. rRNA, small nucleolar RNAs, and snRNAs were unevenly distributed across chromosomes and do not indicate major structural divergence specific to Sumai 3 (Table S3).

3.2 | Transposable element composition and repeats in the Sumai 3 genome

TEs play a significant role in genome structure, evolution, and regulation by contributing to genome expansion and genetic diversity. TEs constitute a major fraction of the Sumai 3 genome (84.88%) (Table S4), consistent with other wheat assemblies. The overall TE composition closely mirrors that of previously reported cultivars, with retrotransposons dominating repeat landscape (Aury et al., 2022; Sato et al., 2021; T. Zhu et al., 2021). The LTR retrotransposons account for the largest portion (70.23% of all TEs), particularly Copia (15.73%) and Gypsy (52.58%) elements, reinforcing their central role in genome expansion and structural organization in wheat. Subgenome-level differences were observed, with the A genome showing slightly higher LTR content (71.87%), suggesting reduced turnover or greater retention of repetitive elements while the D genome (67.90%) exhibited comparatively lower LTR abundance, potentially reflecting higher rates of recombination or TE removal (Kent et al., 2017; Tiley & Burleigh, 2015). DNA transposons (Class II TEs) constitute a minor fraction (10.15%) of the Sumai 3 with CACTA elements (8.84%) being the most prevalent. Other superfamilies such as Mutator DNA transposon (DTM), Harbinger DNA transposon (DTH), Mariner DNA transposon (DTT), hAT DNA transposon (DTA), and Helitron DNA transposon (DHH) were present at lower frequencies (<1% each) (Table S4) but may play roles in structural regions such as centromeres and telomeres. Overall, the repeat landscape of Sumai 3 is highly consistent with other wheat genomes, supporting the conclusion that its global genome architecture is conserved (Athiyannan et al., 2022; Aury et al., 2022; Kale et al., 2022; Sato et al., 2021; T. Zhu et al., 2021).

3.3 | Synteny analysis and structural variants between Sumai 3 and Chinese Spring

A synteny analysis was conducted between Sumai 3 and Chinese Spring. The comparison confirmed strong collinearity between the homoeologous chromosomes of the two cultivars, as evidenced by the prominent diagonal line spanning the plot, consistent with previous wheat studies (Athiyannan et al., 2022; Aury et al., 2022; Z. Liu et al., 2024). Each chromosome pair demonstrated a high degree of synteny, with minimal structural rearrangements observed. However, minor deviations from perfect collinearity, visible as scattered points outside the diagonal, may indicate small-scale structural variants or potential assembly or annotation differences between the genomes (Figure 2; Figure S3).

Comprehensive structural variant (SV) analysis identified deletions, insertions, duplications, contractions, inversions, and translocations across all chromosomes. Structural variants analysis between Sumai 3 and CS T2T assembly revealed deletions and insertions as the most dominant variant types, with 23.36% being less than 500 bp. Chromosome 2A exhibits the highest number of deletions (2773) and insertions (2645), with a net increase of 1.89 Mb compared to CS T2T (Table 2; Figure 3). In contrast, chromosome 4D had the lowest deletion (299) and insertion (275) counts, with a net decrease in 270 kb compared to CS T2T. Among inversions, chromosome 3B showed the highest frequency (359), while chromosome 4D had the fewest (seven), suggesting localized chromosomal rearrangements that could impact gene regulation and recombination in this region. Duplications and contractions followed similar trends, with chromosome 2A containing the highest number of duplications (337) and chromosome 5B with the highest number of contractions (122), whereas chromosome 3D and chromosome 6D generally exhibited the lowest counts across these categories (Table 2; Figure 3). Notably, no translocations were detected across any chromosome, indicating that interchromosomal rearrangements between Sumai 3 and CS T2T are minimal. Overall, the relatively balanced number of insertions and deletions across chromosomes suggests a stable genomic structure, with vari-

