Theor Appl Genet (2015) 128:2301–2316
DOI 10.1007/s00122-015-2586-x

ORIGINAL ARTICLE

High-density mapping of the major FHB resistance gene *Fhb7* derived from *Thinopyrum ponticum* and its pyramiding with *Fhb1* by marker-assisted selection

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Received: 3 April 2015 / Accepted: 16 July 2015 / Published online: 29 July 2015

Abstract

Key message **Wheat lines with shortened *Th. ponticum* chromatin carrying *Fhb7* and molecular markers linked to *Fhb7* will accelerate the transfer of *Fhb7* to breeding lines and provide an important resource for future map-based cloning of this gene.**

Abstract Fusarium head blight is a major wheat disease globally. A major FHB resistance gene, designated as *Fhb7*, derived from *Thinopyrum ponticum*, was earlier transferred to common wheat, but was not used in wheat breeding due to linkage drag. The aims of this study were to (1) saturate this FHB resistance gene region; (2) develop and characterize secondary translocation lines with shortened *Thinopyrum* segments carrying *Fhb7* using *ph1b*; (3) pyramid *Fhb7* and *Fhb1* by marker-assisted selection. *Fhb7* was mapped in a 1.7 cM interval that was flanked by molecular markers *XsdauK66* and *Xcfa2240* with SSR, diversity arrays technology, EST-derived and conserved markers. KS24-2 carrying *Fhb7* was analyzed with molecular markers and genomic in situ hybridization, confirming it was a 7DS.7el$_2$L Robertsonian translocation. To reduce the *Thinopyrum* chromatin segments carrying *Fhb7*, a BC_1F_2 population (Chinese Spring *ph1bph1b**2/KS24-2) was developed and genotyped with the markers linked to *Fhb7*. Two new translocation lines (SDAU1881 and SDAU1886) carrying *Fhb7* on shortened alien segments (approximately 16.1 and 17.3 % of the translocation chromosome, respectively) were developed. Furthermore, four wheat lines (SDAU1902, SDAU1903, SDAU1904, and SDAU1906) with the pyramided markers flanking *Fhb1* and *Fhb7* were developed and the FHB responses indicated lines with mean NDS ranging from 1.3 to 1.6 had successfully combined *Fhb7* and *Fhb1*. Three new molecular markers associated with *Fhb7* were identified and validated in 35 common wheat varieties. The translocation lines with shortened alien segments carrying *Fhb7* (and *Fhb1*) and the markers closely linked to *Fhb7* will be useful for improving wheat scab resistance.

Communicated by H. Buerstmayr.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-015-2586-x) contains supplementary material, which is available to authorized users.

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Introduction

Wheat (*Triticum aestivum* L., $2n = 6X = 42$, genome AABBDD) scab, also called Fusarium head blight (FHB), is predominantly caused by the fungus *Fusarium graminearum* Schwabe [telomorph, *Gibberella zeae* (Schw.) Petch] and is one of the major diseases of wheat in many areas of the world (Bai and Shaner 2004; Goswami and Kistler 2004; West et al. 2012). It causes significant yield losses as well as reduced grain quality and functionality due to contamination with mycotoxins, such as deoxynivalenol (DON) and nivalenol (NIV) (D'mello et al. 1999; Cetin and Bullerman 2005). Climate change is predicted to increase

the incidence of FHB on wheat (Chakraborty and Newton 2011; West et al. 2012). No single strategy has proven effective in mitigating the effects of FHB, but one promising avenue is the development of more resistant wheat cultivars, limiting both fungal growth and accumulation of mycotoxins.

Sources of FHB resistance used in current wheat breeding programs can be traced to very few parents, including Sumai 3 and its derivatives, Wangshuibai and Wuhan 1 (Bai and Shaner 2004; Buerstmayr et al. 2009). Development of only one or a few sources of resistance over large crop production areas poses vulnerability to resistance breakdown and disease epidemics. Resistance to FHB in wheat is a quantitative trait that is governed by multiple genes and modulated by environmental conditions. Numerous quantitative trait loci (QTL) have been reported as associated with FHB resistance (reviewed by Buerstmayr et al. 2009). A few of these FHB resistance-associated loci have been designated with a gene name: i.e., *Fhb1* derived from *T. aestivum* cv. Sumai 3, *Fhb2* derived from *T. aestivum* cv. Sumai 3, *Fhb3* derived from *Leymus racemosus*, *Fhb4* derived from *T. aestivum* cv. Wangshuibai, *Fhb5* derived from *T. aestivum* cv. Wangshuibai, and *Fhb6* derived from *Elymus tsukushiensis* have been mapped and designated previously (Cuthbert et al. 2006, 2007; Liu et al. 2006; Qi et al. 2008; Xue et al. 2010, 2011; Cainong et al. 2015). In addition, it is rare to find wheat cultivars with high levels of resistance to FHB, and no source of immunity has been identified (Bai and Shaner 2004; Yu et al. 2008; He et al. 2014). Therefore, discovery, development, and characterization of new resistance sources will provide breeders with a wider choice of germplasm.

Kim et al. (1993) described a wheat line carrying a wheatgrass, *Thinopyrum ponticum* (Podp.) Barkworth & D.R. Dewey ($2n = 10X = 70$) chromosome $7el_2$, substituted for wheat chromosome 7D. This highly resistant line carrying a FHB resistance gene, previously named as *Fhblop* (and designated as *Fhb7* in the present study) and closely linked to molecular markers *Xcfa2240* and *XBE445653*, was mapped on the long arm of chromosome $7el_2$. It explained 15.0–32.5 % of the phenotypic variation in FHB response (Shen et al. 2004; Shen and Ohm 2007; Zhang et al. 2011). However, due to linkage drag, the original translocation line, KS24-2, carrying a pair of Robertsonian translocation chromosomes identified as $7DS.7el_2L$ (Kim et al. 1993), has not been used for FHB resistance breeding. In addition, the DNA marker density of the *Fhb7* region is far lower than that required for positional cloning. Therefore, development of new molecular markers is needed for saturation mapping of *Fhb7*.

Diversity arrays technology (DArT), based on the use of restriction enzymes to digest genomic DNA to reduce genome complexity and enrich low copy sequences for marker development, has been widely used in plant genetic studies, genetic map construction, and selection of molecular markers linked to genes controlling agronomic traits in wheat, barley, sorghum, and other crop species (Wenzl et al. 2004; Akbari et al. 2006; Semagn et al. 2006). The large amount of crop genomic sequences, such as rice (Goff et al. 2002), *Brachypodium distachyon* (Vogel et al. 2010) and sorghum (Paterson et al. 2009), which are now publicly available, are rich resources for molecular marker development and comparative genomics analysis. Recently, based on synteny between wheat and rice, *B. distachyon* or Sorghum, comparative mapping has been widely used for construction of high-resolution genetic maps of interesting wheat genes or positional cloning of these genes (Liu and Anderson 2003; Wang et al. 2014a).

The objectives of the present study were to (1) saturate this FHB resistance gene region using SSR, DArT, and conserved markers; (2) develop and characterize small segment translocation lines carrying *Fhb7* by reducing the amount of *Th. ponticum* chromatin using *ph1b*-induced homoeologous chromosome pairing (Qi et al. 2007; Marais et al. 2010; Niu et al. 2011); and (3) pyramid *Fhb7* with *Fhb1* by marker-assisted selection.

Plant materials and methods

Plant materials

A set of 156 $F_{8:9}$ recombinatn inbred lines (RILs) derived from a cross between the FHB susceptible line K11463 ($7el_1$[7D]) and the FHB resistant line K2620 ($7el_2$[7D]) were utilized to saturate the 7el genetic linkage map of *Fhb7* (Zhang et al. 2011). K11463 and K2620 were two wheat-*Th. ponticum* substitution lines, in which wheat chromosome 7D was substituted by *Th. ponticum* chromosome $7el_1$ and $7el_2$, respectively. Wheat variety Ning 7840 was used as the resistant control.

Wheat-*Th. ponticum* translocation line KS24-2 carrying FHB resistance gene *Fhb7* on *Th. ponticum* chromatin in common wheat cv. Thatcher was used as the donor of the FHB resistance gene. This translocation was identified as $7DS.7el_2L$ (Kim et al. 1993). The Chinese Spring *Ph1b* mutant and wheat cv. Jimai 22 were used as crossing and backcrossing parents. Chinese Spring (CS), Thatcher, and Jimai 22 were used as susceptible checks and Ning 7840 was used as the resistant check. CS nulli-tetrasomic lines N7AT7B, N7BT7D, and N7DT7A, and CS deletion lines 7AL16, 7AL1, 7DL3, 7BL2, 7BL7, 7BL10, 7DL2, and 7DL5 were used in molecular marker analyses. Thirty-five wheat cultivars were used for validation of newly developed markers along with the SSR marker *Xcfa2240* (Table S1).

Comparative genomics analysis and conserved primer development

The FHB resistance gene *Fhb7* was previously mapped on the long arm of the *Th. ponticum* chromosome 7el (Shen et al. 2004; Shen and Ohm 2007; Zhang et al. 2011). STS markers derived from wheat ESTs were developed and mapped to the long arm of the *Th. ponticum* chromosome 7el by Shen and Ohm (2007). The corresponding EST sequences of the polymorphic EST markers flanking the FHB resistance gene *Fhb7* were used to perform a BLAST search against the genome sequence databases of rice (http://rice.plantbiology.msu.edu/), *B. distachyon* (http://www.Brachypodium.org/) and sorghum (http://www.plantgdb.org/SbGDB/cgi-bin/blastGDB.pl) to identify orthologous genomic regions in rice, *B. distachyon* and sorghum with cutoff parameters of *E* value $\leqq 1E^{-10}$, identity ≧70 % and a minimum of a 100-bp match length. When several significant hits were found, only the best hit was adopted.

Coding sequences (CDS) of the putative genes in the regions were used as queries to search the NCBI database (http://www.ncbi.nlm.nih.gov) with cutoff parameter of *E* value $\leqq 1E^{-10}$ for homologous *Triticeae* ESTs to develop new polymorphic markers linked to *Fhb7*. The conserved primers were designed with the online software version (http://probes.pw.usda.gov/cgi-bin/ConservedPrimers/ConservedPrimers.cgi) as described by You et al. (2009). The primer design parameters were as follows: amplification product size of 800–1500 bp with an optimum size 1000 bp, primer length of 18–24 bp, Tm of 55–65 °C, and GC content of 30–70 %.

