crops

MDPI

*Article*

# Marker Development and Pyramiding of *Fhb1* and *Fhb7* for Enhanced Resistance to Fusarium Head Blight in Soft Red Winter Wheat

**Binod Gyawali [1], Steven R. Scofield [2] and Mohsen Mohammadi [1,*]**

[1] Department of Agronomy, Purdue University, West Lafayette, IN 47907, USA; bgyawali@purdue.edu
[2] Crop Production and Pest Control Research Unit, USDA-Agricultural Research Service, West Lafayette, IN 47907, USA; steve.scofield@usda.gov
* Correspondence: mohamm20@purdue.edu; Tel.: +1-765-496-6851

**Abstract:** Fusarium head blight (FHB) is a devastating fungal disease of hexaploid wheat (*Triticum aestivum*). Several genetic loci were previously identified that control FHB resistance in wheat, including *Fhb1*. *Fhb7*, a major QTL conferring resistance to FHB, controlling for mycotoxin deoxynivalenol (DON) production, has been introgressed into soft red winter wheat (SRWW). As an exotic QTL, *Fhb7* is associated with linkage drag, affecting agronomic and end-use quality performance. This study outlines a breeding strategy for introducing and pyramiding *Fhb7* into SRWW breeding populations that already possessed *Fhb1* and harbored some additional disease-resistance genes. In addition to the *Fhb1-Fhb7* pyramiding, we developed gene-based markers for both genes and examined them on 57 SRWW breeding lines. Our data showed that 15 out of 57 breeding lines possessed both *Fhb1* and *Fhb7* resistant alleles. Two years of phenotypic data from the inoculated and misted irrigation field showed that the combination of *Fhb1-Fhb7* lowers mycotoxin DON accumulation in kernels, which provides protection for end-users and the milling industry. The Fhb gene-pyramided lines, with the additional regionally important disease resistance genes, produced in this breeding pipeline showed reasonable agronomic traits and can be used in crossing programs for the widespread introgression in elite wheat cultivars.

**Keywords:** FHB; *Fhb7*; GST; QTL; *Thinopyrum*



**Citation:** Gyawali, B.; Scofield, S.R.; Mohammadi, M. Marker Development and Pyramiding of *Fhb1* and *Fhb7* for Enhanced Resistance to Fusarium Head Blight in Soft Red Winter Wheat. *Crops* **2023**, *3*, 320–332. https://doi.org/10.3390/crops3040028

Academic Editor: Il-Ryong Choi

Received: 27 September 2023
Revised: 26 October 2023
Accepted: 24 November 2023
Published: 7 December 2023



## 1. Introduction

Fusarium head blight (FHB), caused by the fungal pathogen *Fusarium graminearum* (Fg), is a serious threat to wheat production worldwide [1], which substantially reduces the grain yield and quality. Since *F. graminearum* is a hemi-biotrophic fungus, it penetrates the plant as biotrophs but later becomes necrotrophic. Because of its necrotrophic nature, the plant's effector triggered immunity (ETI) cannot provide complete defense against *F. graminearum*. Resistance to FHB in wheat is quantitative and is controlled by minor genes; thus, it is very difficult, if not impossible, to develop complete resistance against *F. graminearum*. Infection by Fg starts with the bleaching of spikelets, that further results in the sterility or production of discolored and shriveled kernels, also referred to as Fusarium-damaged kernels (FDKs) [2,3]. Deoxynivalenol (DON) and nivalenol (NIV) are the primary trichothecene mycotoxins produced by *F. graminearum* that promote the fungal spread in wheat. Both DON and NIV are poisonous to humans and animals [2,4,5], and thus cause devastating economic losses.

The US Food and Drug Administration (FDA) has established guidelines for DON content in food products. FDA specifies a maximum of 1 part per million (ppm) DON in finished wheat products, e.g., flour, bran, and germ, and a maximum of 5 ppm DON in grains and grain by-products destined for swine consumption. In addition to the grain contamination, yield is also reduced by the FHB infection. In other cases, the presence of

higher levels of DON in wheat grains leads to discounts in wheat price, causing financial loss for wheat growers [3], or even worse, the scabby wheat grains will not be purchased by millers for flour [4]. Continued crop failures due to FHB have driven many Minnesota wheat and barley farmers into bankruptcy [6]. Various approaches such as resistant cultivars and management practices can be deployed to lower the disease severity and DON contamination. The introgression of genes for biotic/abiotic stress resistance from exotic sources has been a common practice in wheat breeding for many years [7]. However, the introgression of genes from other species is associated with linkage drag for yield and end-use quality [8]. While existing germplasm in the soft red winter wheat region harbors multiple FHB-resistance quantitative trait loci (QTL), they still are not sufficient to maintain DON at a very low level [9]

Among the many QTL associated with wheat responses to FHB, *Fhb1*- and *Fhb7*-controlling type II resistance, which is the resistance to disease spread within the spike, have been intensively studied [10–13]. The *Fhb1* resistance allele [14] from the Chinese cultivar 'Sumai 3' has been widely introduced and incorporated in diverse genetic backgrounds, including common and durum wheats worldwide [10,11,15–19]. The wild type (WT) *Fhb1* gene encoding a putative nuclear-localized, histidine-rich calcium-binding protein corresponds to the locus TraesCS3B02G01990 in the reference genome sequence of Chinese Spring (CS), which also carries a 786 bp open reading frame [20,21]. The mutant *Fhb1* gene (designated as *Fhb1*-R in this study) previously characterized in the cultivars Sumai 3 and Wangshuibai, carries a 752 bp deletion from the WT *Fhb1* gene [20,21]. Studies suggest that *Fhb1*-R probably evolved only recently in East Asian countries (China and Japan) [20–22] and its frequency in worldwide common wheat germplasm has been estimated to be 5.2% or 24.9% [20–22]. Rawat et al. [23] found that the wheat *PFT* gene, predicted to encode a putative pore-forming toxin-like chimeric lectin protein, was suggested to be responsible for *Fhb1*-mediated FHB resistance [23]. However, a recent study [20] cannot confirm the role of this gene as the many wheat lines with functional *PFT* gene are susceptible to Fg infection. Together, these findings highlight the complexities in the function of *Fhb1*, which requires further studies to be resolved [24].

As wheat relatives, *Th. elongatum* and *Th. ponticum* are important genetic resources that can be used to improve wheat FHB resistance [13]. *Thinopyrum* species have been used to improve tolerance against abiotic stresses [25] as well as biotic stresses [26,27]. Many wheat–*Thinopyrum* derivatives have been developed and used as bridge materials for transferring valuable genes from *Thinopyrum* into common wheat. The *Fhb7* locus on the distal end of 7E chromosome from *Thinopyrum ponticum* was introgressed in the 7D chromosome of wheat [28] and was validated by several studies [12,29]. The QTL mapped on the distal region on long arm of chromosome 7E [12], which was translocated to chromosome 7D of wheat [30] resulted in resistance to FHB. The introgressed alien chromatin region is not able to recombine when crossed with wild-type chromatin due to the lack of homologous pairing [31], and consequently, the long non-recombinant stretch of DNA might cause yield drag or quality deterioration that are associated with the chromosome segment inherited from *Thinopyrum* [32]. A series of wheat–*Th. elongatum* substitution, ditelosomic, and addition lines were reported and used for locating a novel FHB resistance gene on chromosome 7EL [26,33].

A recent study [30] sequenced the genome of *Th. elongatum* and cloned the glutathione S-transferase-encoding *Fhb7* by genetic mapping. That study indicated that the *Fhb7* transfer was without yield drag. The *Fhb7* gene encoding a glutathione S-transferase was also involved in the xenobiotic detoxification of the trichothecene compounds as observed in *Fhb1* [30]. Research efforts have been made to integrate *Fhb7* gene into the wheat D genome through 7D–7E translocation from *Th. elongatum* and *Th. ponticum*, respectively [12,33,34]. Also, *Fhb7* resistance has been successfully introduced into the wheat A genome via 7A–7E translocation originating from *Th. ponticum* [35]. The gene's polymorphic nature highlights its adaptability to diverse environmental conditions and its potential role in fine-tuning FHB resistance responses. Moreover, the distribution

patterns of GST-*Fhb7* across different Triticeae species provide insights into its evolutionary significance and potential applications in breeding for FHB resistance [36].