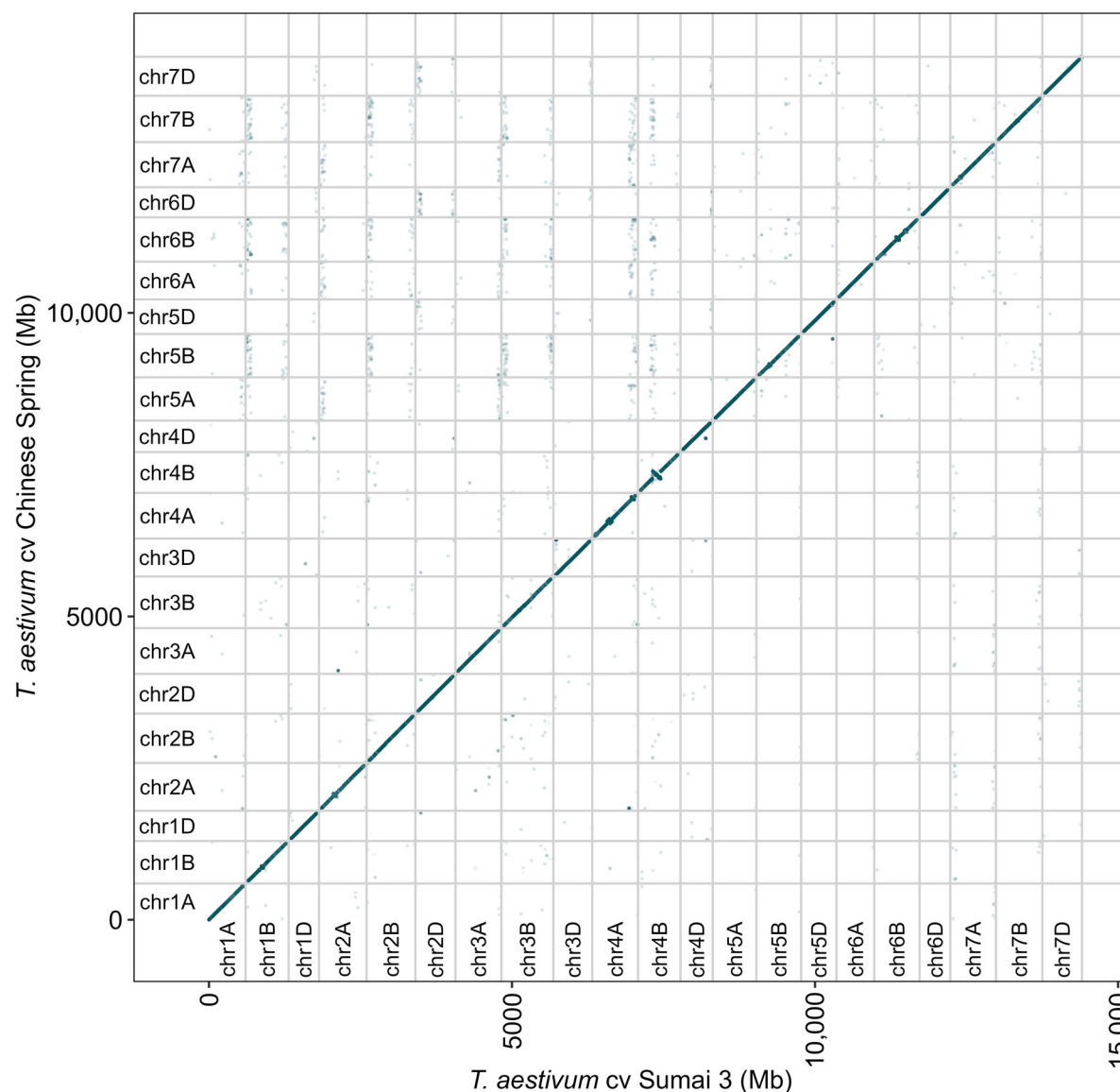


FIGURE 2 Whole genome comparison of Sumai 3 with Chinese Spring IWGSC RefSeq v2.1 (International Wheat Genome Sequencing Consortium [IWGSC] RefSeq v2.1).

ation primarily driven by localized structural changes rather than extensive genome-wide reorganization.

The use of a telomere-to-telomere reference minimizes biases associated with fragmented or short-read-based assemblies, providing a more accurate representation of structural variant. The identified SVs, particularly inversions and duplications likely influence gene expression and functional diversity, potentially influencing important agronomic traits such as disease resistance and stress adaptation (Maccaferri et al., 2019). Together, these results highlight both the overall conservation and targeted divergence between Sumai 3 and Chinese Spring, with specific chromosomal regions likely contributing to phenotypic differences between the two cultivars.

