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Characterization of the wheat-tetraploid *Thinopyrum elongatum* 7E(7D) substitution line with Fusarium head blight resistance

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Abstract

Background Fusarium head blight (FHB), a devastating disease of wheat production, is predominantly elicited by *Fusarium graminearum* (*Fg*). The tetraploid *Thinopyrum elongatum* is a tertiary gene resource of common wheat that possesses high affinity and displays high resistance traits against multiple biotic and abiotic stress. We aim to employ and utilize the novel FHB resistance resources from the wild germplasm of common wheat for breeding.

Results Durum wheat-tetraploid *Th. elongatum* amphiploid 8801 was hybridized with common wheat cultivars SM482 and SM51, and the F_5 generation was generated. We conducted cytogenetically in situ hybridization (ISH) technologies to select and confirm a genetically stable 7E(7D) substitution line K17-1069-5, which showed FHB expansion resistance in both field and greenhouse infection experiments and displayed no significant disadvantage in agronomic traits compared to their common wheat parents in the field. The F_2 segregation populations (K17-1069-5 × SM830) showed that the 7E chromosome conferred dominant FHB resistance with dosage effect. We developed 19 SSR molecular markers specific to chromosome 7E, which could be conducted for genetic mapping and large breeding populations marker-assisted selection (MAS) during selection procedures in the future. We isolated a novel *Fhb7* allele from the tetraploid *Th. elongatum* chromosome 7E (Chr7E) using homology-based cloning, which was designated as *TTE7E-Fhb7*.

Conclusions In summary, our study developed a novel wheat-tetraploid *Thinopyrum elongatum* 7E(7D) K17-1069-5 substitution line which contains stable FHB resistance.

Keywords Fusarium head blight, Tetraploid *Thinopyrum elongatum*, Common wheat substitution line, *Fhb7* gene resistance

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Background

Fusarium head blight (FHB), caused by multiple pathogenic fungi *Fusarium* spp., seriously damages wheat yield and quality in temperate regions worldwide. It reduced more than half of production during the epidemic years [1, 2]. In China, FHB caused 10–50% wheat yield losses from moderate to severe epidemic years, which undermined about 10 million hectares of wheat production [3]. The FHB-infected spikes are deficient in nutrients, cause reddish grain color, reduce grain size, and produce mycotoxins in the seeds, which are hazardous to human and animal bodies [4, 5]. Deoxynivalenol (DON) is a prevalent mycotoxin and potentially restrain protein synthesis. It can induce diarrhea, anorexia, vomiting, and gastrointestinal bleeding in animals, and it is difficult to eliminate through chemical and physical treatments or temperature changes with long-term storage [6, 7].

To maintain grain quality and productivity, resistant varieties and new sources of resistance are necessary to identify and develop in germplasm. *Fhb1-Fhb9* were currently nine formally nominated FHB resistance genes [8–16]. *Fhb1*, *Fhb2*, *Fhb4*, *Fhb5*, and *Fhb8* are resistance fungal expansion genes (type II resistance) and were identified in wheat cultivar Sumai 3 and/or landrace Wangshuibai [8–12], *Fhb9* was derived from Ji5265 [13], *Fhb3*, *Fhb6*, and *Fhb7* were originated from wild Triticeae species *Leymus racemosus* (Lam.) Tzvelev, *Campeiostachys kamoji* (Ohwi) B.R.Baum, J.L.Yang & C.Yen (Synonyms *Elymus tsukushiensis* Honda), and *Thinopyrum poticum* (Podp.) Barkworth & D.R.Dewey, respectively [14–16]. Currently, *Fhb1* and *Fhb7* have been successfully identified based on the positional cloning. They present different FHB resistance mechanisms, of which *Fhb1* encodes a histidine-rich calcium-binding protein, and *Fhb7* encodes a glutathione S-transferase [16–18]. Because of this exceptional rarity of wheat main FHB resistance QTLs, the discovery of novel FHB-resistant gene resources is very important for wheat disease resistance breeding.

Plentiful FHB resistance from wild Triticeae relatives has been evaluated, such as *Thinopyrum*, *Roegneria*, *Leymus*, *Pseudoroegneria*, and *Agropyron* [19, 20]. *Thinopyrum elongatum* (Host) D. R. Dewey (Triticeae, Poaceae), with E as the designed genome symbol, contains 2×, 4×, and 10× ploidys [21]. According to whole genome comparison, the common wheat D sub-genome and the *Th. elongatum* E genome has high genetic affinity, indicating the potential that *Th. elongatum* could be used in wheat breeding through chromosome engineering [16]. Through distant hybridization and chromosome engineering, the alien segments of the *Thinopyrum* species with desirable genes deployed successfully in wheat over the last few decades. For instance, the wheat × *Th. elongatum* derivative lines have been reported carrying genes showed FHB resistance (*Fhb7*), rust resistance (*Sr24* and *Lr24*), and powdery mildew resistance (*Pm51*), as well as the abiotic stresses resistance to salt salinity, chilling, and drought [22–29]. There are many successful examples of transferring chromosome segments of diploid *Th. elongatum* and decaploid *Th. ponticum* to wheat [27, 30, 31], however, the FHB resistance and other excellent agronomy traits in the tetraploid *Th. elongatum* still needs further exploration and utilization.

Previously, we hybridized durum wheat-tetraploid *Th. elongatum* amphiploid 8801 with common wheat cultivar Shumai 482 (hereafter SM482), and crossed the F_1 with common wheat cultivar Shumai 51 (SM51). The generation was advanced to the F_5 generation. The present study aims to (1) select a wheat-tetraploid *Th. elongatum* substitution line and characterize the genome constitution; (2) investigate the FHB resistance phenotype and the agronomic characters of the substitution line; (3) generate and verify the specificity of molecular markers for the alien chromosome; (4) analyze the genetic effect of the alien chromosome.