We previously performed genome-wide association studies (GWAS) and evaluated FHB resistance in a SRWW breeding population, which showed the presence of QTL on chromosome 7D that regulates the DON level [9]. That study showed that the resistance coming from *Thinopyrum elongatum* in some germplasm was ambiguously located in one of the two QTL regions namely *Q7D.1*, and *Q7D.2* on chromosome 7D [9]. However, we were unable to determine which one was *Fhb7* and therefore could not identify which breeding lines possess the *Fhb7* gene. The primary objective of this study was to shed light on the ambiguity of previous germplasm screening and identify the breeding lines that harbor both *Fhb1* and *Fhb7*, by designing reproducible gene-based markers. The secondary objective was to further screen the lines for the leaf and stem rust resistance block (*Lr19/Sr25*) as well as barley yellow dwarf virus resistance gene (*Bdv3*) in the lines that are positive for both *Fhb1* and *Fhb7*.

## 2. Materials and Methods

Germplasm development. Two heterogeneous early-generation breeding populations (07469 and 07117) segregating for *Qfhs.pur-7EL*, later named *Fhb7* [12] and a type II FHB resistance QTL [28,29] were crossed with 'Wheater' (Figure 1). The wheat line 'Wheater', which carries a *7EL-7DL* translocation from tall wheatgrass (*Th. ponticum*), which harbors a linkage block *Lr19/Sr25* [37] conferring leaf and stem rust resistance. The 07469 population also carried a second *7EL-7DL* translocation from intermediate wheatgrass (*Th. intermedium*), which harbors the barley yellow dwarf virus resistance gene, *Bdv3*. The heterogenous F1 materials were crossed with six adapted breeding lines or varieties, '1026A', '1065RA', 'P25R62', '1070RA', '106A', and 'Roane', that possess the resistant allele at *Fhb1* (Figure 1). The crosses underwent five successive generations of backcrossing with the adapted recurrent parent to rebuild the regional adaptation and to remove the potential linkage drags on grain yield.

Sequence analysis and development of gene-based markers. To design the gene-based marker for *Fhb7*, approximately 57.5 kb long sequence of single BAC clone of B3227-3 [30] containing *Fhb7* region was retrieved and subjected to the NCBI open reading frame (ORF) Finder available at https://www.ncbi.nlm.nih.gov/orffinder/ (accessed on 15 December 2022) with default parameters. Among the ORFs predicted, we selected the 846 bp long ORF, which encodes glutathione S-transferase (GST)—the reported candidate gene for *Fhb7* [30]. Primers were designed to amplify the 822 bp of nucleotide from the GST ORF (Figure 2A).

To design the gene-based marker for *Fhb1*, the previously cloned sequence of the susceptible allele *TaHRC-S* as 2650-bp sequence from NIL-S (GenBank accession MK450309) and the resistant allele *TaHRC-R* as 2041-bp sequence from NIL-R (GenBank accession MK450312) were retrieved from Su et al. [20]. Clustal Omega software [38] was used for sequence alignment. For the amplification of the resistant allele, forward primer was anchored starting from 1138 bp of the 'Ning7840', which had 8 bp nucleotides mismatched in the 'Clark' allele (Figure 2B(a)). For the amplification of the susceptible allele, forward primer was anchored to 1424 bp of the susceptible allele (Clark), which had a 1 bp similarity and 18 bp deletion in the Ning7840 (Figure 2B(b)). Reverse primer for both resistance (Ning7840) and susceptible (Clark) sequences was designed by selecting a region that is common in both resistance (Ning7840) and susceptible (Clark) sequences (Figure 2B). Primers for both the *Fhb1* and *Fhb7* alleles were designed using the Primer3 tool [39]. The location of the primer sequences of the resistant allele of *Fhb7* are depicted in Figure 2A. In addition, primer sequences for resistant as well as susceptible alleles for *Fhb1* are depicted in Figure 2B(a)and Figure 2B(b), respectively.

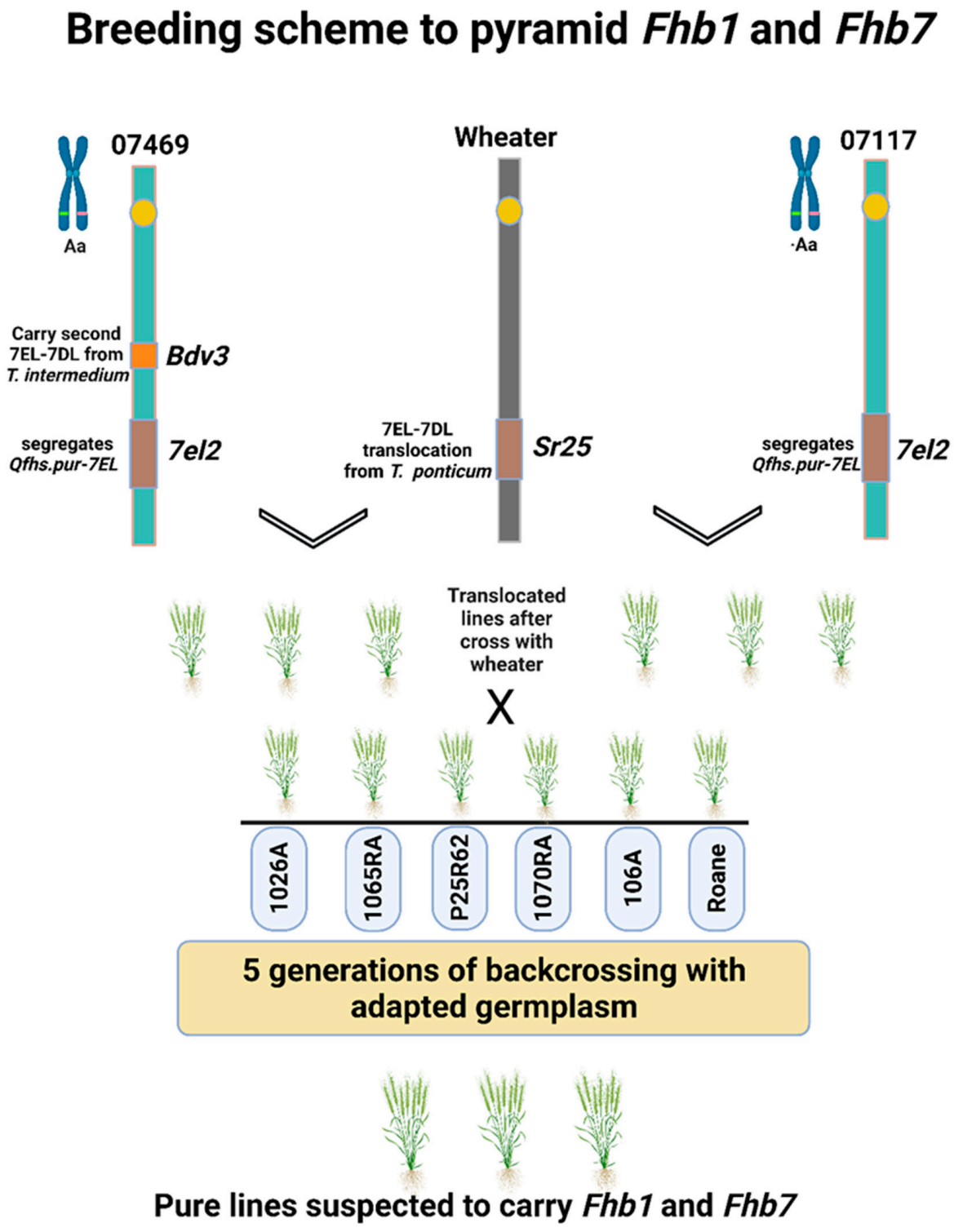


**Figure 1.** The breeding history of integration of multiple disease resistance genes in soft red winter wheat germplasm already harboring *Fhb1*.