SSR marker development

Fourteen scaffolds, i.e., Scaffold48913, scaffold89301, scaffold35216, scaffold38207, scaffold72072, scaffold70783, scaffold5083, scaffold11459, scaffold21010, scaffold23, scaffold76033, scaffold21011, scaffold92632, and scaffold32834, in the distal region of *Ae. tauschii* 7DL were downloaded and used for SSR marker development (Jia et al. 2013). SSR Finder (http://www.fresnostate.edu/ssrfinder/) was used to identify simple sequence repeat regions in the scaffolds, and sequences flanking the SSR region were selected to design SSR primers using Primer3 Input Version 0.4.0 (http://bioinfo.ut.ee/primer3-0.4.0/) under general settings.

The developed SSR and conserved primers were then tested on CS, CS nulli-tetra lines N7AT7B, N7BT7D, and N7DT7A and CS deletion lines 7AL16, 7AL1, 7DL3, 7DL2, and 7DL5, and screened for polymorphisms among wheat lines K11695, K11463, K2620, and KS24-2. Polymorphic molecular markers were used to genotype the 156 RILs. PCR products were separated on 8 % non-denaturing polyacrylamide gels (acrylamide:bisacrylamide = 39:1) and visualized with silver staining (Kong et al. 2008).

DArT marker development and genotyping

DArT service was provided by DArT Pty Ltd (DArT P/L) (Canberra, Australia; http://www.diversityarrays.com/), with genome profiling service. A bread wheat array was adopted for the two parental lines, K11463 and K2260 and its derived 156 RILs and the data were analyzed, as described by Akbari et al. (2006).

Genomic DNA isolation

Total genomic DNA was isolated from young leaves using the CTAB protocol (Zhang et al. 2011) with minor modifications (Supporting information). The concentration of each DNA sample was adjusted to 50–100 ng/μl after quantification on a ND-1000 Spectrophotometer V3.5 (NanoDrop Technologies, Inc., USA).

Molecular marker analysis

PCR amplifications were performed in 15 μl total volume containing 1× PCR buffer, 1.5 mM $MgCl_2$, 200 μM dNTP, 0.7 μM of each primer, 1 unit of Taq polymerase, and 100 ng of template DNA. The amplification profile consisted of 1 cycle at 94 °C for 5 min, followed by 35 cycles of 50 s at 94 °C, 30 s at 50–60 °C (annealing temperature depending on specific primers) and 50 s at 72 °C, with a final extension of 10 min at 72 °C. PCR products were separated in 8 % non-denaturing polyacrylamide gels. Gels were silver stained and photographed (Zhang et al. 2011).

Chromosome manipulation

Wheat plants homozygous *ph1bph1b* and monosomic for wheat chromosome 7D and the translocated chromosome 7DS.7el$_2$L were developed to enable meiotic recombination between 7D and 7DS.7el$_2$L (Fig. 1). These plants were produced by crossing the CS *Ph1* mutant (CS *ph1b*) to KS24-2 and backcrossing the F_1 plants to the *ph1bph1b* line. BC_1F_1 plants were genotyped using the *ph1b*-specific molecular markers *Xpsr128*, *Xpsr574*, and *XAWJL3* (Roberts et al. 1999) and SSR markers *Xcfa2240* and *Xgwm333*, which were specific for chromosome 7el$_2$ (Shen and Ohm 2007; Zhang et al. 2011). A BC_1F_2 population derived from BC_1F_1 plants that were monosomic for chromosomes 7D and 7DS. 7el$_2$L was developed and screened by *Fhb7*-linked molecular markers (*Xcfa2240*, *Xgwm333*, *Xswes130*, *XsdauK66*, and *Xmag1759*) (Fig. S1) and genomic in situ hybridization (GISH). The overall

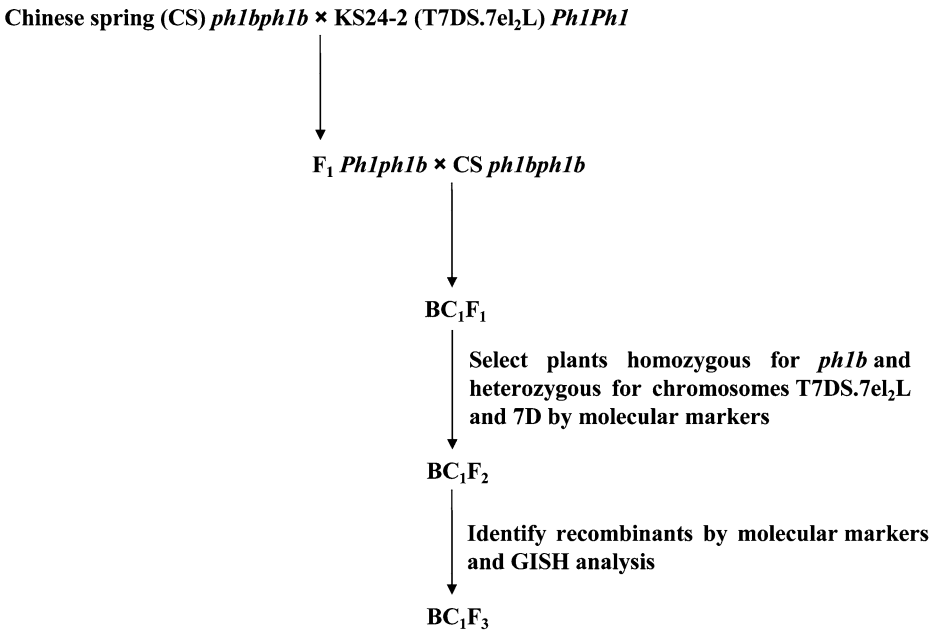

Fig. 1 Procedure for producing wheat-*Thinopyrum* translocation lines using the CS *Ph1* mutant

procedure is summarized in Fig. 1. The progenies of the plants with homozygous putatively shortened *Th. ponticum* chromatin segments were examined by GISH and evaluated for FHB response. The sizes of the *Th. ponticum* chromatin segments in the selected lines were expressed as average percentages of the length of *Th. ponticum* chromatin segment/total length of the translocation chromosome, measured in at least 20 cells with good-quality mitotic metaphases.

Genomic in situ hybridization (GISH)

Seed germination and collection and the pretreatment and fixation of root tips were performed as described by Han et al. (2009) with minor modifications (Supporting information). Total genomic DNA of *Th. ponticum* (accession RM001128, from the Chinese Crop Germplasm Resources Information System) isolated from young leaves, was labeled with DIG-11-dUTP by nick translation and used as a probe. Sheared genomic DNA from Chinese Spring was used for blocking. Preparation of chromosome spreads and visualization of probe hybridization were performed as described by Han et al. (2009).

Validation of new molecular markers linked to *Fhb7* on shortened *Th. ponticum* chromosome segments

Genomic DNA for identification and validation of molecular markers linked to *Fhb7* on the shortened *Th. ponticum* segments in the new lines was extracted from seedling plants. Molecular marker and GISH analysis localized the shortened *Th. ponticum* chromatin segment on the distal region of chromosome 7DL in the new wheat lines. Then molecular markers located in the distal region of chromosome 7elL were selected and used to screen for polymorphism among Thatcher, CS, KS24-2, K2620 and the newly developed introgression lines with shortened *Th. ponticum* segments carrying *Fhb7*.

To validate molecular markers linked to *Fhb7* on the shortened *Th. ponticum* chromosome segments, polymorphic molecular markers that were identified following the above procedures were used to genotype 35 common wheat cultivars (Table S1). Marker analyses were performed as described above.

Pyramiding of FHB resistance genes *Fhb1* and *Fhb7*

Common wheat cv. Ning 7840 carrying *Fhb1* was crossed to the new introgression lines. Markers *Xgwm493* and *Xgwm533* (Cuthbert et al. 2006; Buerstmayr et al. 2009), which are closely linked to *Fhb1* and the SSR markers *XsdauK66* and *Xcfa2240*, which are closely linked to *Fhb7* were used to select homozygous F_2 plants. These homozygous plants were evaluated for FHB response in the greenhouse (Fig. S2A). PCR amplifications were performed as described by Cuthbert et al. (2006). Amplification products were then separated in 2 % agarose gels and visualized by ethidium bromide staining.

Introgression of *Fhb7* into Chinese major winter wheat cultivar Jimai 22

Jimai 22 was crossed to the new translocation lines and the hybrids were backcrossed to Jimai 22 for three generations. BC_3F_1 plants with the shortened *Th. ponticum* chromatin segments were selected by the markers flanking *Fhb7* and then self-fertilized. BC_3F_2 plants homozygous for the marker were evaluated for FHB response in the greenhouse (Fig. S2B).

Determination of FHB response

Type II FHB resistance, defined as inhibition of disease spread from a point infection, was determined in a greenhouse at Shandong Agricultural University over four seasons, 2012 Winter (2012 W), 2013 Winter (2013 W), 2014 Spring (2014 S), and 2014 Winter (2014 W). All experimental lines, that is KS24-2, Thatcher, CS *ph1b*, new *Th. ponticum* derivatives, lines combining markers flanking *Fhb1* and *Fhb7*, Jimai 22, Jimai 22 backcross derivatives homozygous for markers flanking *Fhb7*, and Ning 7840, were screened. Eight to ten spikes of each line were inoculated when each spike reached 50 % anthesis. One basal flower in the 4th or 5th spikelet from the tip of the spike was inoculated by injecting 10 μl micro-conidial suspension (50,000 spores/ml) between the lemma and palea of a floret with a syringe. Inoculated spikes were covered for 72 h with a plastic bag to maintain high humidity. The number of diseased spikelets (NDS), including the inoculated spikelet, were counted at 21 days post-inoculation. Data were analyzed with SAS software version 9.0. The mean values of putatively resistant lines were compared with Thatcher, Chinese Spring *ph1bph1b*, Ning 7840 and Jimai 22. Duncan's multiple range test was used to test for significant differences.

Genetic linkage map construction

All DArT, SSR, and conserved markers polymorphic between K11463 and K2620 were mapped in the K11463/K2620 mapping population of 156 RILs (Zhang et al. 2011). The linkage map was constructed using the software Joinmap version 4.0 (Van Ooijen 2006) with an initial theshold of LOD ≧ 10.0. After a final order of markers was reached, a representative marker from each group of cosegregating markers (termed as a recombination block) was selected and a map was constructed with LOD ≧ 3.0 by using the Kosambi mapping function (Kosambi 1943). The computer program QTL Icimapping V4.0 (Wang et al. 2014b) was used for mapping the FHB resistance QTL with a LOD threshold 3.0 using the data collected by Zhang et al. (2011).