Results

Characterizing the genomic constitution and cytogenetic stability of the 7E(7D) substitution line

To generate the 7E(7D) substitution line, we performed hybridization between durum wheat-tetraploid *Th. elongatum* amphiploid 8801 (♀ parent) and SM482 (♂ parent) and the F_1 plants were further hybridized with SM51 and selfed to F_5 generation. The substitution line was selected from the F_5 generation based on GISH analysis using the gDNA of tetraploid *Th. elongatum* as probes. The result revealed that one plant K17-1069-5 possessed 42 chromosomes, including two E genome signals (in magenta) (Fig. 1A). To distinguish the homoeology of E chromosomes in common wheat genetic background, oligo-FISH painting probes Chr1E–Chr7E was performed. It displayed that the alien chromosomes were labeled by the Chr7E probe (in green) (Fig. 1B). The chromosomal composition of K17-1069-5 was characterized with non-denature FISH (ND-FISH), by comparing with the cytogenetic karyotype of CS [32] and tetraploid *Th. elongatum* [33]. The result showed that K17-1069-5 was absent in a 7D chromosome pair of common wheat and replaced by a 7E chromosome pair (Fig. 1C). Thus, we confirmed that K17-1069-5 was a 7E(7D) substitution line. The cytological stability of K17-1069-5 was evaluated using 40 selfed seeds of K17-1069-5 randomly selected for GISH analysis. All 40 plants contained the same genomic constitution as K17-1069-5 (Fig. 1D–F). Therefore, K17-1069-5 was identified as a genomic-stable 7E(7D) substitution line.

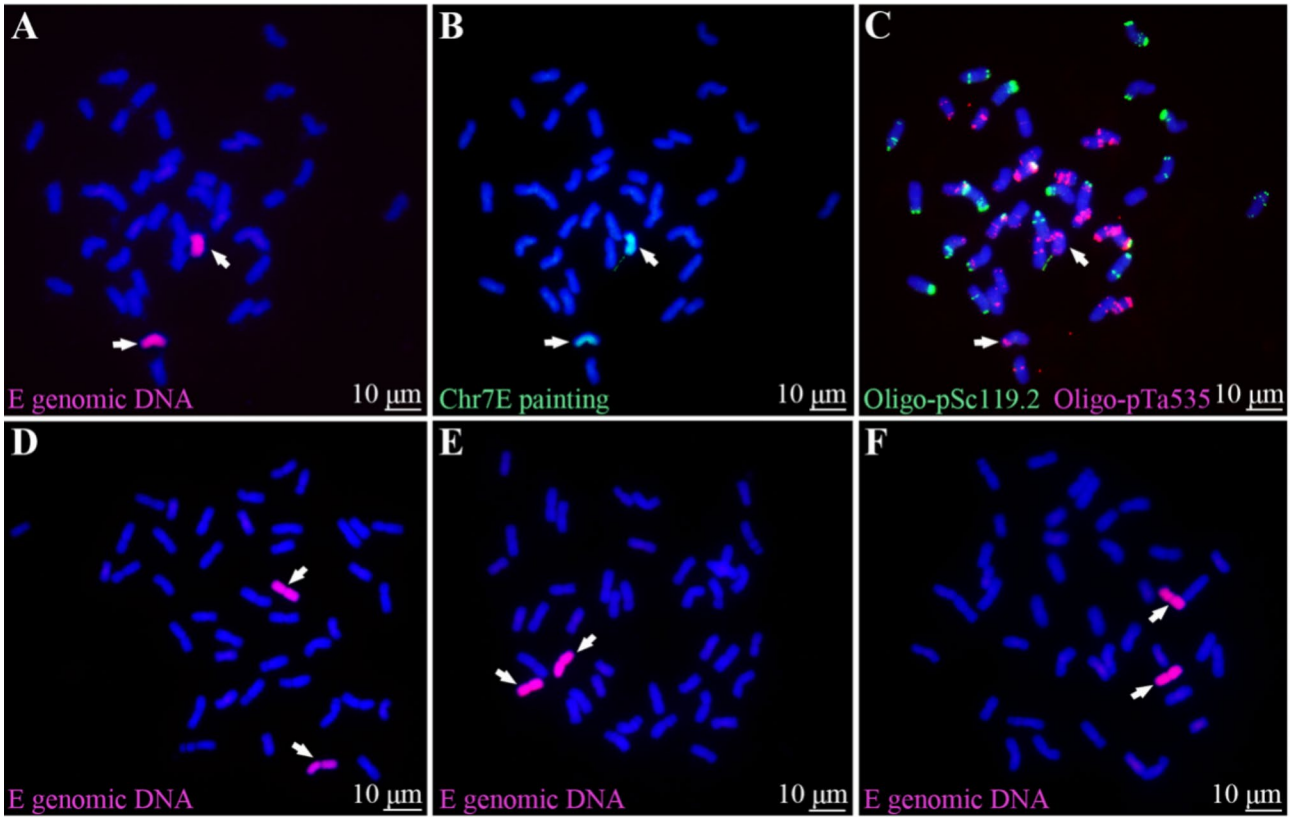

Fig. 1 Characterization of the genome composition of the 7E(7D) substitution line and its offspring by using cytogenetic techniques. **A**, Genomic in situ hybridization (GISH) analysis of K17-1069-5 using tetraploid *Th. elongatum* gDNA as the E genome probe (magenta). **B** Fluorescence in situ hybridization (FISH) painting using Chr7E probe (green). **C** FISH analysis using Oligo-pTa535 (magenta) and Oligo-pSc119.2 (green) probes. **D-F** Cytogenetic stability identification of selfed seeds of K17-1069-5 using GISH (E genome in magenta). White arrows display the alien chromosomes

The FHB resistance and agronomic characters of 7E(7D) substitution line

The 7E(7D) substitution line and common wheat parents (SM482 and SM51) were infected with one of the major pathogen *Fusarium graminearum* (*Fg*) strain PH-1 to assess the severity level of FHB from 2022 to 2023 in the greenhouse and field experimental conditions. After 14 days post-inoculation (dpi) and 21 dpi, it displayed that the susceptible control Roblin and common wheat parents were FHB highly susceptible and the percentage of diseased spikelets (PDS) exceeded 72%, while Sumai3 (resistance control), 8801, and 7E(7D) substitution lines displayed immunity to FHB with expanding resistance to adjacent spikelets and rachis in both greenhouse and field conditions (Fig. 2). In summary, wheat-tetraploid *Th. elongatum* 7E(7D) substitution line displayed excellent Type II FHB resistance to *Fg* strain PH-1.

Agronomic traits investigation exhibited that the 7E(7D) substitution line had a longer flag leaf than the parents. It possessed similar plant height, tiller numbers, spike length, and spikelet numbers to common wheat parents SM482 and SM51, while significantly better than the alien parent amphiploid 8801 (Fig. 3; Additional file 1: Fig. S1; Additional file 2: Table S1). In summary, the tetraploid *Th. elongatum* 7E chromosome displayed less negative effect on common wheat in terms of agronomic characteristics.