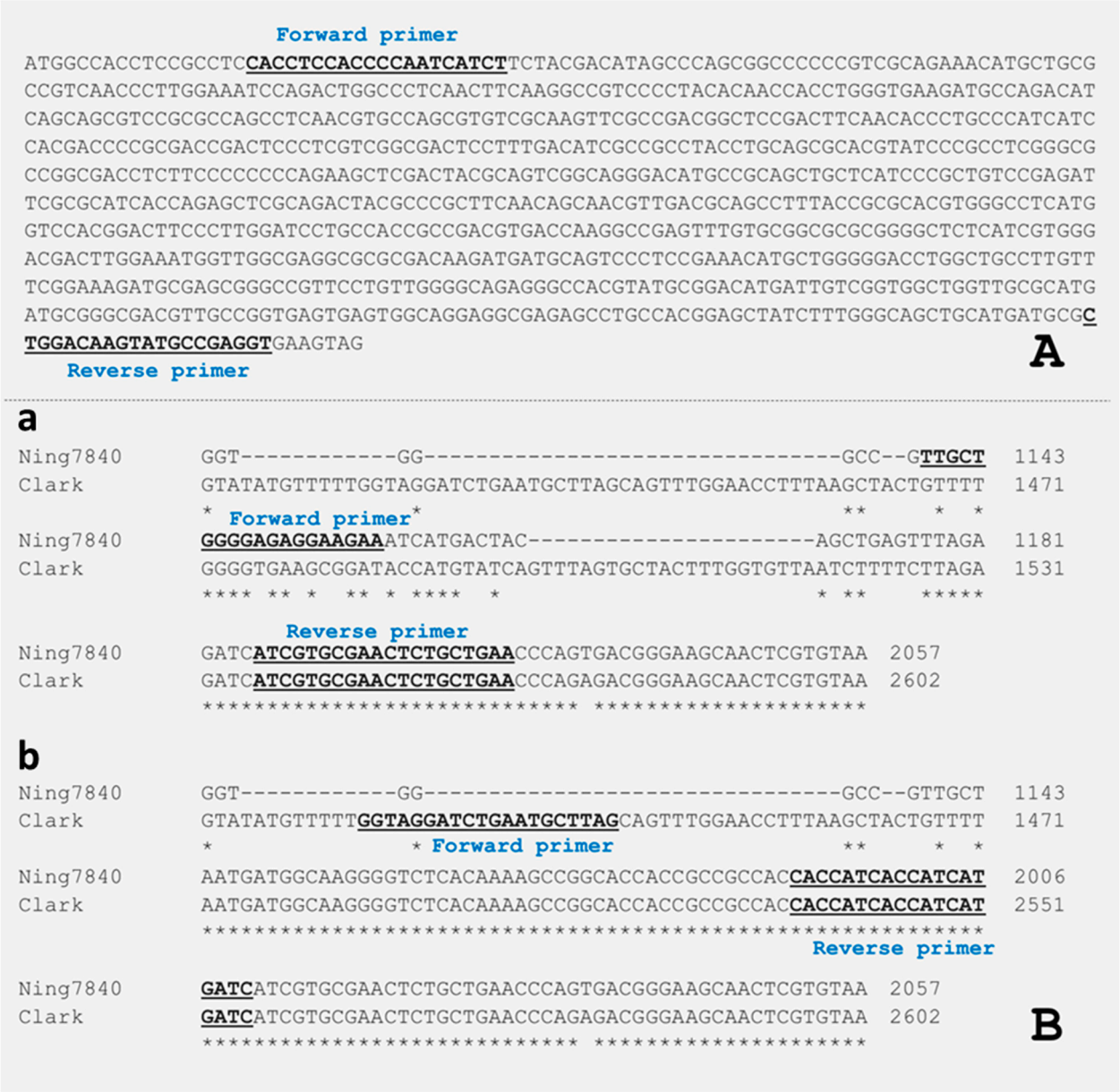


**Figure 2.** Alignment of nucleotide sequences as well as primer designing for the *Fhb7* and *Fhb1*. (**A**). GST ORF as *Fhb7* gene and the location of the primer sequences. (**B**(**a**)). Primer designing and location of the *TaHRC*_R from Ning7840 and its comparison with Clark. (**B**(**b**)). Primer designing and location of the *TaHRC*_S from Clark and its comparison with Ning7840. Sequence of primers for each of the resistant as well as susceptible alleles are underlined within the given sequences. "*" is alignments means homology.

DNA extraction and PCR diagnosis. In this study, 57 advanced breeding lines that had '7D(E)' or 'KS24-2-2(275-4)' in their parentage among 436 SRW wheat germplasm were tested for presence of *Fhb7*. The genomic DNA was extracted from leaves of 7-day-old seedlings following the CTAB method [40]. The isolated DNA was quantified using a NanoDrop-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and diluted to 20 ng/µL. Polymerase chain reactions (PCRs) were performed using a BioRad thermal cycler model 563BR (BioRad, Hercules, CA, USA). Each reaction contained 2 µL of 10X PCR buffer, 0.2 µL of 10 mM dNTPs, and 0.2 µL (1U) of DreamTaq DNA polymerase (Thermo Fisher Scientific), along with 0.4 µL (10 µM) each of the forward and reverse primers. The denaturation was at 95 °C for two minutes, followed by 35 cycles of 94 °C for

30 s, annealing, and 72 °C for extension. Annealing temperatures (Ta), extension time and expected band size of each primer set are shown in Table 1. Amplified PCR products were separated through slow (60 V) agarose gel (2%) electrophoresis in 1X Tris-Acetate-EDTA (pH~8.0) buffer (Thermo Scientific). Primers used for the genotyping of *Fhb1* and *Fhb7* that were listed in Table 1 were designed in the current study, and those used for diagnosis of *Sr25* and *Bdv3* were used by earlier studies [41,42]. The mention of trade names or commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

**Table 1.** Primer oligo sequences and PCR parameters for loci studied.

| Locus | Marker Name | Primers (5′-3′) | Expected Amplicon Size | Ta (°C) | Extension Time | Reference |
|---|---|---|---|---|---|---|
| *Fhb7* | GST | F-CACCTCCACCCCAATCATCT<br>R-ACCTCGGCATACTTGTCCAG | 822 bp | 56 | 1 min | This study |
| *Fhb1* | TaHRC_R | F-TTGCTGGGGAGAGGAAGAAA<br>R-TTCAGCAGAGTTCGCACGAT | 892 bp | 56 | 1 min | This study |
| *Fhb1* | TaHRC_S | F-GGTAGGATCTGAATGCTTAG<br>R-GATCATGATGGTGATGGTG | 1132 bp | 50 | 1 min 20 s | This study |
| *BYD* | Bdv3 | F-CTTAACTTCATTGTTGATCTTA<br>R-CGACGAATTCCCAGCTAAACTAGACT | 164/206/288 bp | 52 | 30 s | [42] |
| *Sr25* | BF145935 | F-CTTCACCTCCAAGGAGTTCCAC<br>R-GCGTACCTGATCACCACCTTGAAGG | 198 bp | 54 | 30 s | [41] |

Agronomic and Fusarium head blight resistance data. Agronomic and FHB phenotypic data for the current study are only the reanalysis of data we reported earlier [43] by comparing the phenotypes of the pyramided *Fhb1-Fhb7* lines with the mean of the entire breeding population, comprising 392 lines. We were unable to make these comparisons in the earlier study due to the uncertainty in the *Fhb7* genotypes. Briefly, experiments were conducted in standard breeding trials with 12 ft length and 4 ft width (~3 m × 1 m) for field testing in the 2017–18 and 2018–19 seasons in West Lafayette, Indiana, USA (40.47° N, 86.99° W, elevation 185 m. Grain yield (YLD), expressed in tons per ha were collected from each plot after adjusting to 13% moisture. The field inoculation was conducted using lab prepared *F. graminearum*-infested corn spawn, which was treated with nine isolates from Indiana, Illinois, and Ohio, as described by Gilbert and Woods [44]. The *F. graminearum*-infested corn spawn was applied at a rate of 40 g/m$^2$ for approximately 2 to 3 weeks before the heading stage [43]. Disease severity (SEV) data were measured 21 days after anthesis as the percentage of infected spikelets within a spike averaged from 10 random spikes in the plot. The deoxynivalenol (DON) concentration was quantified using the gas chromatography–mass spectrometry (GC/MS) [45] method at the University of Minnesota Mycotoxin laboratory. Analysis of variance (ANOVA) for the grain yield was calculated in the R Studio v4.2.2 [46]. Detailed experimental designs about these studies are available in Gaire et al. [43].