3.4 | Comparative analysis of the *Fhb1* region of Sumai 3 on chromosome 3BS with the equivalent regions on CS chromosomes 3BS and 3DS

To evaluate gene conservation and variation at the *Fhb1* locus, we compared the annotated genes in chromosome 3BS of our chromosome-scale Sumai 3 assembly to the previously published Sumai 3 BAC sequence (Rawat et al., 2016), and the Chinese Spring (CS) reference genome, focusing on homoeologous regions on chromosomes 3BS and 3DS (IWGSC RefSeq v2.1; Su et al., 2019). This comparison focuses on an approximately 377 kb-sized interval located at 12.4 Mb containing the *Fhb1* locus (Figure 4a), which allowed us to assess

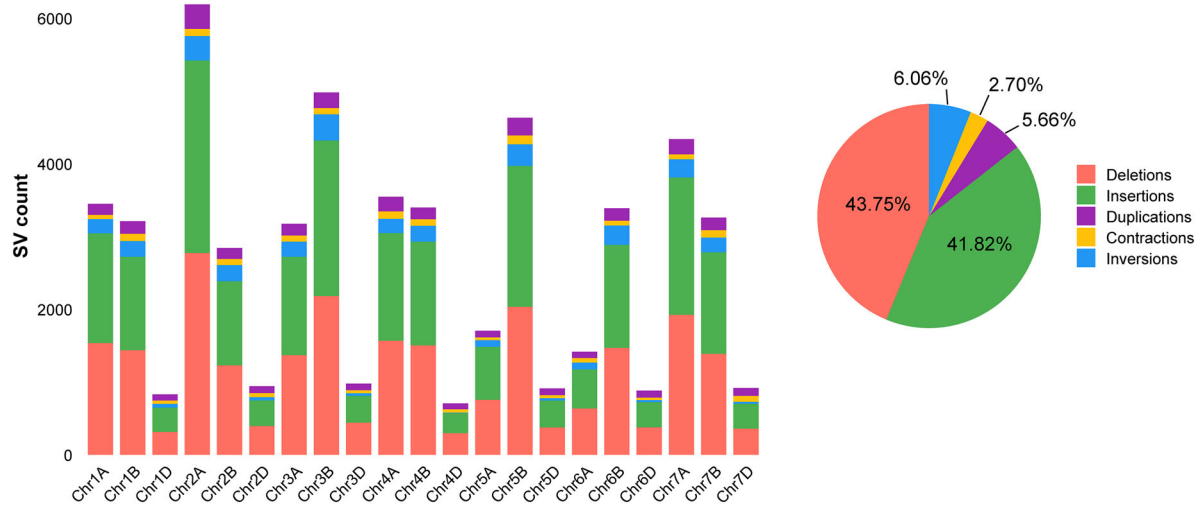


FIGURE 3 Characterization of structural variants (SVs) in Sumai 3 compared to CS T2T across all chromosomes. (a) Number of SVs: deletion, insertion, inversion, contraction, and duplications. (b) Percentage among five types of SVs.