Generation and verification of SSR molecular markers specific for 7E chromosome

To accurately identify the 7E chromosome from a common wheat genetic background, we developed Simple Sequence Repeat (SSR) markers specific to Chr7E which can be applied in the alien chromosome segment identification for large-scale of marker-assisted selection (MAS). First, the diploid *Th. elongatum* 7E reference genome was aligned with Chr1E–Chr6E and reference CS (v2.1), and, 1999 SSR markers were identified specific to Chr7E. In total, 60 SSR primer pairs were arbitrarily picked to confirm their specificity by PCR. A total of 19 SSRs displayed the targeted amplify bands in diploid and tetraploid *Th. elongatum*, 8801, and 7E(7D) substitution line, while absent in common wheat CS, SM482, SM51, and 1E–6E substitution lines. Consequently, these 19 SSRs could be used to detect the Chr7E under a common wheat background (Fig. 4; Table 1).

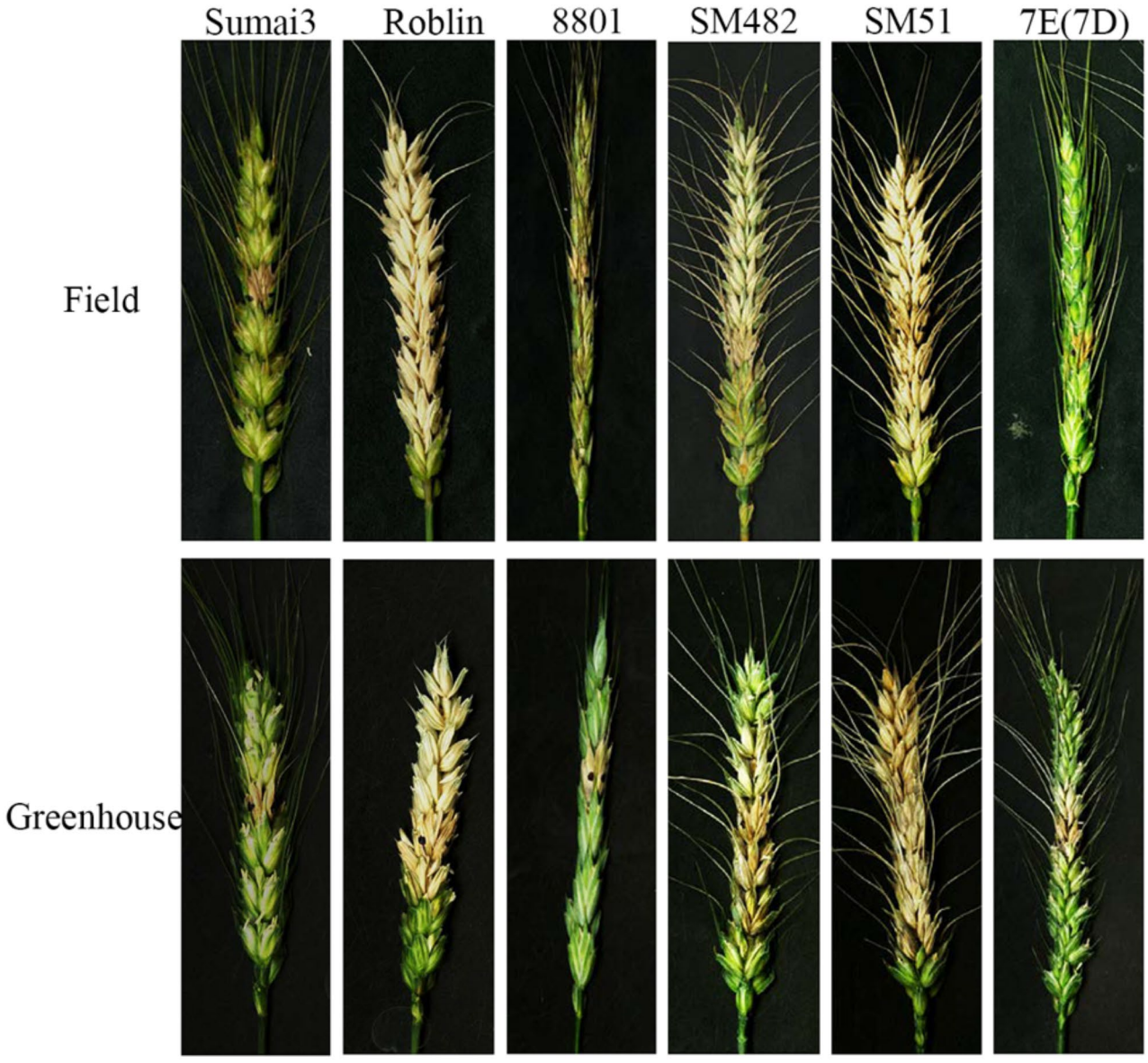

Fig. 2 The FHB resistant evaluation of 7E(7D) substitution line and their common wheat parental materials SM482 and SM51

Transmission and genetic effects of Chr7E and FHB resistance in common wheat genetic background

To analyze the pyramid effect on FHB resistance contributed by Chr7E, we constructed an F_2 population using FHB resistance 7E(7D) substitution line and FHB susceptible wheat SM830. We genotyped 122 F_2 individuals using the Chr7E-specific marker *TTE7E-31* (Fig. 5A–C; Additional file 2: Table S2). Afterwards, we performed cytogenetic GISH analysis to quantity the alien chromosome number. We found a reasonable separation ratio (7D/7D: 7D/7E: 7E/7E) of roughly 1:2:1 in the F_2 population with 36, 59, and 27 plants respectively (Fig. 5D). The FHB severity of F_2 plants was examined with PH-1 inoculation in the greenhouse at the flowering stage (Additional file 2: Table S2). The common wheat parents and all F_2 plants with 7D/7D genotypes were highly susceptible to FHB. In the 7D/7E genotype group, 45 of 59 were resistant to FHB, while 14 of them were susceptible. All the 27 plants in the 7E/7E genotype were highly resistant to FHB. This result implied that the high FHB resistance was provided by the alien Chr7E of tetraploid *Th. elongatum* which considerably reduced the proportion of infected spikelets. Consequently, we deduced that the Chr7E has a dosage-dependent dominant type II FHB resistance locus (s).