Results

Identification of genomic region in rice, *B. distachyon*, and Sorghum with *Th. ponticum* chromosome 7el containing *Fhb7* and comparative genomic analysis

The FHB resistance gene *Fhb7* was previously mapped on the long arm of *Th. ponticum* chromosome 7el and was closely associated with 10 EST-derived STS markers (*XBE404744*, *XBM137749*, *XBE637476*, *XBE445506*, *XBF483039*, *XBG607810*, *XB145935*, *XBE445653*, *Xmag1932* [CD880294], and *XHX2166* [CJ579028]) (Shen and Ohm 2007; Zhang et al. 2011). Based on the corresponding EST sequences of these EST-STS markers, the orthologous genomic regions were identified in rice, *B. distachyon*, and sorghum. The *Th. ponticum* genomic region containing *Fhb7* was orthologous to a distal region of rice chromosome 6 (Fig. 2). Although this gene was not mapped between two EST-derived markers, this orthologous region in rice contained only two PAC clones (AP005750 and AP006616).

Based on the above information and blast analysis, the *Th. ponticum* genomic region containing *Fhb7* was presumably orthologous to rice chromosome 6 (*Os06g51330* and *Os06g51570*), *B. distachyon* chromosome 1 (*Bradi1g29130* and *Bradi1g29510*), and sorghum chromosome 10 (*Sb10g031110* and *Sb10g031310*, Fig. 2; Table 1). Three genomic regions spanning 154.1, 366.2, and 222.1 kb in rice, *B. distachyon* and sorghum, respectively, appeared to be the orthologous genomic regions to *Th. ponticum* harboring the FHB resistance gene *Fhb7*. Detailed comparative analysis revealed that 17 of 25 predicted rice genes on chromosome 6 were orthologous to 15 of 39 predicted *B. distachyon* genes and 14 of 20 predicted sorghum genes in the corresponding genomic regions, which showed high levels of genomic collinearity among these three species (Fig. 2; Table 1).

However, very common phenomena of gene insertion or deletion and gene order inversions or chromosomal rearrangements were also observed in the three species. A segmental inversion, *Bradi1g29390-Bradi1g29510* was present in *B. distachyon* compared to rice and sorghum in the corresponding orthologous genomic region (Fig. 3). Another inversion, *Sb10g031240-Sb10g031265*, was present in sorghum compared to rice and *B. distachyon*. Finally, some specific genomic regions were also observed. A segment *Os06g51520-Os06g51570* was specific to rice and was not present in *B. distachyon* or sorghum. Five segments *Bradi1g29140-Bradi1g29160*, *Bradi1g29200-Bradi1g29230*, *Bradi1g29260-29280*, *Bradi1g29330-Brai1g29380,* and *Bradi1g29434-Brai1g29450* were specific to *B. distachyon* and not present in the corresponding genomic region of rice

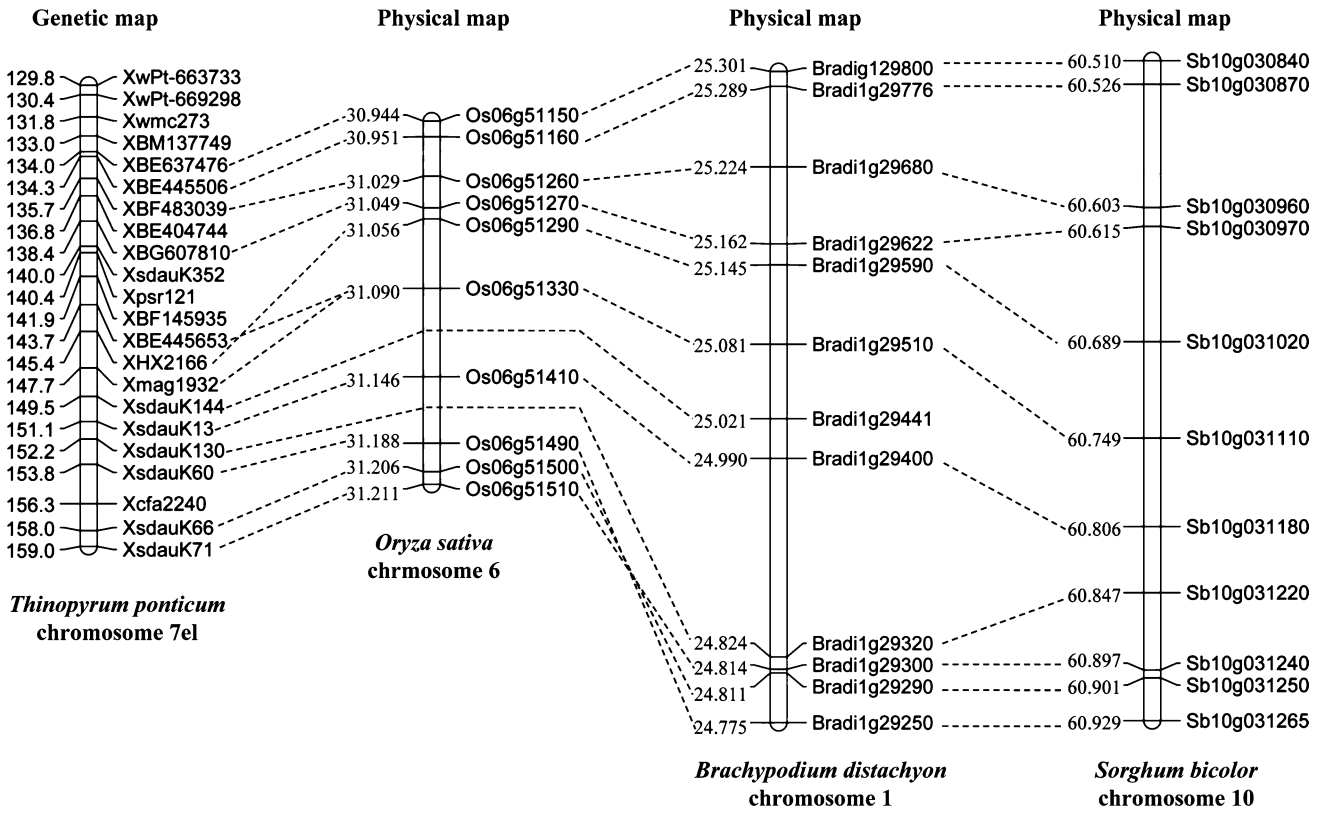

Fig. 2 Comparative genetic linkage map of *Thinopyrum* chromosome 7el, and its syntenic genomic regions on rice chromosome 6, *Brachypodium distachyon* chromosome 1 and sorghum chromosome 10, respectively. The units of these maps are cM, Mb, Mb, and Mb, respectively

and sorghum. Two segments *Sb10g031160-Sb10g031170* and *Sb10g031200-Sb10g031210* were specific to sorghum, which were not present in rice and *B. distachyon* (Table 1; Fig. 3).

A refined genetic map of the region of *Th. ponticum* chromosome $7el_2$ surrounding the FHB resistance gene *Fhb7*

The predicted genes of rice, *B. distachyon,* and sorghum in the corresponding orthologous region of *Th. ponticum* chromosome 7el were used to develop molecular markers linked to the FHB resistance gene *Fhb7*. The putative orthologous or non-orthologous genes between *Os06g51330* and *Os06g51570* in rice, *Bradi1g29130* and *Bradi1g29510* in *B. distachyon,* and *Sb10g031110* and *Sb10g031310* in sorghum were selected to develop markers to narrow the collinearity regions among *Th. ponticum*, rice, *B. distachyon,* and sorghum. The CDS of these genes were used as queries to search for orthologous wheat (*T. aestivum*, *T. turgidum*, and *T. monococcum*), barley and wheatgrass (*Elymus*, *Leymus*, and *Secale*) ESTs. The numbers of orthologs identified in wheat, barley, and wheatgrass were different. Among the 25 rice putative genes investigated, 72 orthologs, 44 orthologs, 5 orthologs, 4 orthologs, and 1 orthologs were identified in *T. aestivum*, *H. vulgare*, *Leymus*, *E. spicatus*, and *T. turgidum*, respectively. None were identified in *T. monococcum* and *S. cereale* (Table 1; Excel S1). When the 39 *B. distachyon* putative genes were queried, 191 orthologs, 71 orthologs, 18 orthologs, 15 orthologs, 15 orthologs, and 2 orthologs were identified in *T. aestivum*, *H. vulgare*, *Leymus*, *T. turgidum*, *E. spicatus,* and *T. monococcum*, respectively. However, none were identified in *S. cereale* (Table 1; Excel S1). With the 20 sorghum putative genes as queries, 90 orthologs, 51 orthologs, 7 orthologs, 16 orthologs, 7 orthologs, 1 orthologs, and 1 orthologs were identified in *T. aestivum*, *H. vulgare*, *Leymus*, *T. turgidum*, *E. spicatus*, *S. cereale*, and *T. monococcum*, respectively (Table 1; Excel S1). Then, 10 rice, 15 *B. distachyon*, and 4 sorghum putative genes were used to develop conserved markers. Finally, 26 conserved markers were produced by the software ConservedPrimer 2.0 (Table S2). Out of them, 6 conserved markers, *XsdauK13*, *XsdauK60*, *XsdauK66*, *XsdauK71*, *XsdauK130*, and *XsdauK144* were polymorphic between the two parents, K11463 and K2620. DArT was also used to produce polymorphic markers between K11463 and K2620 and 499 polymorphic DArT markers were produced. In order to produce more markers

Table 1 Orthologous gene pairs among the putative collinear genomic regions of rice, *Brachypodium*, and sorghum and number of corresponding orthologs in wheat and its relatives