## 3. Results

The aim of this study was to develop the gene-based markers and precisely screen the adapted SRWW that were created with the exotic *Fhb7* and the prevalent *Fhb1* gene. Among the 392 lines tested by [9], only 57 lines had '7D(E)' or 'KS24-2-2(275-4)' in their parentage in their pedigrees. These lines were tested by PCR using gene-based markers for *Fhb7* and *Fhb1*. The PCR diagnosis test by using PCR primer pairs developed in this study showed that 15 out of 57 advanced lines possessed *Fhb7* (Table 2). The re-analysis provided below is only about the 15 selected lines from a previous study [43]. Additionally, the two pairs of primer pairs for the diagnosis of the *Fhb1* resistance allele (specific to Ning7840), and presence of *Fhb1* susceptible allele (specific to Clark) were successfully tested (Figure 3).

**Table 2.** Presence (+) and absence (−) of *Fhb1*, *Fhb7*, *Bdv3*, and *Sr25* in the breeding lines along with their two-year average yield, severity, and DON data.

| Line ID | *Fhb1* | *Fhb7* | *Bdv3* | *Sr25* | Yield (tons/ha) | SEV% | DON (ppm) |
|---|---|---|---|---|---|---|---|
| *PU10535-1* | + | + | + | + | 4.16 ± 0.57 ab | 39.15 ± 6.11 ab | 3.75 ± 1.67 bc |
| *PU10461-1* | + | + | + | + | 3.21 ± 0.88 ab | 34.99 ± 0.97 ab | 4.28 ± 0.84 bc |
| *PU10534-1* | + | + | + | + | 4.22 ± 0.31 ab | 26.06 ± 4.96 ab | 2.84 ± 1.21 c |
| *PU10535-2* | + | + | + | + | 5.28 ± 0.39 a | 52.98 ± 1.55 ab | 1.92 ± 0.35 c |
| *PU10534-2* | + | + | + | + | 3.79 ± 0.58 ab | 38.79 ± 13.38 ab | 1.04 ± 0.54 c |
| *PU10642-1* | + | + | + | + | 2.77 ± 0.52 ab | 51.34 ± 0 | 4.6 ± 0 bc |
| *PU10642-2* | + | + | + | + | 3.37 ± 0.81 ab | 43.79 ± 11.62 ab | 6.32 ± 2.19 bc |
| *PU10642-3* | + | + | + | + | 2.27 ± 0.19 b | 35.56 ± 0 ab | 4.05 ± 0 c |
| *PU10548-1* | + | + | + | − | 2.23 ± 0.50 b | 40.51 ± 0.58 ab | 3.31 ± 0.98 bc |
| *PU10642-4* | − | + | + | + | 3.64 ± 0.82 ab | 38.2 ± 0 ab | 4.29 ± 0 bc |
| *PU10534-3* | + | + | + | + | 4.12 ± 0.39 ab | 45.37 ± 18.09 ab | 3.29 ± 0.78 bc |
| *PU10461-2* | + | + | + | + | 2.94 ± 0.66 ab | 33.51 ± 6.45 ab | 7.16 ± 0 abc |
| *PU10461-3* | + | + | + | − | 3.96 ± 0.30 ab | 27.12 ± 9.4 b | 2.99 ± 1.19 c |
| *PU10461-4* | + | + | + | + | 5.45 ± 0.45 a | 30.58 ± 0.45 ab | 6.36 ± 2.19 abc |
| *PU10535-5* | + | + | + | + | 2.23 ± 0.31 b | 45.56 ± 0 ab | 4.89 ± 2.13 bc |
| *PU99646-7* | + | − | NA | NA | 3.89 ± 0.43 ab | 56.58 ± 3.55 a | 12.78 ± 0.55 a |
| *PU96134-1* | + | − | NA | NA | 5.62 ± 0.63 a | 63.89 ± 1.89 a | 10.21 ± 2.50 ab |

Sr genes are stem rust resistance genes that are resistant against the TTKS Ug99 race of stem rust. *Bdv3* is the resistance gene against the barley yellow dwarf virus of wheat. Grain yield is expressed in tons/ha. FHB severity is expressed in percentage. DON content is expressed in parts per million. Lines sharing same letters have no significant differences. Lines not sharing letters showed significant differences at *p*-value = 0.05.

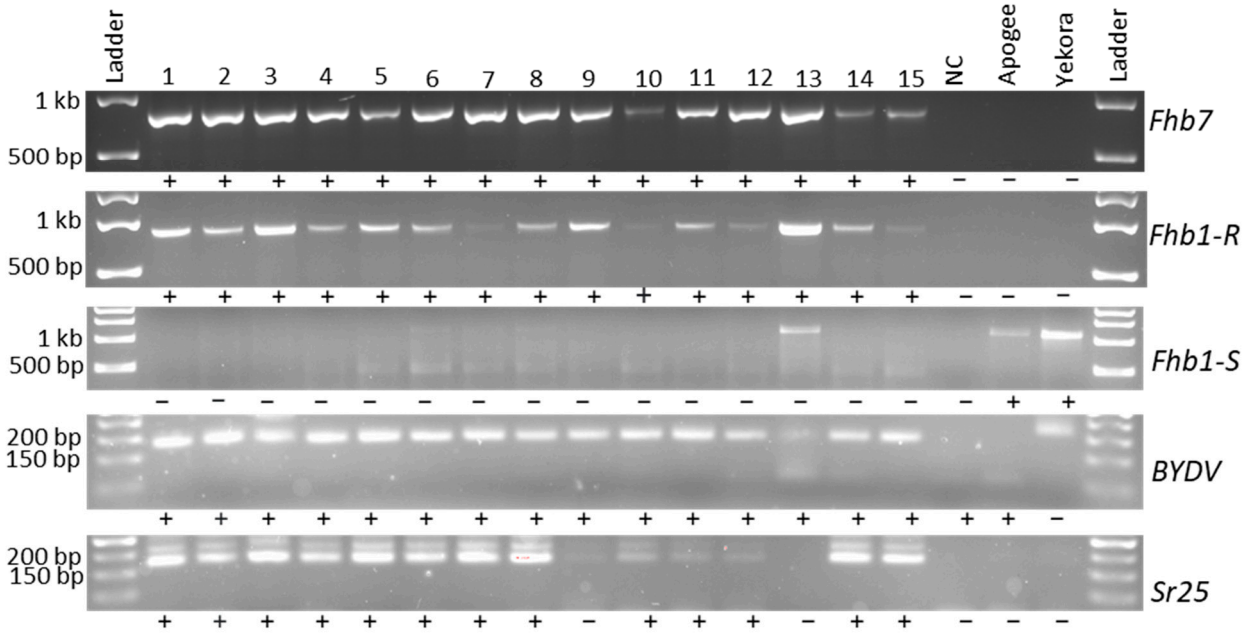


**Figure 3.** Gel electrophoresis images for the amplification of the five alleles (*Fhb7*, *Fhb1*_R, *Fhb1*_S as well as *Bdv3* and *Sr25*) in our soft red winter wheat (SRWH) lines already harboring *Fhb1-R* allele. 15 tested lines are represented from number 1 to 15. The presence of the genes followed by gel electrophoresis is indicated by the (+) sign, while absence is indicated by the (−) sign. Water template was used as negative control (NC), while the wheat lines Apogee and Yekora were negative control for the *Fhb7* as well as *Fhb1*-R, but the positive control for the *Fhb1*-S which is known to have a susceptible allele of the *TaHRC*.