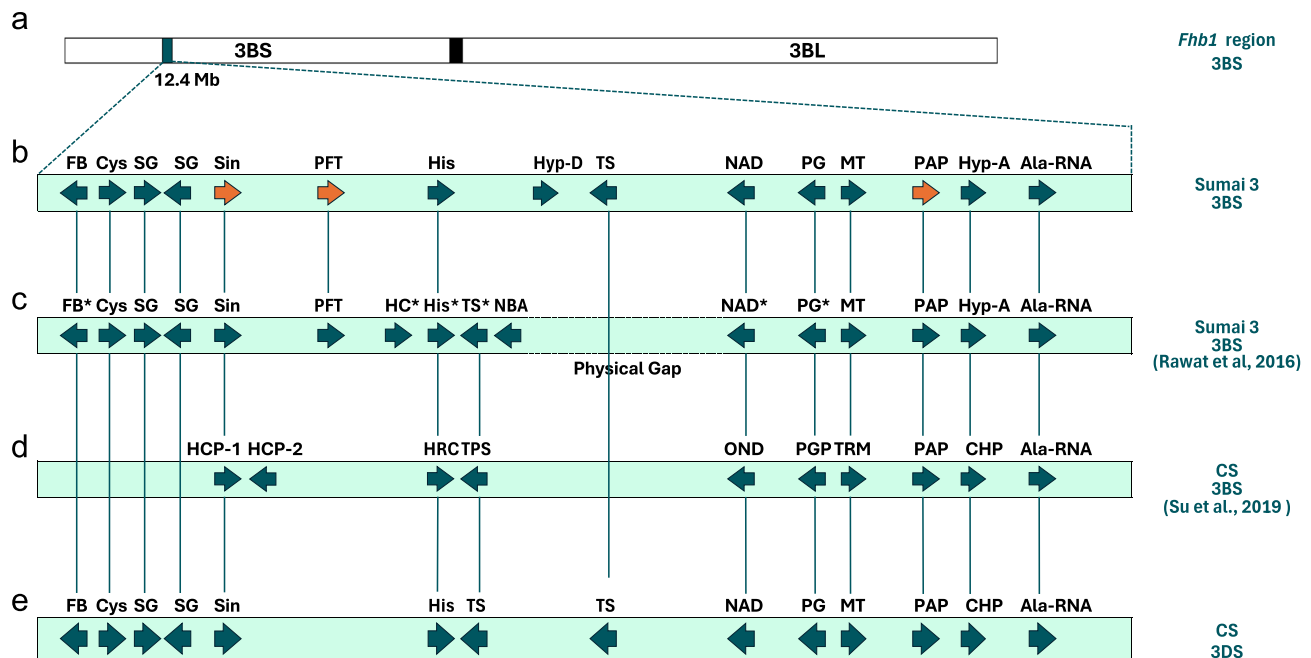


FIGURE 4 Synteny analysis of *Fhb1* region. (a) Chromosome location of the *Fhb1* region on 3BS of Sumai 3; (b) *Fhb1* region of Sumai 3, containing 15 open reading frames (arrows) and their respective positions within the interval; (c) and (d) comparison of gene region in Sumai 3 3BS (Rawat et al., 2016) and CS 3BS (Su et al., 2019); and (e) comparison of homoeologous region in CS 3DS. Arrows represent gene orientation. Genes highlighted in orange were identified as upregulated in resistant lines based on RNA-seq analysis. Solid lines connecting orthologs indicate sequence identity >90%. Genes without connecting lines were not detected or did not meet alignment thresholds in the corresponding genome. Asterisks above gene abbreviations indicate genes previously ruled out as *Fhb1* candidates based on transformation studies (S. Liu et al., 2008; Pumphrey, 2007). Ala-RNA, alanyl-tRNA synthetase; CHP/HCP/Hyp, hypothetical protein; cys, cystatin; FB, F-box domain containing protein; HC, high-confidence or HCBT-like defense response protein; His/HRC; histidine-rich calcium-binding protein; MT/TRM, tRNA methyltransferase; NAD/OND, oxidoreductase NAD-binding; NBA, Nb-ARC domain containing protein; PAP, plastid-lipid associated protein; PFT, pore-forming toxin-like protein; PG/PGP, polygalacturonase; SG, serine-glycine-asparagine-histidine plant lipase-like protein; Sin, sina superfamily protein; TS/TPS, terpene synthase.

TABLE 2 Structural variant summary of Sumai 3 compared to CS T2T.

Chromosomes	Deletions	Insertions	Duplications	Contractions	Inversions	Translocations
Chr1A	1535	1508	151	58	196	0
Chr1B	1437	1285	173	99	216	0
Chr1D	314	334	81	46	53	0
Chr2A	2773	2645	337	97	337	0
Chr2B	1228	1157	151	84	222	0
Chr2D	396	352	94	55	44	0
Chr3A	1368	1353	161	83	209	0
Chr3B	2180	2140	214	86	359	0
Chr3D	442	369	90	42	34	0
Chr4A	1567	1480	201	101	197	0
Chr4B	1503	1427	160	89	218	0
Chr4D	299	275	80	46	7	0
Chr5A	756	727	91	37	92	0
Chr5B	2033	1939	243	122	295	0
Chr5D	377	369	90	43	32	0
Chr6A	639	533	86	63	94	0
Chr6B	1468	1418	170	66	265	0
Chr6D	378	347	94	34	28	0
Chr7A	1922	1889	209	67	251	0
Chr7B	1386	1398	172	102	202	0
Chr7D	360	343	106	82	26	0