Rice PACs	Os chromosome 6	Bd chromosome 1	Sb chromosome 10	Number of orthologs in wheat and its relatives
AP005750	*Os06g51330*	*Bradi1g29510*	*Sb10g031110*	Ta (12), L (2), Hv (4)
	Os06g51340	–	–	–
	–	*Bradi1g29500*	–	Ta (7), L (6)
	Os06g51350	*Bradi1g29490*	*Sb10g031125*	Ta (1), Hv (1)
	Os06g51360	*Bradi1g29480*	*Sb10g031130*	Ta (2), L (1), Hv (4)
	Os06g51370	*Bradi1g29470*	–	Ta (4), Hv (1)
	Os06g51380	–	*Sb10g031135*	Ta (2), Es (1)
	Os06g51390	*Bradi1g29460*	*Sb10g031140*	Ta (9), Hv (2)
	–	*Bradi1g29450*	–	–
	–	*Bradi1g29441*	–	Ta (6), Tt (2)
	–	*Bradi1g29434*	–	Ta (4), Hv (2), Tt (2)
	Os06g51400	*Bradi1g29420*	*Sb10g031150*	Ta (2)
	–	*Bradi1g29410*	–	Ta (6), Es (2), Hv (3)
	–	–	*Sb10g031160*	Ta (7), Es (3), L (1), Hv (1)
	–	–	*Sb10g031170*	Hv (1)
	Os06g51410	*Bradi1g29400*	*Sb10g031180*	Ta (6), L (1), Hv (3)
	Os06g51420	*Bradi1g29390*	*Sb10g031190*	Ta (7), Hv (6)
	–	–	*Sb10g031200*	Ta (5), Es (1), Hv (7), Sc (1), Tt (15), Tm (1)
	–	–	*Sb10g031210*	Ta (6), Es (2), L (1)
	–	*Bradi1g29380*	–	–
	–	*Bradi1g29370*	–	Ta (7), Hv (5)
	–	*Bradi1g29360*	–	–
	–	*Bradi1g29350*	–	Ta (9), L (3), Es (5)
	–	*Bradi1g29340*	–	Ta (6), Hv (4), Tt (10), Tm (2)
	–	*Bradi1g29330*	–	–
	–	*Bradi1g29320*	*Sb10g031220*	Ta (3), Hv (3)
	–	*Bradi1g29310*	–	–
	–	–	*Sb10g031230*	Ta (6), Hv (2)
	Os06g51430	*Bradi1g29130*	*Sb10g031280*	L (1), Hv (1)
	Os06g51440	–	–	–
	–	*Bradi1g29140*	–	Ta (5), Es (2)
	–	*Bradi1g29150*	–	–
	–	*Bradi1g29160*	–	Ta (9), Es (1), Hv (6)
	Os06g51450	*Bradi1g29170*	*Sb10g031300*	Ta (1), Hv (4)
	–	*Bradi1g29180*	–	Ta (27), L (4), Es (2), Hv (2)
AP006616	*Os06g51460*	*Bradi1g29190*	–	Ta (3), Es (1), Hv (6)
	Os06g51470	–	–	Ta (1), Es (2), Hv (1)
	–	*Bradi1g29200*	–	–
	–	*Bradi1g29210*	–	Ta (5)
	–	*Bradi1g29220*	–	Ta (29), Hv (2)
	–	*Bradi1g29230*	–	–
	Os06g51480	*Bradi1g29240*	*Sb10g031310*	Ta (7), Hv (3), Tt (1)
	Os06g51490	*Bradi1g29250*	*Sb10g031265*	Ta (3), Hv (1)
	–	*Bradi1g29260*	–	Es (1), Hv (1)
	–	*Bradi1g29270*	–	–
	–	*Bradi1g29280*	–	Hv (1)

Table 1 continued

Rice PACs	Os chromosome 6	Bd chromosome 1	Sb chromosome 10	Number of orthologs in wheat and its relatives
	Os06g51500	*Bradi1g29290*	*Sb10g031250*	Ta (8), Hv (5)
	Os06g51510	*Bradi1g29300*	*Sb10g031240*	Ta (3)
	Os06g51520	–	–	Ta (1), Hv (2)
	Os06g51530	–	–	–
	Os06g51540	–	–	–
	Os06g51550	–	–	–
	Os06g51560	–	–	–
	Os06g51570	–	–	–

Hv, *Hordeum vulgare*; L, *Leymus*; Es, *Elymus spicatus*; Tt, *T. turgidum*; Sc, *Secale sereale*; Tm, *T. monococcum*; Os, *Oryza sativa*; Bd, *Brachypodium distachyon*; Sb, *Sorghum bicolor*; Ta, *Triticum aestivum*

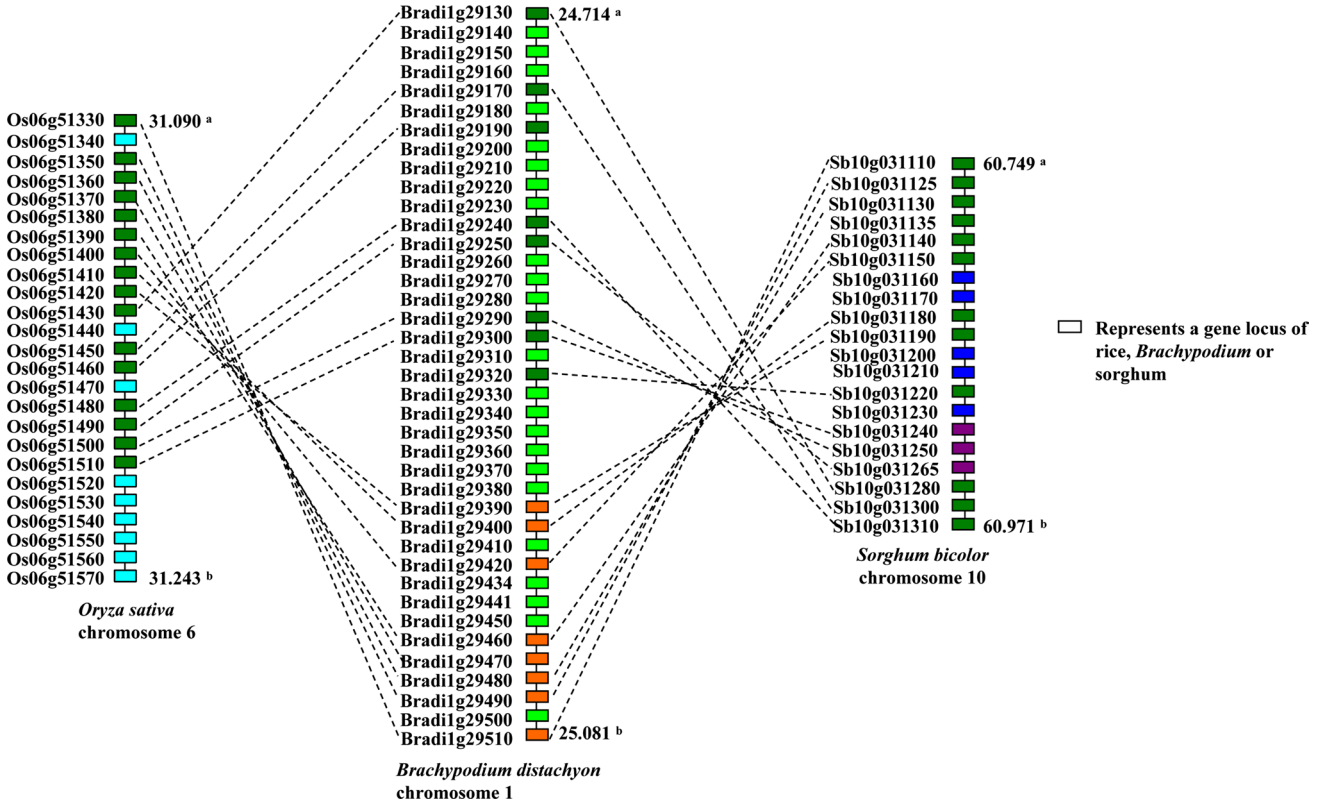

Fig. 3 Structure variations of the *Fhb7* orthologous genomic regions in rice, *B. distachyon,* and sorghum. *Green* regions indicate the same gene order among rice, *B. distachyon*, and sorghum. *Orange* region represents *B. distachyon* inversion in gene order compared to rice and sorghum. *Purple* region and *black* region represent sorghum inversion in gene order compared to rice and *Brachypodium*, respectively. *Light blue* regions represent rice-specific regions compared to *B. distachyon* and sorghum. *Light green* regions represent *B. distachyon*-specific regions compared to rice and sorghum. *Blue* regions represent sorghum-specific regions compared to rice and *B. distachyon*. *Superscript letter* a represents the start position of putatively syntenic regions in rice *B. distachyon* and sorghum corresponding to *Th. ponticum* chromosome 7el regions harboring *Fhb7*. *Superscript letter* b represents the end position of putatively syntenic regions in rice *B. distachyon* and sorghum corresponding to *Th. ponticum* chromosome 7el regions harboring *Fhb7* (color figure online)

surrounding *Fhb7*, fourteen scaffolds, i.e., Scaffold48913, scaffold89301, scaffold35216, scaffold38207, scaffold72072, scaffold70783, scaffold5083, scaffold11459, scaffold21010, scaffold23, scaffold76033, scaffold21011, scaffold92632, and scaffold32834, in the distal region of *Ae. tauschii* 7DL (Jia et al. 2013) were downloaded and used for SSR marker development. Finally, 14 SSR markers were developed (Table S2) and one SSR marker, *XsdauK352*, showed polymorphisms between K11463 and K2620. In total, 506 polymorphic markers, including 1 SSR, 499 DArT and 6 conserved markers, were used to construct a linkage map of *Th. ponticum* chromosome 7el combining the map of Zhang et al. (2011) with the present results. After elimination of unlinked markers and other small linkage groups, the high-density genetic map contained 167 markers covering 158.97 cM, with an average density of one marker per 0.95 cM (Excel S2). Of the six conserved markers identified above, *XsdauK60* and *XsdauK130* were assigned to deletion bin 7BL10-0.78-1.0, and *XsdauK13* and *XsdauK66* were assigned to deletion bin 7DL10-0.82-1.0. These markers permitted mapping of *Fhb7* within a 1.7 cM interval flanked by molecular markers *XsdauK66* and *Xcfa2240* (Fig. 4). Thus, *Fhb7* was most closely linked to *Os06g51490* and *Os06g51500* in rice representing a 18.8 kb genomic region and corresponding to a 37.1 kb genomic region in *B. distachyon* and a 27.0 kb genomic region in sorghum.

Development and characterization of *Fhb7*-carrying wheat lines with reduced *Th. ponticum* chromatin segments induced by *ph1b* with marker-assisted introgression and genomic in situ hybridization

For characterization of the original translocation line KS24-2, all molecular markers except DArT markers mapped on the 7el chromosome (Excel S2) were screened among Chinese Spring, KS24-2, K2620, Thatcher, and *Th. ponticum* and 12 markers were polymorphic among them (Table S4). Finally, markers *Xcfa2240* and *XsdauK66* mapped in wheat chromosome bin 7DL3-0.82-1.0 and closely linked to *Fhb7* as described above, *Xgwm333* previously mapped in wheat chromosome bin 7BL2-0.33-7BL7-0.63 Sourdille et al. (2004), *Xmag1759* mapped in wheat chromosome bin 7BL7-0.63-7BL10-0.78, and *Xswes130* showing polymorphic between KS24-2 and wheat checks Thatcher and Chinese Spring were selected for further analysis (Figs S1, 4; Table S4). The SSR marker *Xcfa2240* mapped to the long arms of chromosomes 7A and 7D with CS 7L-deletion lines (7AL1, 7AL16, 7DL2, 7DL3, and 7DL5) (Fig. 5d). GISH results confirmed that the interchanged chromosome in line KS24-2 is a 7DS.7el$_2$L Robertsonian translocation (Fig. 6b).