The re-analysis of ANOVA for the selected 15 lines showed significant effect of lines for grain yield ($F = 4.042$, $p < 0.005$). Unlike the claim of no yield penalty made by Wang et al. [30], the lines identified as positive for *Fhb7* in this study showed a substantially lower yield than the highest yielding lines in the 2018–19 trials. We further calculated

the average value of plant's group with *Fhb7* (3.57 tons/hac) as well as average value of plant's group without *Fhb7* (4.43 tons/hac). When conducting the one-way ANOVA for yield, two groups were on the border of significant differences (F1 = 4.119, $p$ = 0.05201). The best agronomic performance in the 2018–19 trials was 9 tons per ha [43]. The grain yields of the 15 *Fhb7*-carrying lines are listed in Table 2. For comparison, we used two lines, i.e., 'PU99646-7′ and 'PU PU96134-1', as an *Fhb7*-check, with *Fhb1* and without *Fhb7*, and compared their SEV with these 15 lines. Tukey comparison was only able to identify PU10461-3 as being a better accession than the *Fhb7*-checks for SEV (Table 2). However, when we compared lines for DON content, there were few lines that exhibited significantly smaller amount of DON compared with *Fhb7*-checks (Table 2). Since these numbers are only preliminary, multilocation trails are required to further explore the effects of *Fhb7* on grain yield.

In addition, these 15 lines showed superior FHB trait performance (Table 2). The combined year analysis of severity and DON for the 15 *Fhb7*-positive lines along with susceptible lines 'PU99646-7′ as well as 'PU PU96134-1′ are summarized in Table 2. PU10535-2, with the maximum yield, contained 1.92 ppm DON when grown and inoculated in an FHB-misted irrigation farm. Severity, FDK, and DON values for these lines are also at the lower tail of distribution observed from the 392 lines in the combined-year data analysis. The individual year severity and DON as well as the combined-year FHB data for the 15 *Fhb7*-positive lines are summarized in Table 2. Among the *Fhb7*-positive lines, PU10534-2 had the best resistance, with an average DON value of 1.04 ppm followed by PU10535-2, with a DON value of 1.92 ppm. Mean comparisons of SEV showed that both susceptible checks were significantly different with the rest of the 15 tested lines, whereas for DON, only the check PU99646-7 showed significant difference with tested lines.

Furthermore, the presence of alien stem rust and leaf rust resistance gene block *Sr25/Lr19* originally from wheat relative *Th. ponticum* [47] and inherited from the Australian spring wheat variety 'Wheater' was verified in 13 of these lines (Table 2, Figure 3). PCR tests showed all 15 lines were positive for the presence of the *Bdv3* gene (Figure 3). In addition, 13 lines carried the *Sr25* gene, which is an additional disease package offered by these lines.

## 4. Discussion

The United States is one of the top three wheat exporter countries, accounting for approximately 6–7% of the global wheat exports. The soft red winter wheat class is grown on 6.86 million acres in the eastern USA, with a total production of 337 million bushels [48] of grains. FHB is a serious wheat disease particularly in the eastern USA that reduces yield, lowers quality, deteriorates kernels, and results in the accumulation of the mycotoxin DON. From the year 2003 to 2014, the annual average levels of DON found in USA wheat delivered to milling facilities was close to 0.5 mg/kg (or ppm), while DON levels in individual samples even exceeded 2 mg/kg [49], while the FDA has set the upper limit of 1 ppm (1 mg/kg) for finished wheat products. In the United States, a total of USD 1.176 billion in economic losses caused by FHB were reported in 2015 and 2016 [50]. These losses are anticipated to escalate due to more frequent and severe FHB outbreaks caused by rising temperatures and humidity levels.

There has been significant progress in improving resistance to FHB in winter wheat [51] over a 20-year period (1998–2018). This suggests that ongoing investment in wheat breeding for FHB resistance is effective and will continue to be important for decreasing FHB levels for growers and DON levels for end-users. Genetic Trend analyses on breeding trial data are helpful to track the progress of breeding programs [52], including FHB resistance, and determine if the investments in breeding are producing the desired results. To further lower the impacts of FHB disease, researchers have been extensively studying new genes that provide resistance to FHB from wild relatives of wheat, to improve the diversity and effectiveness of resistance to FHB in wheat [30,53,54]. This is the first study to report the pyramiding of *Fhb1* with *Fhb7* genes in soft red winter wheat in the eastern United

States. The expectation is that the stack of *Fhb1-Fhb7* in our lines will provide a better resistance package against FHB. These lines are also unique as they are the only source of *Fhb7* germplasm adapted to the SRWW region and breeding history.

*Fhb1* (syn *Qfhs.ndsu-3BS*), the most significant quantitative trait locus (QTL), was shown to provide a reasonably high level of genetic resistance against FHB [11,55], which has been effective across various genetic backgrounds [56]. Another study [15] showed that the *Fhb1* locus decreased the severity of the FHB disease by 23%. Both in greenhouse and field circumstances, the *Fhb1* resistance allele had a consistent impact on lowering the severity of FHB, with most *Fhb1*-carrying lines displaying consistent resistance to FHB [11]. After introducing *Fhb1* from 'Ning 7840′ (a 'Sumai 3′ derivative), into the susceptible SRWW line IL89-7978, the percentage of scabby spikelets reduced dramatically from 70–80% to 30–40% [16]. Zhang et al. [17] found that for backcross progenies carrying the *Fhb1* resistance allele, the mean number of diseased spikelets was 8.1%, and the disease index was 28.4% lower than in the recurrent parent. While successful in many genetic backgrounds, not all *Fhb1*-R-carrying lines exhibit type II FHB resistance. Nearly 36% of them are susceptible to FHB disease, probably caused by inhibitor genes [24]. Previous studies [15,18] show that transferring *Fhb1* into commercial wheat varieties does not always result in FHB resistance. Su et al. [20] reported that about 10.6% of common wheat lines carrying *Fhb1*-R allele did not show the expected type II FHB resistance, indicating the need for incorporating novel resistance genes in addition to *Fhb1*.

It was shown that the *Fhb7* significantly reduced the amount of Fusarium biomass in blighted kernels resulting in the reduction in DON contamination by up to 85% as compared to susceptible sibs [35]. A previous study from our group also showed that the pyramiding of *Fhb1* and *Fhb7* loci had no additional benefit for the severity; however, it resulted in fewer symptoms of FDK and DON [9]. This study shows additional benefits on severity as the *Fhb7* and *Fhb1* pyramided lines were significantly different than those susceptible checks for severity. A study conducted by Wang et al. [30] indicated that *Fhb7* transfer was without yield drag. Recently, by backcrossing the FHB-resistant lines with the main cultivar Jimai 22, three wheat–*Th. elongatum* translocation lines, Zhongke 1878, Zhongke 166, and Zhongke 545, were successfully applied in wheat breeding without yield penalty [13]. We observed small degrees of yield penalty in some of the lines that were *Fhb7*-positive, although yield in the group that had *Fhb7* as well as the group that did not have *Fhb7* were not significantly different. This yield drag can be partially, if not totally, minimized by multiple backcrosses [33]. Techniques such as *ph1b* mutants [57] and disomic substitution lines [58,59] mediated by the gene *Ph1b/Ph2* has been frequently used to enable non-homologous recombination in situations like this [34,60]. However, given the agronomic status of the lines produced in this breeding program, producing large breeding populations by crossing these 15 lines to the highest yielding lines of the region should be the first line of action to offset the yield penalty.