The Chr7E of tetraploid *Th. elongatum* contains a novel *Fhb7* allele

To detect whether the 7E(7D) substitution line contained *Fhb7* from the 7E of diploid *Th. elongatum* or

Fig. 3 Agronomic performance of the 7E(7D) substitution line, common wheat parental materials SM482 and SM51

its homologs, we used two functional markers of *Fhb7* (*Fhb7*^{Thp} and *Fhb7*^{The2}). The result showed that the 7E(7D) substitution line might contain a functional *Fhb7* allele. Then we used the homology-based cloning method to clone the *Fhb7* allele from tetraploid *Th. elongatum*, which were named as *TTE7E-Fhb7.* Nucleotide alignment displayed that the sequence in the 7E(7D) substitution line displayed 6 bp deletion compared to both *Fhb7*^{The2} and *Fhb7*^{Thp}, as well as 5 and 10 SNPs compared to *Fhb7*^{The2} and *Fhb7*^{Thp}, respectively (Fig. 6A). Thus, we confirmed that *TTE7E-Fhb7* in the tetraploid *Th. elongatum* 7E possessed an *Fhb7* homologous gene. Comparative analysis of protein primary sequences indicated that *TTE7E-Fhb7* lack a serine and threonine at the N-terminal, in addition, two nonsynonymous mutants from SNPs differences made alanine to serine, leucine to phenylalanine (Fig. 6B). However, whether these sequence differences could influence the function of gene or not needs further exploration.

Discussion

Employment of wild germplasm resources in wheat breeding

Interspecific hybridization and chromosome engineering are two crucial methods for wheat breeders to introduce new traits from wild germplasm into elite modern cultivars. The 1RS·1BL and 6VS·6AL are two translocation lines that are effectively employed in wheat breeding program with desirable disease resistance and great agronomic performance [34, 35]. Genes from wheatgrasses (*Thinopyrum* spp.) have been transferred into cultivated *Triticum* spp by employing amphidiploids, and even small alien fragment introgressions. In this study, the 7E(7D) substitution line presented less negative effect on common wheat, and hence it is a potential germplasm for FHB resistance breeding.

To introduce the alien material with FHB resistance gene(s) into the wheat genetic background and reduce the redundancy traits on alien chromosomes, several chromosome engineering methods have been developed: the use of Chinese Spring *ph1b* mutant (CS *ph1b*) [36, 37], gamma irradiation [38, 39], and breakage-fusion mechanism [40, 41]. By using these methods translocations and/or deletions can be induced in the substitution line, and can narrow down the physical interval of alien segments, to have a better compensation effect and genetic balance for breeding [34, 35, 42]. All these three strategies could further explore the FHB resistance *TTE7E-Fhb7* haplotype and mechanism, and deploy FHB resistance from the tetraploid *Th. elongatum* 7E chromosome to wheat breeding.

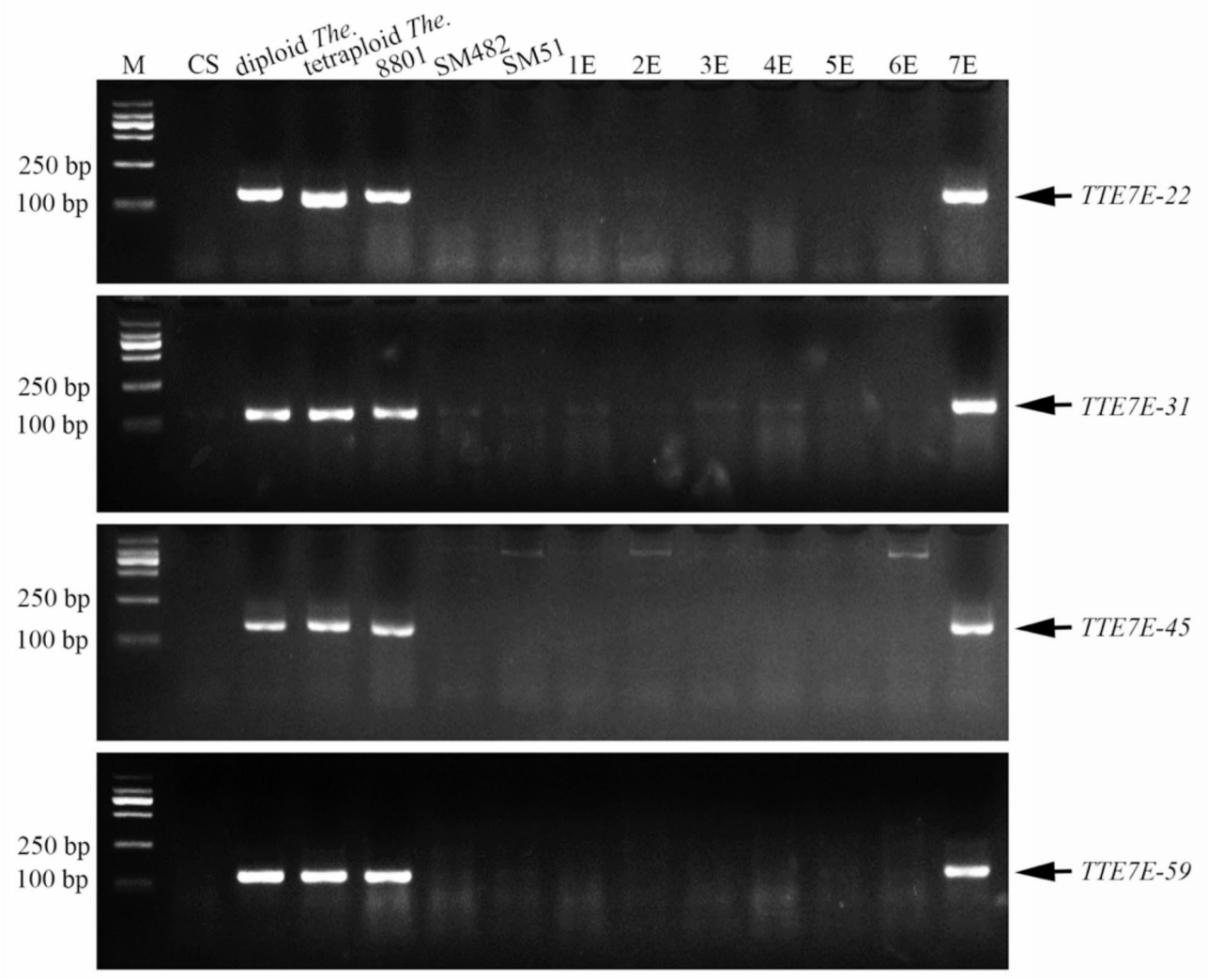

Fig. 4 Generation and verification of the 7E-chromosome unique SSR primers. SSR molecular markers *TTE7E-22*, *TTETE-31*, *TTETE-45*, and *TTETE-59*

The application of *Th. elongatum* in common wheat disease resistance

It has been proved that the chromosome 7E group included the genes/loci resistance to biotic and abiotic stress [43, 44]. For instance, gene *Lr19* was resistance to leaf rust [45], gene *Sr25* was stem rust resistant to Ug99 races [46, 47], and a yellow pigment content (YPC) *Yp* gene which are closely linked and localization at the distal region of 7E#l chromosome [43, 44]. Tounsi et al. [48] reported that the 7E chromosome enhanced the tolerance to salinity stress in the durum wheat. More recently, two quantitative trait loci (QTLs) for FHB resistance were identified from the homologous group 7 in different *Thinopyrum* species [16, 49]. In this research, we generated an FHB expansion-resistant resource7E(7D) substitution line, without negatively compromising the agronomic characteristics of common wheat. Hence, *Thinopyrum* spp. has the potential to be utilized for wheat FHB resistance breeding as an appropriate bridge breeding material.