When the T7DS.7el$_2$L × CS *ph1bph1b* F_1 was used as the female parent in crossing with CS *ph1bph1b*, 61 plants had the translocation chromosome (96.8 %) and two did not (3.2 %). However, in the reciprocal cross 32 plants (53.3 %) had the translocation chromosome and 28 (46.7 %) lacked it. Clearly, there was preferential transmission of the T7DS.7el$_2$L translocation chromosome by female gametes whereas male transmission was normal.

To identify introgression lines carrying *Fhb7* on smaller alien segments, 208 BC_1F_2 individuals were genotyped for five markers; i.e., *Xcfa2240*, *Xgwm333*, *Xswes130*, *XsdauK66*, and *Xmag1759* (Figs. S1, 1; Table S4); 34 carried wheat CS *ph1b* alleles at all five SSR loci (Plant type 8; Table 2), 15 carried *Th. ponticum* alleles at 1 or 2 SSR loci (Plant types 4, 5, 7, and 9) and the remaining 159 plants retained *Th. ponticum* alleles at three or more marker loci (Plant types 1, 2, 3, 6, and 10). *Fhb7* was located on chromosome 7el$_2$L and was flanked by molecular markers *Xcfa2240* and *XsdauK66* as described above. GISH was utilized to examine the two BC_1F_2 plants with only the *Th. ponticum* alleles at the *Xcfa2240* and *XsdauK66* locus. Lines 338-29 and 338-63 (Type 4, Table 2) carrying much smaller translocated *Th. ponticum* segments were presumed to have the structure T7DS.7DL-7el$_2$L (Fig. 6). In addition, six molecular markers (*Xbarc76*, *Xwmc273*, *XBM137749*, *XHX2166*, *XsdauK352*, and *Xpsp3003*) between *Xmag1759* and *Xcfa2240* were selected to screen the above two short lines identified (Table S5). Our results indicated the 7elL-7DL translocation might be between *Xwmc273* and *XBM137749*.

The two lines with putatively shortened segments and homozygous for the *Th. ponticum Xcfa2240* and *XsdauK66* alleles were evaluated for FHB response using the parental lines and the resistant and susceptible varieties as controls. Plants were classified as resistant or susceptible. FHB response differences represented by the NDS between the parental lines KS24-2 and CS *ph1b* were significant at $p = 0.01$. The two new lines with terminal 7el$_2$L fragments, designated as SDAU1881 and SDAU1886, were resistant (Table 3; Figs. 5d, 6b, c), with mean NDS of 1.4 and 1.5, respectively, similar to KS24-2. In contrast, three other derivatives; i.e., SDAU1885, SDAU1893, and SDAU1894 (all belonging to plant type 8), had mean NDS of 5.8, 5.2, and 5.7, respectively. The FHB responses were not significantly different from CS *ph1b* (Table 3)

Based on the FHB responses, GISH, and molecular maker analysis, the shortened *Th. ponticum* chromatin segments in SDAU1881 and SDAU1886 were located in the distal region of chromosome 7DL (Table 3; Figs. 5d, 6c, d). The sizes of the *Th. ponticum* chromatin segments in the entire chromosomes were 16.1 % (25 cells) for SDAU1881 and 17.3 % (30 cells) for SDAU1886 compared to 49.0 % (20 cells) for line KS24-2. Therefore, approximately 65 % of the *Th. ponticum* chromatin proximal to *Fhb7* was removed in the two new translocation lines after *ph1b*-induced homoeologous recombination.

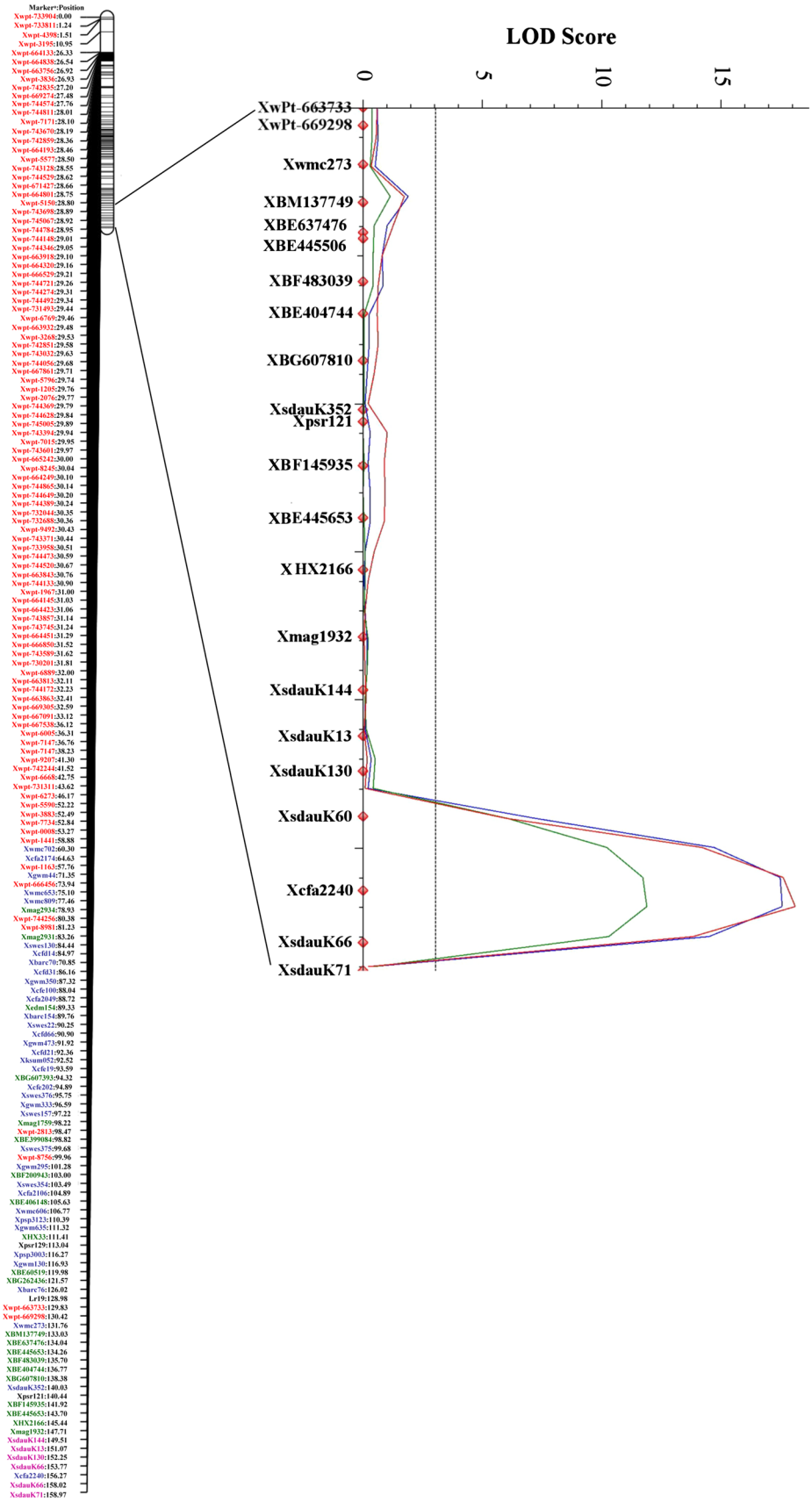

Fig. 4 A high-density genetic map of *Thinopyrum* chromosome 7el constructed with DArT, SSR, EST-derived, and conserved markers and genetic detection of FHB resistance gene *Fhb7* derived from *Th. ponticum*. FHB response data from the greenhouse (2005 USA) at Purdue University are presented in *red*. FHB response data from the greenhouse (2008 China) at Shandong Agricultural University are presented in *green*. The mean response data from both locations are presented in *blue*. Composite interval mapping was performed with Icimapping V4.0. The DArT markers are painted *red*; SSR markers are painted *blue*; EST-derived markers are painted *green*; conserved markers are painted *pink*; and the rest are painted *black* (color figure online)