Abbreviations: Chr, chromosome; IWGSC, International Wheat Genome Sequencing Consortium.

coding sequence identity and to contextualize the annotation within previously published resources.

In the chromosome-scale Sumai 3 assembly, we annotated a total of 15 genes spanning the *Fhb1* interval. These included functionally characterized or predicted genes such as alanyl-tRNA synthetase (Ala-RNA), hypothetical protein (Hyp/CHP/HCP)-A, plastid-lipid-associated proteins (PAP), tRNA methyltransferase (MT/TRM), polygalacturonase (PG), oxidoreductase NAD-binding (NAD/OND), terpene synthase (TS), Hyp-D, histidine-rich calcium-binding protein (His/HRC), PFT, sina superfamily protein (Sin), two copies of serine-glycine-asparagine-histidine plant lipase-like protein, cystatin (Cys), and F-box domain-containing protein (Figure 4b). Several genes, including Ala-RNA, Hyp-A, PAP, MT, PG, and His, showed high coding sequence (CDS) identity (>90%) and full or near-full alignment lengths when aligned to the Sumai 3 BAC (Figure 4c; Table S5), CS 3BS (Figure 4d; Table S5), and CS 3DS (Figure 4e; Table S5), indicating strong conservation across B-genome and D-genome. While Sumai 3 BAC represents the same 3BS haplotype, not all annotated genes showed 100% identity. This is likely due to a combination of factors, including differences in sequencing and assembly approaches (BAC-based Sanger sequencing versus long-read HiFi assembly), potential base-calling or polishing discrepancies, and minor structural

or allelic variation within the region. In addition, the BAC sequence represents a single clone spanning a limited interval, whereas the chromosome-scale assembly integrates genome-wide context and may resolve regions that were previously collapsed, truncated, or misassembled in the BAC. NAD aligned well to BAC and CS 3BS sequences, with 100.0% and 97.0% identity across 805 bp, respectively. Alignment to CS 3DS showed slightly lower identity at 96.6% over a shorter 675 bp region, reflecting typical divergence between B and D subgenomes. For TS, two tandem copies were previously annotated in CS 3DS. The Sumai 3 annotation aligned with high identity (98.2%) to the TS copy of CS 3DS at 7.10 Mb, whereas the Sumai 3 BAC and CS 3BS annotations aligned to the second TS copy at 7.03 Mb of CS 3DS, with 94.2% and 94.9% identity and alignment lengths of 1546 and 1000 bp, respectively. Importantly, the TS copy present in the Sumai 3 annotation, along with Hyp-D, was located within the ~76 kb physical gap previously reported in the Sumai 3 BAC sequence (Rawat et al., 2016). Hyp-D did not align to the BAC, CS 3BS, or CS 3DS, further supporting its position within the BAC gap. BLAST analysis revealed that Hyp-D encodes a protein with a zf-RVT (zinc finger-reverse transcriptase) domain and an RNase H-like domain, suggesting it is derived from a retrotransposon. While its function remains unclear, the specific localization within previously