Wheat FHB resistance is considered a quantitative trait, that governed by one or several QTLs (probably major or minor effects), additionally, it is associated with genotypes and environment interactions [50]. It has been reported that the average percentage of infected spikes of the lines carrying resistance genes was lower than lines without the resistance genes, and the effect was most obvious in the case of gene *Fhb1* and the combination of *Fhb1*+*Fhb2* and *Fhb1*+*Fhb2*+*Fhb4* [51]. The FHB resistance was stronger when *Fhb1* and *Fhb9* were combined than the single *Fhb1* or *Fhb9* gene, indicating a positive dosage effect between the two QTLs [13]. In this research, F_2 segregation population plants with single alien 7E chromosomes varied for FHB resistance from resistance to susceptible, while homozygous 7E chromosome plants were highly resistant to FHB, indicating the significant dosage effect.

Table 1 The specific SSR primer pairs of the Chr7E identified in this study

Primers	Sequences of the Special Primers (5′–3′)	
	Forward	Reverse
TTE7E-17	ACCTACTCTGACCGCCTGAA	GAAATATCATGCCGGGAGAC
TTE7E-20	AAAAGGAGTGGGAGGAGGAG	CGGCGCTTACCTAAACTTTG
TTE7E-21	GTCAACTCGCCAGGTCTCTC	CAAAACCTCCGCCTTAGGTA
TTE7E-22	CTGCGCTACCAGCTACCTCT	TAGCGCTCTTCCGCTACTTC
TTE7E-23	ATGCCTCGATCCATACCTTG	GACGGTGGTAGAGCTTCTGG
TTE7E-26	TATATCGCCTGCTCGAACG	ATTTATGTGCGGCAAAAAGG
TTE7E-27	CCAGGCCAGTTTACTCAAAGA	GAGAGGATGGATGCCACTTT
TTE7E-30	GTGAGGTTCCACCGGTTTT	CGTATTTGGAAAAGTTTCAGTGG
TTE7E-31	AGCCGGAGCCTATTCTTTTT	GACGCAGCTGATCAATAGGA
TTE7E-34	TTGTTGTTGCAAAATGCACTC	CGGAAGGCAACTTCTTCTTG
TTE7E-38	TCCGGTGGCTTCTTCTTCT	TGACCAGCACGGAACAATAG
TTE7E-41	AAGGCGTAAAAAGTGGAGCA	TACCCATTGCAGCCACTAAA
TTE7E-43	CCTCACTCCCTCTCTGATCG	GCAACCTTGACCATGTTCCT
TTE7E-45	TTATGTCATCACGCGCCTAC	TCAATACGCGATGAACATTTTT
TTE7E-46	GAAATTGAACCACGCATCCT	AGTTCCTCGTTGGCATCACT
TTE7E-47	TCAAAAGCGCCCTACTCATT	ATCCTCTAGCCTCGCCTTTT
TTE7E-50	CATGGAAGGGTTCGTAAGGA	TCCACTCCCAAAATCCACTC
TTE7E-58	GAACTAGATGGGCGTCTCCA	AGGGCAAGGAAACTGTCTCA
TTE7E-59	TCTGGCAATGGATGAATGAA	ATATACCCTCCCCGGCACTA

High allelic multiplicity of resistance genes differing in pathogen resistance in plants

Plants recognize pathogens through nucleotide-binding leucine-rich repeat receptors (NLRs) intracellularly [52]. Variations have led to multiple functional alleles within the same locus in syntenic regions, likely enabling the recognition of different effector alleles to confer resistance or exhibit different resistance magnitudes. There are multiple examples of allelic diversity that give rise to functional diversity in resisting various pathogens. For example, powdery mildew (Pm) resistance CNLs *Pm60a*, *Pm60*, and *Pm60b* cloned from *Triticum urartu* are allelic to *PmG16*/*MlIW18*/*MlIW172* from wild emmer wheat [53]. In *Arabidopsis*, abundant intraspecies diversity possesses highly variable NLRs (hvNLRs), provides new pathogen-recognition specificities, and is associated with allelic diversity and genome at a population level [54]. Consistent with our previous wheat-*Elymus repens* 7St FHB-resistance translocation line work [55], in this research, we confirmed that the FHB expansion resistance was conferred by *TTE7E-Fhb7* loci in the Chr7E of tetraploid *Th. elongatum*. Meanwhile, three *Fhb7* alleles alignment revealed that the sequence of *TTE7E-Fhb7* exhibits 6 bp deletion as well as 5 and 10 SNPs with $Fhb7^{The2}$ and $Fhb7^{Thp}$, respectively. This indicates multiple FHB resistance alleles may exist in this homoeologous group. Whether *TTE7E-Fhb7* has the same detoxification function as $Fhb7^{Thp}$, and the relationship between $Fhb7^{The2}$ and $Fhb7^{Thp}$ need further study.

Strategies unveiling the candidate genes associated with aimed traits

Wild relatives are crucial germplasm resources for wheat improvement and breeding. Traditionally, cytogenetic ISH experiments were conducted to discriminate the alien chromosomes. Recently, a newly developed oligo-painting FISH technique has been designed for chromosome-specific identification, which can be applied to alien homoeologous chromosome groups [56, 57]. Generally, cytogenetic-based methods directly visualize the alien segments introduced into the wheat genetic background [58]. Nevertheless, it costs labor and time, especially with small alien fragments or sequence introgression that may display undetectable fluorescence signals due to highly condensed mitotic chromosomes and the limited resolution of the microscope.

Molecular markers were conducted to precisely track alien sequences with targeted traits in a wheat genetic background, and significantly improve the efficiency of the MAS during the breeding cycle [59, 60]. In the past, a series of molecular markers in *Thinopyrum elongatum* Chr1E–7E have been continuously generated, such as expressed sequence tags (EST), PCR-based landmark unique gene (PLUG) markers, specific-locus amplified fragment sequencing (SLAF-seq), GBS (genotyping-by-sequencing). Gong et al. [61] confirmed the three 4E chromosome long arm translocation lines at the centromeric region by 55 K SNP arrays since the most of the SNPs in the translocation regions were detected as deletions.