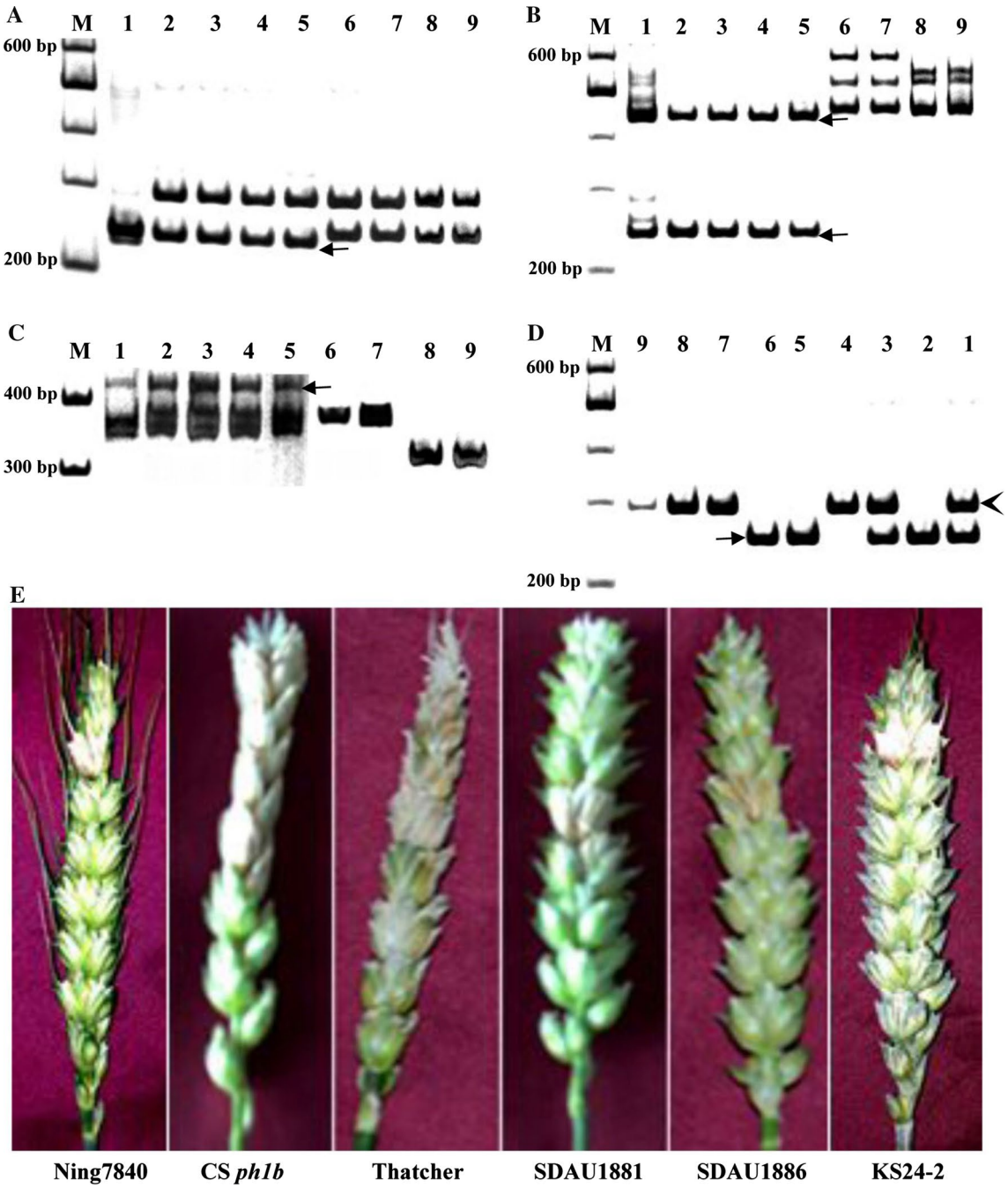

Fig. 5 Characterization of two new wheat lines (SDAU1881 and SDAU1886) carrying *Fhb7* in small segments of *Thinopyrum* chromatin. **a**, **b**, and **c** Gel images of the PCR products of three co-dominant markers *Xcfa2240*, *XsdauK352*, and *XsdauK66* run on 8 % non-denaturing polyacrylamide gels, respectively. The *numbers* at the *top* of the gels are *lane numbers*: M, 100 bp ladder; 1, *Th. ponticum*; 2, $7el_2(7D)$; 3, KS24-2; 4, SDAU1881; 5, SDAU1886; 6, Chinese Spring (CS); 7, Thatcher; 8, K11463; 9, K11695. *Arrows* indicate *Thinopyrum*-specific bands linked to *Fhb7*. **d** Gel image of the PCR products of *Xcfa2240* run on 8 % non-denaturing polyacrylamide gels. The *numbers* at the top of the gels are *lane numbers*: M, 100 bp ladder; 1, CS; 2, N7AT7B; 3, N7BT7D; 4, N7DT7A; 5, 7AL16; 6, 7AL1; 7, 7DL5; 8, 7DL2; 9, 7DL3. *Arrows* indicate 7D-specific bands in bin 7DL3-0.82-1.0. *Arrowhead* indicates 7A-specific bands in bin 7AL16-0.86-1.0. **e** FHB reactions

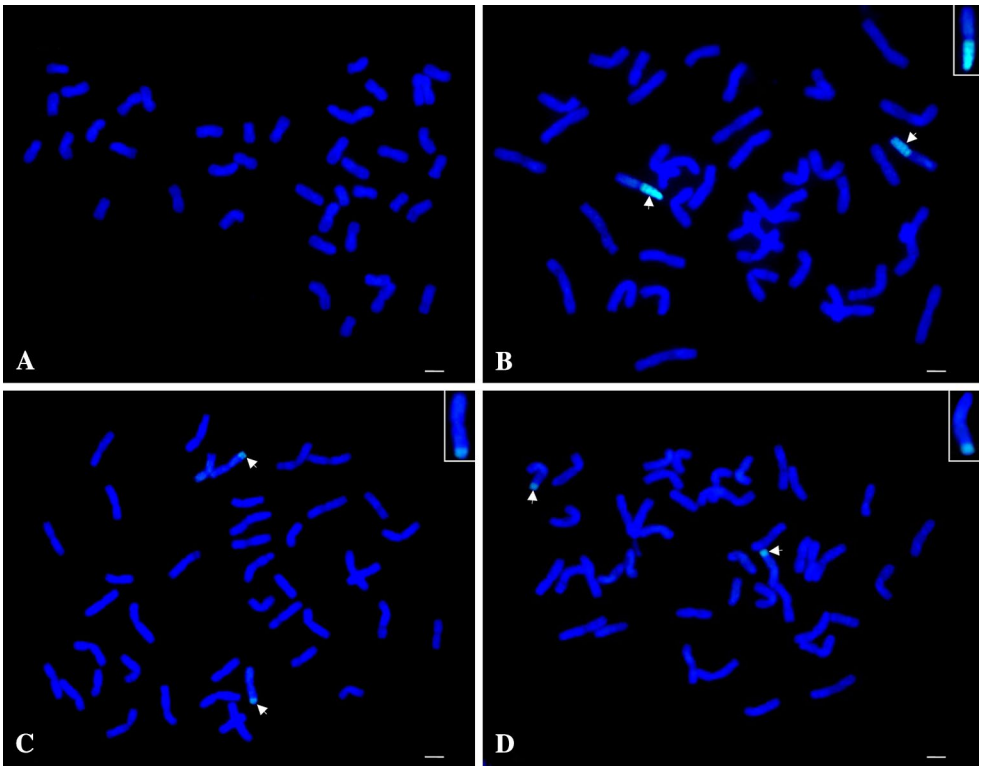

Fig. 6 Images from GISH analysis of wheat-*Thinopyrum* translocation lines carrying FHB resistance gene *Fhb7*. GISH was performed using *Th. ponticum* genomic DNA as the probe and Chinese Spring (CS) genomic DNA as the blocker on **a** CS **b** KS24-2 **c** SDAU1881 **d** SDAU1886. *Bars* 10 μm. *Thinopyrum* chromatin is painted *green* and indicated *arrows*. Wheat chromosomes are painted *blue* (color figure online)

Table 2 Markers identified in 208 BC_1F_2 plants derived from backcrossing of Chinese Spring (CS) *ph1bph1b**2/KS24-2

Plant type	No. of plants	Haplotypes of SSR marker[a]				
		Xswes130	*Xgwm333*	*Xmag1759*	*Xcfa2240*	*XsdauK66*
1	8	T	T	T	W	W
2	32	T	W	T	T	T
3	9	W	W	T	T	T
4	2	W	W	W	T	T
5	9	W	W	T	W	W
6	1	W	T	T	T	T
7	1	T	W	W	W	W
8	34	W	W	W	W	W
9	3	W	T	W	W	W
10	109	T	T	T	T	T

[a] Marker haplotypes: W, homozygous for wheat alleles; T, *Th. ponticum* alleles and wheat alleles

Validation of new molecular markers linked to *Fhb7* on the shortened *Th. ponticum* chromosome segments

The GISH patterns and physical position of the SSR marker *Xcfa2240* on chromosome 7D indicated that *Fhb7* was located in the deletion bin 7DL3-0.82-1.0 (Fig. 5d). The three co-dominant markers; i.e., *XsdauK352*, *Xcfa2240*, and *XsdauK66*, were selected and validated with 35 wheat accessions (Table S1). The co-dominant marker *XsdauK352* derived from the scaffold76033 amplified two fragments of approximately 210 and 483 bp in KS24-2, $7el_2$(7D), SDAU1881 and SDAU1886, and amplified one 490 bp fragment in Thatcher and CS *ph1b* (Figs. 4, 5b, Table S1). The SSR marker *Xcfa2240* amplified

Table 3 Evaluation of two putative short translocation lines (bold script), non-*Thinopyrum*-containing lines, pyramided lines with *Fhb1* and *Fhb7* (italic script), and control wheat lines in the greenhouse

Line	No. of damaged spikelets (NDS)[a]			
	2012 W	2013 W	2014 S	Mean
SDAU1881	1.4	1.4	1.4	1.4^{C}
SDAU1886	1.6	1.3	1.6	1.5^{C}
SDAU1885	5.5	5.9	6.1	5.8^{C}
SDAU1893	5.6	5.3	4.6	5.2^{B}
SDAU1894	5.7	6.0	5.4	5.7^{A}
SDAU1902	1.9	1.4	1.6	1.6^{C}
SDAU1903	1.7	1.3	1.3	1.4^{C}
SDAU1904	1.3	1.5	1.4	1.4^{C}
SDAU1906	1.8	1.4	1.4	1.5^{C}
CS *ph1b*	5.4	6.1	6.5	6.0^{A}
Thatcher	5.7	5.9	6.3	6.0^{A}
KS24-2	1.6	1.4	2.4	1.8^{C}
Ning 7840	1.5	1.3	1.3	1.4^{C}

[a] Responses marked with the same uppercase letter are not significantly different at $p = 0.05$

Table 4 Evaluation of *Fhb7* introgression lines (bold script) and non-*Fhb7* lines in Jimai 22 background and their parental lines in the greenhouse

Line	No. of damaged spikelets (NDS)[a]			
	2013 W	2014 S	2014 W	Mean
SDAU2028	**1.8**	**1.6**	**1.5**	**1.6^{D}**
SDAU2003	**1.4**	**1.6**	**1.4**	**1.5^{D}**
SDAU1081	5.9	6.4	6.4	6.2^{A}
SDAU1082	6.0	5.7	6.8	6.2^{A}
SDAU1083	5.3	5.7	5.5	5.5^{B}
SDAU1084	4.7	5.3	5.1	5.0^{C}
SDAU1881	1.4	1.4	1.3	1.4^{D}
Ning 7840	1.3	1.3	1.1	1.2^{D}
Jimai 22	6.8	6.0	5.3	6.1^{A}

[a] Responses marked with the same uppercase letter are not significantly different at $p = 0.05$

two fragments of 240 and 280 bp in KS24-2, $7el_2$(7D), SDAU1881 and SDAU1886, and two fragments of 245 and 280 bp in Thatcher and CS *ph1b* (Figs. 4, 5a; Table S1). The conserved marker, *XsdauK66*, derived from the rice gene *Os06g51500* (Fig. 4), amplified three fragments of approximately 358, 360, and 420 bp in KS24-2, $7el_2$(7D), SDAU1881, and SDAU1886, and amplified one 360 bp fragment in Thatcher and CS *ph1b* (Figs. 4, 5c, Table S1).