Since the cloning of *Fhb7* in 2020 [30], gene-based markers are not available for the rapid and easy screening of the lines suspected to carry *Fhb7*. In this study, we retrieve the coding sequences of GST and designed the marker within the ORF. The development of gene-based *Fhb7* markers will aid in the deployment of *Fhb7* in wheat breeding programs to improve FHB resistance as well as marker-assisted selection. Furthermore, for the efficient amplification and the screening of *Fhb1*, Su et al. [61] proposed the markers for both *Fhb1*-R and *Fhb1*-S, considering *TaHRC* as the candidate For *Fhb1* [20,21], but those markers are hard to reproduce as they have their own technical difficulties. Because of the unavailability of the efficient and highly reproducible gene-based markers for *TaHRC* (*Fhb1*), we designed *Fhb1* markers for both resistant and susceptible alleles. The designing of the markers for *Fhb1* will further aid in the rapid screening of the germplasm as well as the marker-based selection in the wheat breeding programs to improve FHB resistance.

Barley yellow dwarf virus (BYDV) disease is caused by Luteovirus genus transmitted by aphids, *Rhopalosiphum padi* and is a globally important disease of wheat [62]. BYDV causes wheat plants to produce fewer tillers, fewer seeds per tiller, and lighter seeds [63,64].

Wheat yield losses have been found to range widely between 11 and 33 percent [65], as high as 40 to 50 percent [66], and sporadically as high as 80 percent [65]. A base level of BYDV infection that causes an unperceived loss of 1% to 5% appears to exist even in years where there is not a significant BYDV outbreak in the US [6]. Additionally, the *Sr25* [37] gene was introduced into wheat from *Thinopyrum ponticum*, which possesses the resistant linkage block *Lr19/Sr25* [37]. The presence of these disease resistance genes along with the *Fhb1-Fhb7* stack should provide useful resistance to a suite of diseases that tend to reduce wheat yield. To appreciate the value of this level of disease resistance protection, it is worth noting that in the US, a 1% decrease in wheat yield could result in a loss of USD 123 million at the current price.

## 5. Conclusions

In this study, we successfully developed the gene-based markers for both *Fhb1* and *Fhb7*, which are the key wheat genes for the resistance to the Fusarium head blight. These markers will help in the deployment of the *Fhb1* as well as *Fhb7* for the wheat breeding associated with the FHB management. We further screened our soft red winter wheat lines for the pyramiding of both genes and found that 15 of the lines had both genes. In addition, we also identified additional disease resistance packages having *Bdv3* and *Sr25* genes in our germplasm.

**Author Contributions:** Conceptualization, M.M.; methodology, M.M. and B.G.; software, M.M. and B.G.; validation, M.M., B.G. and S.R.S.; formal analysis, B.G. and M.M.; investigation, M.M. and B.G.; resources, M.M. and S.R.S.; data curation, B.G. and M.M.; writing—original draft preparation, B.G. and M.M.; writing—review and editing, M.M. and S.R.S.; visualization, M.M. and B.G.; supervision, M.M. and S.R.S.; project administration, M.M.; funding acquisition, M.M. and S.R.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the USWBSI agreement 59-0206-0-142 and USDA Hatch grant 1013073 via Purdue College of Agriculture; U.S. Wheat and Barley Scab Initiative grant number 17000549. Also, SRS is supported by USDA-ARS number: 5020-21220-014-000D. The USDA is an equal opportunity employer.

**Data Availability Statement:** Data are contained within the article.

**Acknowledgments:** Additional financial aid and research support through College of Agriculture, USDA Small Grains Genotyping Laboratory, Raleigh, NC and the University of Minnesota DON Testing Lab is greatly appreciated.

**Conflicts of Interest:** Authors have no conflict of interest to report.

## References

1. Wegulo, S.N.; Baenziger, P.S.; Nopsa, J.H.; Bockus, W.W.; Hallen-Adams, H. Management of Fusarium head blight of wheat and barley. *Crop Prot.* **2015**, *73*, 100–107. [CrossRef]
2. Parry, D.W.; Jenkinson, P.; McLeod, L. Fusarium ear blight (scab) in small grain cereals—A review. *Plant Pathol.* **1995**, *44*, 207–238. [CrossRef]
3. McMullen, M.; Jones, R.; Gallenberg, D. Scab of wheat and barley: A re-emerging disease of devastating impact. *Plant Dis.* **1997**, *81*, 1340–1348. [CrossRef]
4. McMullen, M.; Bergstrom, G.; De Wolf, E.; Dill-Macky, R.; Hershman, D.; Shaner, G.; Van Sanford, D. A unified effort to fight an enemy of wheat and barley: Fusarium head blight. *Plant Dis.* **2012**, *96*, 1712–1728. [CrossRef] [PubMed]
5. Panthi, A.; Hallen-Adams, H.; Wegulo, S.N.; Hernandez Nopsa, J.; Baenziger, P.S. Chemotype and aggressiveness of isolates of *Fusarium graminearum* causing head blight of wheat in Nebraska. *Can. J. Plant Pathol.* **2014**, *36*, 447–455. [CrossRef]
6. USDA, ARS. FHB Epidemic in Wheat and Barley. 2016. Available online: https://www.ars.usda.gov/midwest-area/stpaul/cereal-disease-lab/docs/fusarium-head-blight/fhb-epidemic-in-wheat-and-barley-overview/ (accessed on 20 August 2022).
7. Hajjar, R.; Hodgkin, T. The use of wild relatives in crop improvement: A survey of developments over the last 20 years. *Euphytica* **2007**, *156*, 1–13. [CrossRef]
8. Rubio Teso, M.L.; Lara-Romero, C.; Rubiales, D.; Parra-Quijano, M.; Iriondo, J.M. Searching for Abiotic Tolerant and Biotic Stress Resistant Wild Lentils for Introgression Breeding Through Predictive Characterization. *Front. Plant Sci.* **2022**, *13*, 90. [CrossRef] [PubMed]