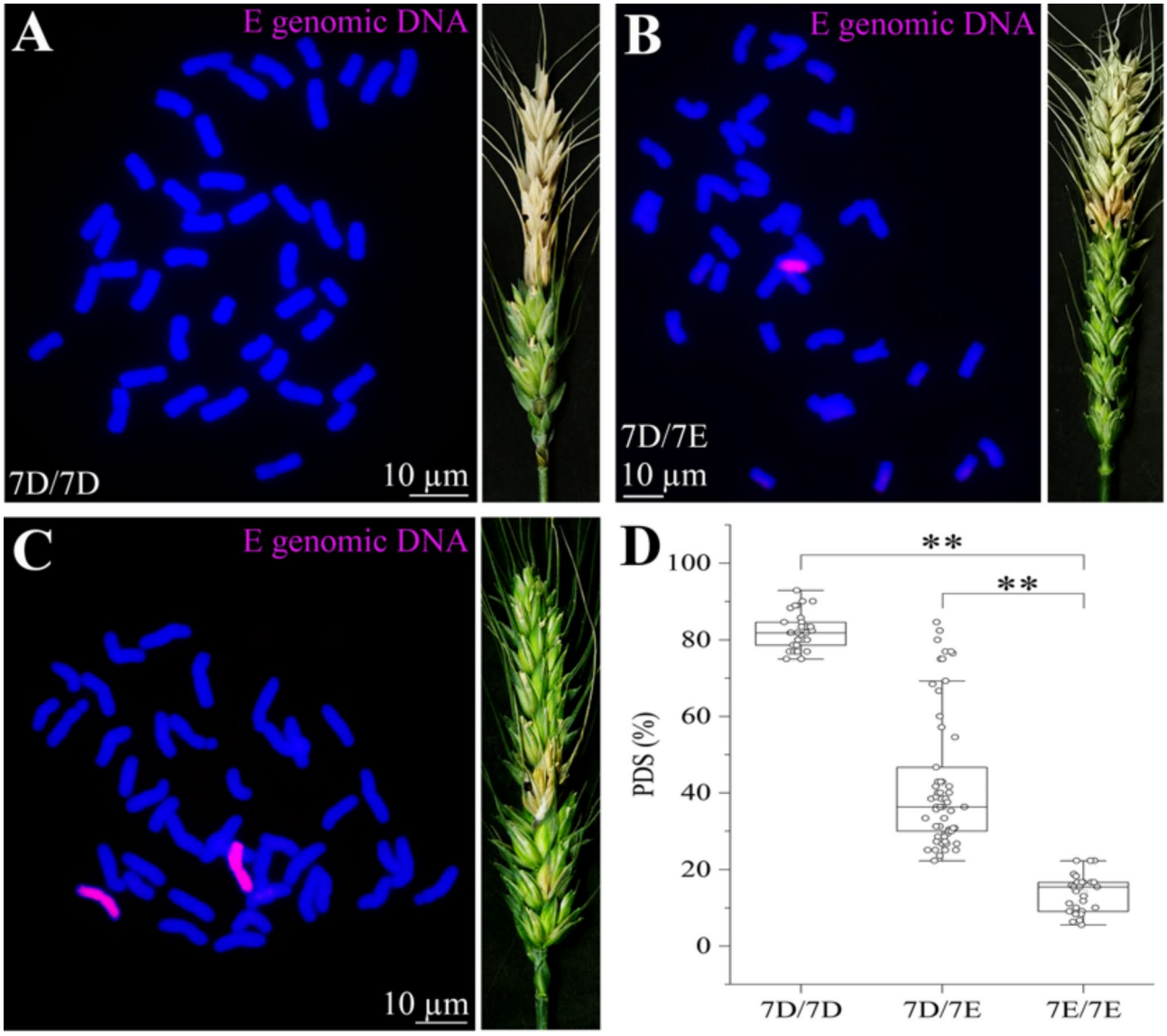

Fig. 5 Genetic effects analysis of Chr7E in common wheat background. **A-C** GISH identification of three F_2 genotypes and FHB resistance (**A** 7D/7D, without alien E chromosome, **B** 7D/7E, containing one 7E chromosome, and **C** 7E/7E, containing two 7E chromosomes) with tetraploid *Th. elongatum* whole genome DNA probe (magenta). **D** The PDS percentage for different genotypes. The statistical significance differences were evaluated using a Student's t-test (* $p < 0.05$, ** $p < 0.01$, ns not significant)

More and more released reference genome and resequencing data offer an advantage for detecting alien genetic information. Based on the sequencing system, Deng et al. [62] generated a wheat–*Thinopyrum elongatum* array for genotyping alien E chromosome of *Thinopyrum* species and derivative. The chip array assists in the cytological characterization of chromosome engineering breeding. Kompetitive allele-specific PCR (KASP) markers provides efficient approach for large population genotyping during breeding [63]. In this study, we generated and verified Chr7E-specific SSR markers for MAS. We plan to obtain a small 7E chromosome translocation line and develop KASP co-dominant markers for identifying the alien fragments with FHB resistance.

More recently, target resistance genes (*R* genes) and specific genomic regions have been efficiently characterized using next-generation sequencing (NGS). However, due to abundant genetic diversity and variations, aimed *R* genes in wild germplasm may not be included in the published reference haplotypes. Additionally, target *R* genes may be suppressed for chromosome recombination and linkage redundancy. Multiple strategies, such as chromosome sorting, chromosome microdissection, bulked segregation analysis, MutMap, QTL-seq, reference genome assembling, or primary long-read sequencing, offer