Confirmation of *Fhb7* in the Chinese major wheat cultivar Jimai 22 and its pyramiding with *Fhb1*

To confirm the FHB resistance conferred by *Fhb7* and its flanking markers, a BC_3F_2 population totaling 200 plants was produced by crossing SDAU1881 to the susceptible cv. Jimai 22 and then backcrossing to Jimai 22 for three generations. Two $BC_3F_{2:3}$ lines that were putatively homozygous for *Fhb7* and four non-*Fhb7* lines were selected by marker-assisted selection using markers *XsdauK66* and *Xcfa2240* flanking *Fhb7*, and were then evaluated for FHB response in the greenhouse. The four non-*Fhb7* lines determined with the above markers, SDAUI081, SDAUI082, SDAUI083, and SDAUI084, had mean NDS of 6.2, 6.2, 5.5, and 5.0, respectively, which was similar to Jimai 22. The two lines that were agronomically attractive predicted to carry *Fhb7* had means NDS of 1.5 and 1.6, which were not significantly different from the donor parent SDAU1881 (Table 4; Fig. S6). The two newly improved lines that were homozygous to *Fhb7* were designated as SDAU2003 and SDAU2028.

For pyramiding *Fhb7* and *Fhb1*, a population totaling 300 F_2 plants was generated from crosses of the new wheat-*Th. ponticum* derivatives and cv. Ning 7840. Four lines homozygous for markers *Xcfa2240* and *XsdauK66* flanking *Fhb7*, and *Xgwm493* and *Xgwm533* flanking *Fhb1*, were selected (Fig. 2). The FHB responses indicated that four $F_{2:3}$ lines were resistant to FHB with mean NDS ranging from 1.3 to 1.6 and not significantly different from KS24-2 or Ning 7840 (Table 3; Fig. S6). The four pyramided lines putatively homozygous for both *Fhb7* and *Fhb1* were designated as SDAU1902, SDAU1903, SDAU1904, and SDAU1906.

Inheritance of FHB resistance gene *Fhb7* derived from *Th. ponticum*

We evaluated 63 F_2 progeny from a cross between Jimai 22 and SDAU1881 and grouped them based on the molecular markers *XsdauK352*, *Xcfa2240*, and *XsdauK66*, which were classified as homozygous for T7DS.7DL-$7el_2$L, heterozygous for 7D/T7DS.7DL-$7el_2$L, and homozygous for 7D. The ratio of the above three groups was 15:34:14, which fitted the expected 1:2:1 ratio ($\chi^2_{1:2:1} = 0.4$, $p = 0.8$). The mean NDS in plants with only one copy of T7DS.7DL-$7el_2$L was not significantly different from lines without T7DS.7DL-$7el_2$L ($LSD_{0.05} = 1.6$), while plants homozygous for T7DS.7DL-$7el_2$L had significantly lower NDS (Table 5).

Table 5 Mean disease severities for genotypes segregating for translocation chromosome T7DS.7DL-7el$_2$L, scored 21 days after point inoculation of *F. graminearum*

Genotype	Number of observed plants	*XsdauK352* and *Xcfa2240* alleles	Generation	NDS[a]
Homozygous T7DS.7DL-7el$_2$L	15	7el$_2$L	F_2	1.9^{c}
Heterozygous 7D/T7DS.7DL-7el$_2$L	34	7el$_2$L	F_2	3.6^{b}
Homozygous 7D	14	7D	F_2	4.7^{ab}
SDAU1881	10	7el$_2$L	–	1.6^{c}
Jimai 22	10	7D	–	5.3^{a}

[a] Responses marked with the same uppercase letter are not significantly different at $p = 0.05$ (LSD)

Discussion

Thinopyrum species, as members of the tertiary gene pool for wheat, harbor many biotic and abiotic stress genes (Wang 2011). To date, at least 3 FHB resistance genes or QTLs have been identified in *Thinopyrum* derivatives. Among them, one gene located on chromosome 1E derived from *Th. elongatum* showed high FHB resistance in the greenhouse, but no visible effect was observed in the field (Jauhar 2014). Another gene *Fhb7*, located on chromosome 7el$_2$ derived from *Th. ponticum* showed high FHB resistance in the greenhouse and was resistant in the field (Shen et al. 2004; Shen and Ohm 2007; Zhang et al. 2011). A third gene located on chromosome 7E derived from *Th. elongatum* also showed FHB resistance, but it was not used due to linkage drag (Shen et al. 2004; Fu et al. 2012). We are developing a backcrossed population between 7E(7D) and wheat the *Ph1* mutant inducing homoeologous chromosome pairing in our ongoing research.

The FHB resistance gene *Fhb7* was identified in the wheat-*Th. ponticum* substitution line K2620 and mapped closely linked to *Xcfa2240* on the long arm of chromosome 7el (Shen and Ohm 2007; Zhang et al. 2011), but the DNA marker density of the *Fhb7* region was far lower than that required for fine mapping and positional cloning. Therefore, development of new molecular markers is needed for saturation mapping of *Fhb7*. Comparative genomics approaches have been widely used to construct high-density genetic maps of important wheat genes based on synteny among wheat, rice, *B. distachyon,* and sorghum. Based on synteny among these species, the FHB resistance gene *Fhb1* and the powdery mildew resistance gene *Pm41* were narrowed to sub-centiMorgan intervals (Liu and Anderson 2003; Cuthbert et al. 2006; Liu et al. 2006; Wang et al. 2014b). In this study, synteny among *Th. ponticum*, rice, *B. distachyon,* and sorghum was established. Subsequently, conserved primers were developed and used to map the FHB resistance gene *Fhb7*, which was flanked by *XsdauK66* and *XsdauK60*, corresponding to 18.8, 37.1, and 27.0 kb genomic regions in rice, *B. distachyon*, and sorghum, respectively. Of the 6 polymorphic markers, 3 were derived from the orthologous genes presented in the three species, which indicated the orthologous genes of the three species were more useful for marker development than others. However, the genes specific for one species or two species could also be useful for high-density genetic linkage map construction (Figs. 2, 4). Previous studies showed that *Thinopyrum* species were closely related to wheat (Dvořák 1980; Dewey 1983; Wang and Lu 2014). We expect that the newly published genome sequences of *T. uratu* (Ling et al. 2013), *Aegilops tauschii* (Jia et al. 2013), and *T. aestivum* (Mayer et al. 2014) will be more useful for marker development and mapping of important genes in *Thinopyrum* being transferred to wheat.

Creation and development of crops with stable or high disease resistance play important roles in sustainable crop production (Duveiller et al. 2007). Both the spectrum of protection against different races of pathogens, and the degree of resistance, can be increased by combining, or pyramiding resistance genes in the same line. This process is greatly aided by the availability of genetic markers because the required genotypes cannot be recognized by disease response alone. In this study, the introgression lines SDAU1881 and SDAU1886 with shortened *Th. ponticum* segments carrying *Fhb7* were identified and developed using the CS *ph1b* mutant, molecular markers, and GISH. Both lines were characterized as T7DS.7DL-7el$_2$L translocations and showed resistance to FHB, with outcomes consistent with the genetic mapping data (Fig. 4). The alien segments in these two lines were shortened by approximately 65 % compared to the original line KS24-2 (49 %), which meant that the distal 35 % of the long arm of chromosome 7el$_2$L or 7DL is common in these two lines, i.e., SDAU1881 and SDAU1886. In addition, co-dominant markers *Xcfa2240*, *XsdauK352*, and *XsdauK66* closely linked to *Fhb7* were identified and validated. MAS based on these markers will expedite transfer of *Fhb7* to other genotypes and the newly developed polymorphic markers would be helpful to select shorter 7el$_2$L chromosome segment carrying *Fhb7* through a second round of homeologous recombination. Two agronomically attractive Jimai 22 backcross derivatives (SDAU2003 and SDAU2028) developed by MAS using markers closely linked to *Fhb7* were highly resistant to FHB. Four lines putatively carrying *Fhb1* and *Fhb7* (SDAU1902, SDAU1903, SDAU1904, and SDAU1906) were developed by MAS and all showed high

resistance to FHB, although none was more resistant than the *Fhb1* donor parent Ning 7840, or the new introgession lines SDAU1881 or SDAU1886. Nevertheless, we believe that the pursuit of resistance gene combinations in breeding is worthwhile because of the likelihood of more stable resistance to multiple pathogenic types and across environments and genetic backgrounds.

The FHB resistance conferred by translocation T7DS.7DL-7el$_2$L in the CS background is novel and the level of resistance is similar to Ning 7840 (Table 3). However, our screening results of a population segregating for T7DS.7DL-7el$_2$L showed that the resistance gene *Fhb7* in 7DL-7el$_2$L is likely homozygous effective (Table 5). We are presently transferring the 7DS.7DL-7el$_2$L translocation to different commercial cultivars, which will allow us to evaluate the effect of 7DL-7el$_2$L on FHB resistance and other agronomical traits in other genetic backgrounds.

In summary, new wheat lines with shortened *Th. ponticum* chromatin segments carrying *Fhb7* were developed by molecular chromosome engineering using the *Ph1* mutant. Identification of molecular markers closely linked to *Fhb7* will accelerate the transfer of *Fhb7* to breeding lines and also provide an important resource for future map-based cloning of this FHB resistance gene.

Author's contribution statement LR Kong and Herbert W. Ohm conceived and designed the experiments. J Guo, XL Zhang, YR Hou, and XR Shen performed the experiments. J Guo, XL Zhang, YR Hou, JJ Cai, and LR Kong analyzed the data. TT Zhou, HH Xu, HW Wang, AF Li, FP Han, and HG Wang contributed reagents/materials/analysis tools. J Guo and LR Kong wrote the paper.

Acknowledgments The authors thank Robert McIntosh, University of Sydney Plant Breeding Institute–Cobbitty, PB4011, Narellan, NSW 2567, Australia, for review of the manuscript before submission. We thank Dr. B. S. Gill, Kansas state university for providing seeds of wheat nulli-tetra and deletion stocks of group 7. We acknowledge financial supports by the National High-Tech R&D Program of China (2011AA100102 and 2012AA101105), the NSF of China (Grant nos. 31171553 and 31471488), Shandong Seed Engineering Project (2015-2019), and the International Collaboration Program (948 Project, 2013-S19).