9. Gaire, R.; Brown-Guedira, G.; Dong, Y.; Ohm, H.; Mohammadi, M. Genome-wide association studies for Fusarium head blight resistance and its trade-off with grain yield in soft red winter wheat. *Plant Dis.* **2021**, *105*, 2435–2444. [CrossRef]
10. Buerstmayr, H.; Ban, T.; Anderson, J.A. QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: A review. *Plant Breed.* **2009**, *128*, 1–26. [CrossRef]
11. Jin, F.; Zhang, D.; Bockus, W.; Baenziger, P.S.; Carver, B.; Bai, G. Fusarium head blight resistance in US winter wheat cultivars and elite breeding lines. *Crop Sci.* **2013**, *53*, 2006–2013. [CrossRef]
12. Guo, J.; Zhang, X.; Hou, Y.; Cai, J.; Shen, X.; Zhou, T.; Xu, H.; Ohm, H.W.; Wang, H.; Li, A.; et al. High-density mapping of the major FHB resistance gene *Fhb7* derived from *Thinopyrum ponticum* and its pyramiding with *Fhb1* by marker-assisted selection. *Theor. Appl. Genet.* **2015**, *128*, 2301–2316. [CrossRef]
13. Guo, X.; Shi, Q.; Liu, Y.; Su, H.; Zhang, J.; Wang, M.; Wang, C.; Wang, J.; Zhang, K.; Fu, S.; et al. Systemic development of wheat–*Thinopyrum elongatum* translocation lines and their deployment in wheat breeding for Fusarium head blight resistance. *Plant J.* **2023**, *114*, 1475–1489. [CrossRef]
14. Anderson, J.A.; Stack, R.W.; Liu, S.; Waldron, B.L.; Fjeld, A.D.; Coyne, C.; Moreno-Sevilla, B.; Fetch, J.M.; Song, Q.J.; Cregan, P.B.; et al. DNA markers for Fusarium head blight resistance QTLs in two wheat populations. *Theor. Appl. Genet.* **2001**, *102*, 1164–1168. [CrossRef]
15. Pumphrey, M.O.; Bernardo, R.; Anderson, J.A. Validating the *Fhb1* QTL for Fusarium head blight resistance in near-isogenic wheat lines developed from breeding populations. *Crop Sci.* **2007**, *47*, 200–206. [CrossRef]
16. Zhou, W.C.; Kolb, F.L.; Bai, G.H.; Domier, L.L.; Boze, L.K.; Smith, N.J. Validation of a major QTL for scab resistance with SSR markers and use of marker-assisted selection in wheat. *Plant Breed.* **2003**, *122*, 40–46. [CrossRef]
17. Zhang, H.; Su, Z.; Bai, G.; Zhang, X.; Ma, H.; Li, T.; Deng, Y.; Mai, C.; Yu, L.; Liu, H.; et al. Improvement of resistance of wheat cultivars to Fusarium head blight in the Yellow-Huai Rivers Valley Winter wheat zone with functional marker selection of *Fhb1* gene. *Acta Agron. Sin.* **2018**, *44*, 505–511. [CrossRef]
18. Salameh, A.; Buerstmayr, M.; Steiner, B.; Neumayer, A.; Lemmens, M.; Buerstmayr, H. Effects of introgression of two QTL for fusarium head blight resistance from Asian spring wheat by marker-assisted backcrossing into European winter wheat on fusarium head blight resistance, yield and quality traits. *Mol. Breed.* **2011**, *28*, 485–494. [CrossRef]
19. Li, G.; Yuan, Y.; Zhou, J.; Cheng, R.; Chen, R.; Luo, X.; Shi, J.; Wang, H.; Xu, B.; Duan, Y.; et al. FHB resistance conferred by *Fhb1* is under inhibitory regulation of two genetic loci in wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* **2023**, *136*, 134. [CrossRef]
20. Su, Z.; Bernardo, A.; Tian, B.; Chen, H.; Wang, S.; Ma, H.; Cai, S.; Liu, D.; Zhang, D.; Li, T.; et al. A deletion mutation in *TaHRC* confers *Fhb1* resistance to Fusarium head blight in wheat. *Nat. Genet.* **2019**, *51*, 1099–1105. [CrossRef]
21. Li, G.; Zhou, J.; Jia, H.; Gao, Z.; Fan, M.; Luo, Y.; Zhao, P.; Xue, S.; Li, N.; Yuan, Y.; et al. Mutation of a histidine-rich calcium-binding-protein gene in wheat confers resistance to Fusarium head blight. *Nat. Genet.* **2019**, *51*, 1106–1112. [CrossRef]
22. Ma, Z.; Xie, Q.; Li, G.; Jia, H.; Zhou, J.; Kong, Z.; Li, N.; Yuan, Y. Germplasms, genetics and genomics for better control of disastrous wheat Fusarium head blight. *Theor. Appl. Genet.* **2020**, *133*, 1541–1568. [CrossRef] [PubMed]
23. Rawat, N.; Pumphrey, M.O.; Liu, S.; Zhang, X.; Tiwari, V.K.; Ando, K.; Trick, H.N.; Bockus, W.W.; Akhunov, E.; Anderson, J.A.; et al. Wheat *Fhb1* encodes a chimeric lectin with agglutinin domains and a pore-forming toxin-like domain conferring resistance to Fusarium head blight. *Nat. Genet.* **2016**, *48*, 1576–1580. [CrossRef] [PubMed]
24. Zheng, N.; Li, G.; Zhang, K.; Zheng, H.; Yang, J.; Yan, K.; Shi, K.; Su, Z.; Chen, F.; Wang, D.; et al. Analysis of *Fhb1* gene and resistance to Fusarium head blight in 3,177 diverse wheat accessions. *J. Cereal Sci.* **2022**, *104*, 103387. [CrossRef]
25. Ali, N.; Rahman, I.U.; Badakshi, F.; Tariq, M.J.; Mujeeb-Kazi, A. Ensuring sustainable food security: Exploiting alien genetic diversity in wheat breeding for adaptation to emerging stresses. In *Climate Change and Food Security with Emphasis on Wheat*; Academi Press: Cambridge, MA, USA, 2020; pp. 31–42.
26. Fu, S.; Lv, Z.; Qi, B.; Guo, X.; Li, J.; Liu, B.; Han, F. Molecular cytogenetic characterization of wheat–*Thinopyrum elongatum* addition, substitution and translocation lines with a novel source of resistance to wheat Fusarium head blight. *J. Genet. Genom.* **2012**, *39*, 103–110. [CrossRef]
27. Li, M.; Yuan, Y.; Ni, F.; Li, X.; Wang, H.; Bao, Y. Characterization of Two Wheat-*Thinopyrum ponticum* Introgression Lines With Pyramiding Resistance to Powdery Mildew. *Front. Plant Sci.* **2022**, *13*, 943669. [CrossRef]
28. Shen, X.; Ohm, H. Molecular mapping of *Thinopyrum*-derived Fusarium head blight resistance in common wheat. *Mol. Breed.* **2007**, *20*, 131–140. [CrossRef]
29. Zhang, X.; Shen, X.; Hao, Y.; Cai, J.; Ohm, H.W.; Kong, L. A genetic map of *Lophopyrum ponticum* chromosome 7E, harboring resistance genes to Fusarium head blight and leaf rust. *Theor. Appl. Genet.* **2011**, *122*, 263–270. [CrossRef]
30. Wang, H.; Sun, S.; Ge, W.; Zhao, L.; Hou, B.; Wang, K.; Lyu, Z.; Chen, L.; Xu, S.; Kong, L. Horizontal gene transfer of *Fhb7* from fungus underlies Fusarium head blight resistance in wheat. *Science* **2020**, *368*, eaba5435. [CrossRef]
31. Gill, B.S.; Friebe, B.R.; White, F.F. Alien introgressions represent a rich source of genes for crop improvement. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 7657–7658. [CrossRef]
32. Balut, A.L.; Clark, A.J.; Brown-Guedira, G.; Souza, E.; Van Sanford, D.A. Validation of *Fhb1* and *QFhs. nau-2DL* in several soft red winter wheat populations. *Crop Sci.* **2013**, *53*, 934–945. [CrossRef]
33. Ceoloni, C.; Forte, P.; Kuzmanović, L.; Tundo, S.; Moscetti, I.; De Vita, P.; Virili, M.; D'ovidio, R. Cytogenetic mapping of a major locus for resistance to Fusarium head blight and crown rot of wheat on *Thinopyrum elongatum* 7EL and its pyramiding