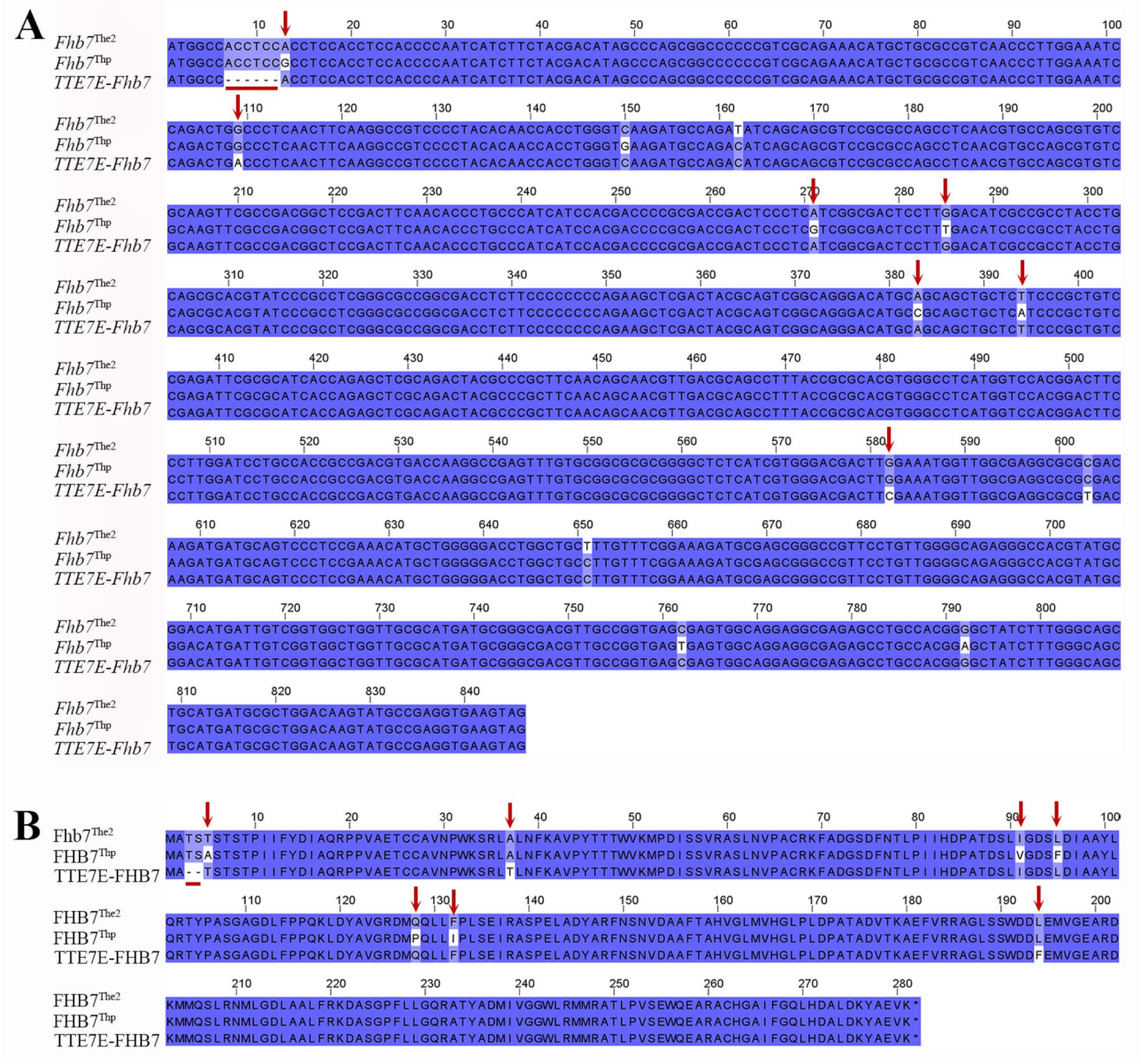

Fig. 6 The detection of the functional marker (*GST*) of *Fhb7* to 7E(7D). **A** Nucleotide sequence alignment. **B** Amino acid sequences alignment. The *Fhb7-Thp* sequence from the BAC clone *NODE_28_length_58203_cov_9148.280322* [16]; The *Fhb7*^{The2} and Fhb7^{Thp} sequence from the *Tel7E01T1020600.1*. The blue arrow pairs indicate the initiation codon and termination codon, respectively. The red lines below indicate deletion. Arrows above the sequences display anonymous SNP mutants

targeted gene cloning and exploring gene mechanisms [64, 65]. To clone and characterize the FHB resistance gene in the Chr7E of tetraploid *Th. elongatum*, we will sequence the partial amphiploid *Trititrigia* 8801 parental by long-read sequencing technology.

Methods

Materials

The tetraploid *Triticum durum*-tetraploid *Th. elongatum* partial amphiploid *Trititrigia* 8801 ($2n=6x=42$, genome constitution AABBEE) was provided by Dr. George Fedak from Ottawa Research and Development Centre, Agriculture and Agri-Food Canada. It displayed broad-spectrum resistance to biotic stress such as rust, FHB, and powdery mildew, and tolerance to abiotic stress such as drought, chilling, and salinity. *Triticum aestivum* cv. Shumai 482 (SM482) and Shumai 51 (SM51) in Sichuan province were susceptible to FHB, which were used as parental lines for 7E(7D) substitution line generation. The F_2 population of the substitution line and Shumai 830 (SM830) was constructed for genetic effect analysis of the alien 7E chromosome. The GISH analysis was conducted

using the gDNA of tetraploid *Th. elongatum* (2n=4*x*=28, EEEE) as the probe, and the fragmented gDNA of *T. aestivum* cv. Chinese Spring 'CS' (2*n*=6*x*=42, AABBDD) as the block. *T. aestivum* cv. Sumai 3 and Robblin were applied as the FHB resistance and susceptible control group, respectively.

Preparation of chromosome spreads

The mitotic metaphase chromosome spreads were carried out according to Aliyeva-Schnorr et al. [66] with adjustments. Shortly, the fixed root-tip meristems were digested for 60 min at 37 °C using enzyme solution (cellulase onozuka R-10: 2% pectolyasein Y-23=4: 2). This was followed by two times washing in 75% ethanol, followed by the addition of 10–15 µL 90% acetic acid (per meristem) in place of the ethanol, and crushing into meristems suspension. The suspension was spread onto slides with a humidity range of 50–55%. Using phase-contrast microscopy, slides with expected chromosome morphology and number were selected once they had dried, and they were kept at −20 °C until used for sequential ISH.

Sequential ISH analyses and microscopy

GISH protocol was conducted according to Wu et al. [67]. The whole gDNA of tetraploid *Th. elongatum* was labeled using dUTP-ATTO-550 (Jena Bioscience, Jena, Germany), meanwhile, the CS gDNA was applied as a block and fragmented by autoclave (121 ℃ for 5 min). The probe: block equals 1:150. In total, 20 µL of hybridization solution was hybridized with each prepared slide (16 µL hybridization mixture, 100 ng/µL labeled probe, 15000 ng/µL block).

To distinguish alien chromosomes of tetraploid *Th. elongatum* and substitutional common wheat chromosomes, we conducted mc-FISH with probes Oligo-pSc119.2 (6-FAM-5') and Oligo-pTa535 (Tamra-5'), and compared with cytogenetic karyotype of wheat and *Th. elongatum* [33, 34].