missing regions may indicate a structural or regulatory role in genome evolution or disease response. The Sumai 3 PFT gene showed 100% identity to the BAC gene, reinforcing annotation consistency in this region. The gene Sin revealed an interesting divergence pattern, while the CDS in Sumai 3 aligned with high identity to BAC sequence (99.7% over 338 bp), CS 3BS (93.6% over 484 bp), and CS 3DS (94.6% over 573 bp), only the CS 3DS alignment covered over 95% of the full gene length (605 bp), compared to ~80% for CS 3BS and ~56% for the BAC sequence. To investigate the transcriptional relevance of these genes, a preliminary RNA-seq analysis was performed using *F. graminearum*-inoculated wheat lines, including resistant lines (Sumai 3, ND2710, and Alsen), sensitive lines (Wheaton and Steele-ND), and more variable, moderately resistant lines (Rollag and Glenn). Differential expression analysis between the R and MR/S groups, applying a threshold of $\log_2\text{FoldChangel} \geq 2$ for upregulation and ≤ -2 for downregulation, revealed that PAP, PFT, and Sin were consistently upregulated across R samples. Importantly, this upregulation was not induced when comparing fungal to mock infection, but observed between the resistance classifications, suggesting that these genes are part of a shared transcriptional pathway immediately available to fight pathogen challenge. No genes within the interval were significantly downregulated across the treatments tested. Although the coding sequence of PAP and Sin did not show complete identity to the previously reported Sumai 3 BAC sequence (Table S5), their high sequence similarity and conserved annotation strongly support that they represent the same orthologous genes. The slight sequence differences are likely attributable to allelic variation, improved sequence resolution in the chromosome-scale assembly, or differences in annotation between the present assembly and the earlier BAC-based sequence. Such minor CDS differences are not unexpected in independently assembled references and do not preclude functional conservation. The corresponding genes identified in the syntenic regions of Chinese Spring 3BS and 3DS were included for comparative sequence analysis only. Because homeolog-specific expression was not evaluated in this study, we cannot determine whether related copies outside the *Fhb1* interval also responded to infection. Nonetheless, the differential expression observed for genes within the *Fhb1* interval supports their candidacy for involvement in resistance-associated responses in Sumai 3.

Together, this comparative analysis of the *Fhb1* region on chromosome 3BS demonstrates strong conservation of gene content between our chromosome-scale assembly, the Sumai 3 BAC, and the Chinese Spring reference genome. The resolution of previously missing genes (TS and Hyp-D) within the ~76 kb BAC gap highlights the improved completeness of our assembly. While most genes exhibited high sequence similarity, moderate divergence in TS, Sin, and NAD underscores the importance of subgenome-specific variation. Notably, the

upregulation of PAP, PFT, and Sin in resistant lines suggests functional relevance of these genes in host defense. Overall, our integration of structural and transcriptomic data provides a refined view of the *Fhb1* locus and strengthens the utility of the Sumai 3 reference for understanding FHB resistance.

AUTHOR CONTRIBUTIONS

Rubylyn D. Mijan: Formal analysis; investigation; methodology; visualization; writing—original draft. **Bikash Poudel:** Data curation; formal analysis; investigation; methodology; writing—review and editing. **Sittal Thapa:** Methodology; resources. **Oluwatayo Ajayi-Moses:** Methodology; resources. **Thomas Lux:** Formal analysis; methodology; resources; software. **Raz Avni:** Formal analysis; investigation; resources; software; writing—review and editing. **Steven Xu:** Resources. **Manuel Spannagl:** Conceptualization; formal analysis; methodology; resources; software; writing—review and editing. **Martin Mascher:** Formal analysis; methodology; resources; software; writing—review and editing. **Jeff Maughan:** Conceptualization; formal analysis; investigation; methodology; resources; software; writing—review and editing. **Jason D. Fiedler:** Conceptualization; formal analysis; methodology; project administration; resources; supervision; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data associated with this genome are deposited in the NCBI under BioProject accession number PRJNA1247523. These include the fully scaffolded pseudomolecules, PacBio reads from the genome (HiFi) and transcription (IsoSeq), as well as short-read data from RNA-Seq and HiC library preparations.

ORCID

Rubylyn D. Mijan  <https://orcid.org/0009-0004-5450-2695>

Bikash Poudel  <https://orcid.org/0000-0002-0117-581X>
 Oluwatayo Ajayi-Moses  <https://orcid.org/0000-0002-7215-1209>
 Raz Avni  <https://orcid.org/0000-0002-0801-5093>
 Steven Xu  <https://orcid.org/0000-0001-7000-2034>
 Martin Mascher  <https://orcid.org/0000-0001-6373-6013>
 Jeff Maughan  <https://orcid.org/0000-0003-3714-3411>
 Jason D. Fiedler  <https://orcid.org/0000-0001-7736-4484>

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SUPPORTING INFORMATION

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