with valuable genes from a *Th. ponticum* homoeologous arm onto bread wheat 7DL. *Theor. Appl. Genet.* **2017**, *130*, 2005–2024. [CrossRef]
34. Gou, L.; Hattori, J.; Fedak, G.; Balcerzak, M.; Sharpe, A.; Visendi, P.; Edwards, D.; Tinker, N.; Wei, Y.-M.; Chen, G.-Y.; et al. Development and validation of *Thinopyrum elongatum*–expressed molecular markers specific for the long arm of chromosome 7E. *Crop Sci.* **2016**, *56*, 354–364. [CrossRef]
35. Forte, P.; Virili, M.E.; Kuzmanović, L.; Moscetti, I.; Gennaro, A.; D'Ovidio, R.; Ceoloni, C. A novel assembly of *Thinopyrum ponticum* genes into the durum wheat genome: Pyramiding Fusarium head blight resistance onto recombinant lines previously engineered for other beneficial traits from the same alien species. *Mol. Breed.* **2014**, *34*, 1701–1716. [CrossRef]
36. Guo, X.; Wang, M.; Kang, H.; Zhou, Y.; Han, F. Distribution, polymorphism and function characteristics of the GST-encoding *Fhb7* in Triticeae. *Plants* **2022**, *11*, 2074. [CrossRef]
37. Sarma, D.; Knott, D.R. The transfer of leaf-rust resistance from *Agropyron* to *Triticum* by irradiation. *Can. J. Genet. Cytol.* **1966**, *8*, 137–143. [CrossRef]
38. Sievers, F.; Higgins, D.G. Clustal Omega for making accurate alignments of many protein sequences. *Protein Sci.* **2018**, *27*, 135–145. [CrossRef] [PubMed]
39. Untergasser, A.; Cutcutache, I.; Koressaar, T.; Ye, J.; Faircloth, B.C.; Remm, M.; Rozen, S.G. Primer3—New capabilities and interfaces. *Nucleic Acids Res.* **2012**, *40*, e115. [CrossRef] [PubMed]
40. Doyle, J.J.; Doyle, J.L. Isolation of plant DNA from fresh tissue. *Focus* **1990**, *12*, 13–15.
41. Liu, S.; Yu, L.X.; Singh, R.P.; Jin, Y.; Sorrells, M.E.; Anderson, J.A. Diagnostic and co-dominant PCR markers for wheat stem rust resistance genes *Sr25* and *Sr26*. *Theor. Appl. Genet.* **2010**, *120*, 691–697. [CrossRef]
42. Kong, L.; Anderson, J.M.; Ohm, H.W. Segregation distortion in common wheat of a segment of *Thinopyrum intermedium* chromosome 7E carrying *Bdv3* and development of a *Bdv3* marker. *Plant Breed.* **2009**, *128*, 591–597. [CrossRef]
43. Gaire, R.; Ohm, H.; Brown-Guedira, G.; Mohammadi, M. Identification of regions under selection and loci controlling agronomic traits in a soft red winter wheat population. *Plant Genome* **2020**, *13*, e20031. [CrossRef] [PubMed]
44. Gilbert, J.; Woods, S. Strategies and considerations for multi-location FHB screening nurseries. In *The Global Fusarium Initiative for International Collaboration: A Strategic Planning Workshop*; CIMMYT: El Batàn, Mexico, 2006; pp. 93–102.
45. Fuentes, R.G.; Mickelson, H.R.; Busch, R.H.; Dill-Macky, R.; Evans, C.K.; Thompson, W.G.; Wiersma, J.V.; Xie, W.; Dong, Y.; Anderson, J.A. Resource allocation and cultivar stability in breeding for Fusarium head blight resistance in spring wheat. *Crop Sci.* **2005**, *45*, 1965–1972. [CrossRef]
46. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2021; Available online: https://www.R-project.org/ (accessed on 4 December 2022).
47. Bariana, H.S.; Brown, G.N.; Bansal, U.K.; Miah, H.; Standen, G.E.; Lu, M. Breeding triple rust resistant wheat cultivars for Australia using conventional and marker-assisted selection technologies. *Aust. J. Agric. Res.* **2007**, *58*, 576–587. [CrossRef]
48. USDA-ERS. Wheat Outlook: October 2022. Available online: https://www.ers.usda.gov/webdocs/outlooks/104961/whs-22j.pdf?v=476.4 (accessed on 15 January 2023).
49. Bianchini, A.; Horsley, R.; Jack, M.M.; Kobielush, B.; Ryu, D.; Tittlemier, S.; Wilson, W.W.; Abbas, H.K.; Abel, S.; Harrison, G.; et al. DON occurrence in grains: A North American perspective. *Cereal Foods World* **2015**, *60*, 32–56. [CrossRef]
50. Wilson, W.; Dahl, B.; Nganje, W. Economic costs of Fusarium Head Blight, scab and deoxynivalenol. *World Mycotoxin J.* **2018**, *11*, 291–302. [CrossRef]
51. Gaire, R.; Sneller, C.; Brown-Guedira, G.; Van Sanford, D.; Mohammadi, M.; Kolb, F.L.; Olson, E.; Sorrells, M.; Rutkoski, J. Genetic Trends in Fusarium Head Blight Resistance from 20 Years of Winter Wheat Breeding and Cooperative Testing in the Northern USA. *Plant Dis.* **2022**, *106*, 364–372. [CrossRef] [PubMed]
52. Rutkoski, J.E. Estimation of realized rates of genetic gain and indicators for breeding program assessment. *Crop Sci.* **2019**, *59*, 981–993. [CrossRef]
53. Zhang, W.; Cai, X. Alien introgression and breeding of synthetic wheat. In *Advances in Breeding Techniques for Cereal Crops*; Burleigh Dodds Science Publishing: Cambridgeshire, UK, 2019; pp. 3–54.
54. Zhang, W.; Danilova, T.; Zhang, M.; Ren, S.; Zhu, X.; Zhang, Q.; Zhong, S.; Dykes, L.; Fiedler, J.; Xu, S.; et al. Cytogenetic and genomic characterization of a novel tall wheatgrass-derived *Fhb7* allele integrated into wheat B genome. *Theor. Appl. Genet.* **2022**, *135*, 4409–4419. [CrossRef]
55. Anderson, J.A.; Glover, K.; Mergoum, M. Successful adoption of spring wheat cultivars with moderate resistance to FHB by growers in the North Central Region. In Proceedings of the 2011 National Fusarium Head Blight Forum, USWBSI, St. Paul, MA, USA, 4–6 December 2011.
56. Bai, G.; Shaner, G. Management and resistance in wheat and barley to Fusarium head blight. *Annu. Rev. Phytopathol.* **2004**, *42*, 135–161. [CrossRef]
57. Sears, E.R. Genetics society of canada award of excellence lecture an induced mutant with homoeologous pairing in common wheat. *Can. J. Genet. Cytol.* **1977**, *19*, 585–593. [CrossRef]
58. Dvorak, J.; Knott, D.R. Disomic and ditelosomic additions of diploid *Agropyron elongatum* chromosomes to *Triticum aestivum*. *Can. J. Genet. Cytol.* **1974**, *16*, 399–417. [CrossRef]
59. Dvořák, J. Homoeology between Agropyron elongatum chromosomes and Triticum aestivum chromosomes. *Can. J. Genet. Cytol.* **1980**, *22*, 237–259. [CrossRef]

60. Roberts, M.A.; Reader, S.M.; Dalgliesh, C.; Miller, T.E.; Foote, T.N.; Fish, L.J.; Snape, J.W.; Moore, G. Induction and characterization of *Ph1* wheat mutants. *Genetics* **1999**, *153*, 1909–1918. [CrossRef] [PubMed]
61. Su, Z.; Jin, S.; Zhang, D.; Bai, G. Development and validation of diagnostic markers for Fhb1 region, a major QTL for Fusarium head blight resistance in wheat. *Theor. Appl. Genet.* **2018**, *131*, 2371–2380. [CrossRef] [PubMed]
62. Jarošová, J.; Beoni, E.; Kundu, J.K. Barley yellow dwarf virus resistance in cereals: Approaches, strategies and prospects. *Field Crops Res.* **2016**, *198*, 200–214. [CrossRef]
63. McKirdy, S.J.; Jones, R.A.C.; Nutter, F.W., Jr. Quantification of yield losses caused by barley yellow dwarf virus in wheat and oats. *Plant Dis.* **2002**, *86*, 769–773. [CrossRef]
64. Perry, K.L.; Kolb, F.L.; Sammons, B.; Lawson, C.; Cisar, G.; Ohm, H. Yield effects of barley yellow dwarf virus in soft red winter wheat. *Phytopathology* **2000**, *90*, 1043–1048. [CrossRef]
65. Walls, J., III; Rajotte, E.; Rosa, C. The past, present, and future of barley yellow dwarf management. *Agriculture* **2019**, *9*, 23. [CrossRef]
66. Larkin, P.J.; Kleven, S.; Banks, P.M. Utilizing *Bdv2*, the *Thinopyrum intermedium* source of *BYDV* resistance, to develop wheat cultivars. *Barley Yellow Dwarf Dis. Recent Adv. Future Strateg.* **2002**, 60–63. Available online: https://books.google.co.uk/books?hl=zh-CN&lr=&id=-CDnnbNKvCMC&oi=fnd&pg=PA60&ots=M-zigfWJOf&sig=RqeSJl5RPtr_hiL1JloaChrv9M4#v=onepage&q&f=false (accessed on 15 January 2023).