To identify the homology of the E chromosome, oligo-FISH probes Chr1E–Chr7E were conducted as sequential bulk oligo-FISH painting [57, 68]. In total, a 10 µL hybridization mixture consisted of 3 µL of mc-FISH probes (200–300 ng), 4 µL of 50% dextran sulfate sodium salt, 2 µL of 20× SSC, and 100% formamide. Sequential in situ hybridization protocol was referred to by Wu et al. [67].

The 4,6-diamino-2-phenylindole solution (DAPI; Vector Laboratories, Burlingame, CA, USA) was applied as a counterstain for in situ hybridization. We observed and recorded the fluorescence signals using a fluorescence microscope Olympus BX63 (Olympus, Tokyo, Japan) equipped with a DP80 CCD camera.

Phenotyping of FHB resistance

The *Fg* strain PH-1 was applied for FHB inoculation. Type II resistance, indicating the ability to resistance fungal spread, is evaluated using single floret inoculation (percentage of damaged florets among infected spikes) in the greenhouse (22–25 °C) and the experimental field of Sichuan Agricultural University (Wenjiang, Sichuan, China), with ten plant replicates per wheat lines. In total, 10 µL mixed *Fg* (1×10^3/mL) conidial suspension was inoculated into two flowering florets in the middle spikelet with a pipette gun or syringe. We sprayed sterile water on the clingfilm and wrapped the inoculated spike for 48 h to ensure inoculation with sufficient humidity. The PSD was recorded 14 days post-inoculation (dpi) and 21 dpi in the greenhouse and field, respectively.

Evaluation of agronomic performance

Investigations were conducted on agronomic traits of amphiploid 8801, 7D (7E) substitution line, SM482, and SM51 in the field (Chengdu, China), including plant height, tillering, flag leaf length, flag width, spike length, grain number per spike, and spikelet number per spike were investigated. Each material was planted in four rows, 15 grains per row (0.3 m* 1.5 m=space* length). Six individual plants of each sample were selected for agronomic traits investigation. We used OriginPro 2021 (OriginLab Corp., Northampton, MA, USA) software to calculate the mean and standard deviation of each group of agronomic traits. One-way ANOVA ($p<0.01$) was conducted to determine if the differences between the 7D (7E) substitution line and parental material were significant, and was visualized in a bar chart.

Locus-specific molecular marker development

MISA (http://pgrc.ipk-gatersleben.de/misa/download/misa.pl) was used to obtain SSR molecular markers from the reference diploid *Th. elongatum* (ASM1179987v1) genome Chr7E. Using bedtools (V2.28.0), we pulled the 150 bp flanking sequences surrounding the SSR sites. To select SSR markers with target fragments, we then performed a mock e-PCR specifical amplification. Afterward, we aligned SSR sequences of the above amplification with Chr1E–Chr6E and the reference 'Chinese Spring' (IWGSC RefSeq v2.1) and eliminated homologous sequences. We randomly selected SSR primers and synthesized them in Sangon Biotech (Chengdu) Co., Ltd. Using 3% agarose gel, we detected the specificity of PCR amplification products. Primers that showed positive amplification in *Trititrigia* 8801 and 7E(7D) substitution lines, yet no amplification in SM482 and SM51, were selected as 7E-specific primers.

Pedigree analysis

We constructed F_2 population (7E(7D) substitution line× SM830 F_2, 122 F_2 plants) to analyse the genetic effect of 7E(7D) substitution line on FHB resistance. Three genotypes will be segregated in the F_2 population, 7E/7E (7EL-homozygous), 7E/7D (7E/7D -heterozygous) and 7D/7D (7E-null). To accurately genotype F_2 plants, 7E-specific SSR marker *TTE7E-31* detection was followed by GISH identification to quantify the alien 7E chromosome number. The phenotypes of FHB resistance of all F_2 plants were identified in the greenhouse according to the 7E(7D) substitution line above. The statistical significance of differences in PDS means between genotypes was analyzed using a two-sample t-test (*, $p<0.05$; **, $p<0.01$; ns, $p>0.05$) by OriginPro 2021 (OriginLab Corp., Northampton, MA, USA) software and was visualized with a boxplot.

Homology-based cloning of *Fhb7* allele and sequence alignment

To determine the *Fhb7* homologous gene in the 7E(7D) substitution line, we conducted PCR amplification using *Fhb7* diagnostic markers, *GST-F/R* and *26,102-F/R* [16]. PCR was performed in a 50 μL reaction mixture: 25 μL 2×Taq PCR PreMix, 2.5 μL per primer, 200 ng/μL DNA, and ddH_2O to the final volume. The PCR amplification was checked using a 1.5% (w/v) agarose gel and cloned into the pMD19-T vector (TaKaRa) according to the manufacturer's instructions. Six randomly chosen positive clones were sequenced in Sangon Biotech (Chengdu) Co., Ltd. The nucleotide and CDS sequences data were blasted with DNAMAN 9.0. The *Fhb7* was assigned the designation $Fhb7^{The2}$ in *Th. elongatum* and $Fhb7^{Thp}$ in *Th. ponticum* [69].

Abbreviations

CS	ChineseSpring
DON	Deoxynivalenol
dpi	Days post-inoculation
EST	Expressed Sequence Tags
FHB	Fusarium Head Blight
FISH	Fluorescence In Situ Hybridization
GBS	Genotyping-By-Sequencing
GISH	Genome In Situ Hybridization
KASP	Kompetitive Allele-Specific PCR
MAS	Marker-Assisted Selection
NGS	Next-Generation Sequencing
NLRs	Leucine-Rich Repeat Receptors
PLUG	PCR-based Landmark Unique Gene
Pm	Powdery Mildew
PDS	Percentage of Diseased Spikelets
QTLs	Quantitative Trait Loci
SLAF-seq	Specific-Locus Amplified Fragment Sequencing
SM482	Shumai 482
SM51	Shumai 51
SM830	Shumai 830
SSC	Saline Sodium Citrate
SSR	Simple Sequence Repeats
YPC	Yellow Pigment Content

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-024-05703-3.

Supplementary Material 1: Additional file 1: fig. S1 Statistical analysis of 7E(7D) substitution and their parental lines for eight agronomic traits. **a** Plant height (cm). **b** Tiller number. **c** Spike length (cm). **d** Spikelets per spike. **e** Grains per spike. **f** Flag leaf length (cm). **g** Flag leaf width (cm). **h** 1000-grain weight (g). Asterisks indicate the level of significance: $*p<0.05$, $**p<0.01$, and $***p<0.001$.

Supplementary Material 2: Additional file 2: table S1 Agronomic traits of 7E(7D) substitution line and their parents. Table S2 Genetic effects of 7E chromosome on FHB resistance in F_2 population.

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Author contribution

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Data availability

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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