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

References

Akbari M, Wenzl P, Caig V, Carling J, Xia L, Yang S, Uszynski G, Mohler V, Lehmensiek A, Kuchel H, Hayden MJ, Howes N, Sharp P, Vaughan P, Rathmell B, Huttner E, Kilian A (2006) Diversity arrays technology (DArT) for high-throughput profiling of the hexaploid wheat genome. Theor Appl Genet 113:1409–1420

Bai G, Shaner G (2004) Management and resistance in wheat and barley to Fusarium head blight 1. Annu Rev Phytopathol 42:135–161

Buerstmayr H, Ban T, Anderson JA (2009) QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: a review. Plant Breed 128:1–26

Cainong JC, Bockus WW, Feng Y, Chen P, Qi L, Sehgal SK, Danilova TV, Koo D-H, Friebe B, Gill BS (2015) Chromosome engineering, mapping, and transferring of resistance to Fusarium head blight disease from *Elymus tsukushiensis* into wheat. Theor Appl Genet 128:1019–1027

Cetin Y, Bullerman LB (2005) Cytotoxicity of *Fusarium* mycotoxins to mammalian cell cultures as determined by the MTT bioassay. Food Chem Toxicol 43:755–764

Chakraborty S, Newton AC (2011) Climate change, plant diseases and food security: an overview. Plant Pathol 60:2–14

Cuthbert PA, Somers DJ, Thomas J, Cloutier S, Brulé-Babel A (2006) Fine mapping *Fhb1*, a major gene controlling Fusarium head blight resistance in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 112:1465–1472

Cuthbert PA, Somers DJ, Brulé-Babel A (2007) Mapping of *Fhb2* on chromosome 6BS: a gene controlling Fusarium head blight field resistance in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 114:429–437

D'mello J, Placinta C, Macdonald A (1999) *Fusarium* mycotoxins: a review of global implications for animal health, welfare and productivity. Anim Feed Sci Technol 80:183–205

Dewey DR (1983) Historical and current taxonomic perspectives of *Agropyron*, *Elymus*, and related genera. Crop Sci 23:637–642

Duveiller E, Singh RP, Nicol JM (2007) The challenges of maintaining wheat productivity: pests, diseases, and potential epidemics. Euphytica 157:417–430

Dvořák J (1980) Homoeology between *Agropyron elongatum* chromosomes and *Triticum aestivum* chromosomes. Can J Genet Cytol 22:237–259

Fu S, Lv Z, Qi B, Guo X, Li J, Liu B, Han F (2012) 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 Genomics 39:103–110

Goff SA, Ricke D, Lan TH, Presting G, Wang R, Dunn M, Glazebrook J, Sessions A, Oeller P, Varma H (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*). Science 296:92–100

Goswami RS, Kistler HC (2004) Heading for disaster: *Fusarium graminearum* on cereal crops. Mol Plant Pathol 5:515–525

Han F, Gao Z, Birchler JA (2009) Reactivation of an inactive centromere reveals epigenetic and structural components for centromere specification in maize. Plant Cell 21:1929–1939

He X, Singh PK, Schlang N, Duveiller E, Dreisigacker S, Payne T, He Z (2014) Characterization of Chinese wheat germplasm for resistance to Fusarium head blight at CIMMYT, Mexico. Euphytica 195:383–395

Jauhar PP (2014) Durum wheat genetic stocks involving chromosome 1E of diploid wheatgrass: resistance to Fusarium head blight. Nucleus 57:19–23

Jia J, Zhao S, Kong X, Li Y, Zhao G, He W, Appels R, Pfeifer M, Tao Y, Zhang X, Jing R, Zhang C, Ma Y, Gao L, Gao C, Spannagl M, Mayer K, Li D, Pan S, Zheng F, Hu Q, Xia X, Li J, Liang Q, Chen J, Wicker T, Gou C, Kuang H, He G, Luo Y, Keller B, Xia Q, Lu P, Wang J, Zou H, Zhang R, Xu J, Gao J, Middleton C, Quan Z, Liu G, Wang J, International Wheat Genome Sequencing Consortium, Yang H, Liu X, He Z, Mao L, Wang J (2013)

Aegilops tauschii draft genome sequence reveals a gene repertoire for wheat adaptation. Nature 496:91–95

Kim NS, Armstrong K, Knott D (1993) Molecular detection of *Lophopyrum* chromatin in wheat-*Lophopyrum* recombinants and their use in the physical mapping of chromosome 7D. Theor Appl Genet 85:561–567

Kong L, Cambron SE, Ohm HW (2008) Hessian fly resistance genes *H16* and *H17* are mapped to a resistance gene cluster in the distal region of chromosome 1AS in wheat. Mol Breed 21:183–194

Kosambi D (1943) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Ling HQ, Zhao S, Liu D, Wang J, Sun H, Zhang C, Fan H, Li D, Dong L, Tao Y (2013) Draft genome of the wheat A-genome progenitor *Triticum urartu*. Nature 496:87–90

Liu S, Anderson JA (2003) Targeted molecular mapping of a major wheat QTL for Fusarium head blight resistance using wheat ESTs and synteny with rice. Genome 46:817–823

Liu S, Zhang X, Pumphrey MO, Stack RW, Gill BS, Anderson JA (2006) Complex microcolinearity among wheat, rice, and barley revealed by fine mapping of the genomic region harboring a major QTL for resistance to Fusarium head blight in wheat. Funct Integr Genomics 6:83–89

Marais G, Kotze L, Eksteen A (2010) Allosyndetic recombinants of the *Aegilops peregrina*-derived *Lr59* translocation in common wheat. Plant Breed. doi:10.1111/j.1439-0523.2009.01713.x

Mayer KF, Rogers J, Doležel J, Pozniak C, Eversole K, Feuillet C, Gill B, Friebe B, Lukaszewski AJ, Sourdille P (2014) A chromosome-based draft sequence of the hexaploid bread wheat (*Triticum aestivum*) genome. Science 345:1251788

Niu Z, Klindworth DL, Friesen TL, Chao S, Jin Y, Cai X, Xu SS (2011) Targeted introgression of a wheat stem rust resistance gene by DNA marker-assisted chromosome engineering. Genetics 187:1011–1021

Paterson AH, Bowers JE, Bruggmann R, Dubchak I, Grimwood J, Gundlach H, Haberer G, Hellsten U, Mitros T, Poliakov A (2009) The *Sorghum bicolor* genome and the diversification of grasses. Nature 457:551–556

Qi L, Friebe B, Zhang P, Gill BS (2007) Homoeologous recombination, chromosome engineering and crop improvement. Chromosome Res 15:3–19

Qi L, Pumphrey M, Friebe B, Chen P, Gill B (2008) Molecular cytogenetic characterization of alien introgressions with gene *Fhb3* for resistance to Fusarium head blight disease of wheat. Theor Appl Genet 117:1155–1166

Roberts MA, Reader SM, Dalgliesh C, Miller TE, Foote TN, Fish LJ, Snape JW, Moore G (1999) Induction and characterization of *Ph1* wheat mutants. Genetics 153:1909–1918

Semagn K, Bjørnstad Å, Skinnes H, Marøy AG, Tarkegne Y, William M (2006) Distribution of DArT, AFLP, and SSR markers in a genetic linkage map of a doubled-haploid hexaploid wheat population. Genome 49:545–555

Shen X, Ohm H (2007) Molecular mapping of *Thinopyrum*-derived Fusarium head blight resistance in common wheat. Mol Breed 20:131–140

Shen X, Kong L, Ohm H (2004) Fusarium head blight resistance in hexaploid wheat (*Triticum aestivum*)-*Lophopyrum* genetic lines and tagging of the alien chromatin by PCR markers. Theor Appl Genet 108:808–813

Sourdille P, Singh S, Cadalen T, Brown-Guedira GL, Gay G, Qi L, Gill BS, Dufour P, Murigneux A, Bernard M (2004) Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). Funct Integr Genomics 4:12–25

Van Ooijen JW (2006) JoinMap® 4.0: software for the calculation of genetic linkage maps in experimental population. Kyazma BV, Wageningen, p. 33

Vogel JP, Garvin DF, Mockler TC, Schmutz J, Rokhsar D, Bevan MW, Barry K, Lucas S, Harmon-Smith M, Lail K (2010) Genome sequencing and analysis of the model grass *Brachypodium distachyon*. Nature 463:763–768

Wang RR (2011) Agropyron and Psathyrostachys. In: Kole C (ed) Wild crop relatives: genomic and breeding resources, cereals, 1st edn. Springer, Berlin, pp 77–108

Wang RR, Lu B (2014) Biosystematics and evolutionary relationships of perennial *Triticeae* species revealed by genomic analyses. J Syst Evol 52:697–705

Wang J, Li H, Zhang L, Meng L (2014a) Users' Manual of QTL IciMapping. The Quantitative Genetics Group, Institute of Crop Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing 100081, China, and Genetic Resources Program, International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 06600 Mexico, D.F., Mexico

Wang Z, Cui Y, Chen Y, Zhang D, Liang Y, Zhang D, Wu Q, Xie J, Ouyang S, Li D (2014b) Comparative genetic mapping and genomic region collinearity analysis of the powdery mildew resistance gene *Pm41*. Theor Appl Genet 127:1741–1751

Wenzl P, Carling J, Kudrna D, Jaccoud D, Huttner E, Kleinhofs A, Kilian A (2004) Diversity arrays technology (DArT) for whole-genome profiling of barley. Proc Natl Acad Sci USA 101:9915–9920

West JS, Holdgate S, Townsend JA, Edwards SG, Jennings P, Fitt BD (2012) Impacts of changing climate and agronomic factors on Fusarium ear blight of wheat in the UK. Fungal Ecol 5:53–61

Xue S, Li G, Jia H, Xu F, Lin F, Tang M, Wang Y, An X, Xu H, Zhang L (2010) Fine mapping *Fhb4*, a major QTL conditioning resistance to Fusarium infection in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 121:147–156

Xue S, Xu F, Tang M, Zhou Y, Li G, An X, Lin F, Xu H, Jia H, Zhang L (2011) Precise mapping *Fhb5*, a major QTL conditioning resistance to Fusarium infection in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 123:1055–1063

You FM, Huo N, Gu YQ, Lazo GR, Dvorak J, Anderson OD (2009) ConservedPrimers 2.0: a high-throughput pipeline for comparative genome referenced intron-flanking PCR primer design and its application in wheat SNP discovery. BMC Bioinformatics 10:331

Yu J, Bai G, Cai S, Dong Y, Ban T (2008) New Fusarium head blight-resistant sources from Asian wheat germplasm. Crop Sci 48:1090–1097

Zhang X, Shen X, Hao Y, Cai J, Ohm HW, Kong L (2011) A genetic map of *Lophopyrum ponticum* chromosome 7E, harboring resistance genes to Fusarium head blight and leaf rust. Theor Appl Genet 122:263–270