Alien chromatin but not *Fhb7* confers Fusarium head blight resistance in wheat breeding

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Abstract

Fusarium head blight (FHB), caused by *Fusarium* species, seriously threaten global wheat production. Three wheat-*Th.elongatum* FHB resistant translocation lines have been developed and used for breeding. Transcriptomic analysis identified a derivative glutathione S-transferase transcript T26102, which was homologous to *Fhb7* and induced dramatically by *Fusarium graminearum*. Homologs of *Fhb7* were detected in several genera in Triticeae, including *Thinopyrum*, *Elymus*, *Leymus*, *Pseudoroegeria* and *Roegeria*. Several wheat-*Thinopyrum* translocation lines carrying *Fhb7* remain susceptible to FHB, and transgenic plants overexpressing the T26102 on different backgrounds did not improve the FHB resistance. Taken as a whole, we show the application of the chromatin derived from diploid *Thinopyrum elongatum* successfully conferring wheat with high level FHB resistance independent of the *Fhb7*.

One Sentence Summary

Thinopyrum elongatum chromatin from 7EL was successfully applied to wheat FHB resistance breeding, but the resistant gene other than the reported *Fhb7* remained unknown.

Introduction

Fusarium head blight (FHB), colloquially known as scab, is caused by *Fusarium* species and remain one of the most devastating wheat diseases in many areas around the world (*1*). Scab causes significant yield losses and reduces grain quality because of mycotoxins generated during the course of infection, such as deoxynivalenol (DON) and nivalenol (NIV) (*2, 3*). Due to climate

change, residue incorporation and frequent crop rotations, FHB now occurs at greater and greater frequencies, making the task of developing FHB resistance wheat cultivars an urgent priority for breeders (*4-6*). While a few wheat accessions with high levels of FHB resistance have been exploited worldwide, most of them produce small heads, late maturity and other undesirable agronomic traits that hampered incorporating the resistance to elite cultivars (*3*). Cultivars Sumai 3 and its derivatives carrying *Fhb1* showed a good combination FHB resistance and yield traits and have been successfully applied to wheat breeding worldwide (*3, 7*). However, in spite of a successful clone of the major resistance gene *Fhb1*, it is difficult to combine the high FHB resistance with other necessary traits in wheat breeding practice, especially for winter and facultative wheat (*3, 7, 8*).

The genus *Thinopyrum* contains numerous resistance genes for biotic and abiotic stress and has been considered as an important genetic resource for wheat improvement (*9*). Until now, two genes conferring wheat FHB resistance were mapped on the homologous group seven in *Thinopyrum* species. Our previous research showed that the wheat-*Th. elongatum* 7E disomic addition line and substitution lines exhibit high resistance to FHB, and the further analysis confirmed the location of the resistant gene on the chromosome arm 7EL (*10*). Functioning as a glutathione S-transferase (GST) and derived from *Th. ponticum*, *Fhb7* was reported to be located on 7E2 and conferred broad resistance to *Fusarium* species by detoxifying trichothecenes via de-epoxidation (*11, 12*).

Many translocation lines have been developed from wild relatives of wheat and applied to wheat breeding. Some of the most successful example of transferring alien genes to common wheat include the wheat-rye 1BL/1RS translocation lines with fused centromere (*13*). These translocation lines were employed in wheat breeding because of their excellent stripe rust and powdery mildew resistance (*14*). Here, we produced a bountiful number of wheat-*Th. elongatum* translocation lines by radiating the pollens of the ditelosomic addition line 7EL and successfully applied the FHB resistant translocation lines to wheat breeding without yield penalty. In the process of screening the FHB resistance genes, we found *Fhb7* and its homologs are not responsible for FHB resistance.

Results

Development of wheat-*Th. elongatum* translocation lines

Previously, the long arm of chromosome 7E of *Th. elongatum* was found to harbor a new resistance gene capable of suppressing FHB spreading on wheat spikes (*10*). Irradiation was performed on the pollens of wheat-*Th. elongatum* addition line 7EL at early flowering stage. Following that, fresh pollens were pollinated to the emasculated spikes of the recurrent parent. In total, 8400 M_1 seeds were obtained and cytological analyses were performed on all germinated seeds. Consequently, 671 wheat-*Th. elongatum* translocation lines were identified, accounting for 7.99% of all developed lines (Table 1). The translocation lines were classified into terminal and intercalary types by the position of alien chromosome fragments (fig. S1). By the size of alien chromosome fragments, the terminal translocation lines were furtherly classified into short, medium and long alien segmental translocation lines (fig. S1). Totally, we obtained 184 short alien segmental translocation lines, 141 medium alien segmental translocation lines, 247 long alien segmental translocation lines and 99 intercalary translocation lines (Table 1).

Application of wheat-*Th. elongatum* translocation lines in FHB resistance breeding

Wheat cultivar Jimai 22 is widely grown in the northern part of China because of its broad adaptation and high yield potential. In order to improve the integrated agronomic traits of our developed translocation lines, we back crossed them with Jimai 22 and evaluated FHB resistance using the single floret inoculation method. Finally, 81 translocation lines were identified with good resistance to FHB (fig. S2). After backcrossing with Jimai 22 for at least three generations, homozygous translocation lines were selected from the self-crossed progenies. Out of these, the short alien segmental translocation lines Zhongke 1878 and Zhongke 166 as well as the long alien segmental translocation line Zhongke 545 were developed with good integrated agronomic traits and no significant grain yield penalty (Fig. 1, A and C, and fig. S3, A and B). All the three lines showed high resistance to FHB, with less diseased spikes after infection in nature and higher yield

than the control cultivar Jimai 22 after spraying *Fusarium graminearum* (Fig. 1, B and C, and fig. S3, C and D). In the national wheat yield contest, the grain yield of Zhongke 166 exceeded that of the national control Zhoumai 18 by an average of 6.26% in 23 locations (fig. S3E). Cytological analysis revealed that the translocation occurred on the long arm of chromosome 6D in line Zhongke1878, and on the long arm of chromosome 7D in line Zhongke 166 and Zhongke 545 (fig. S4).

Screening specific transcripts for disease-resistant interval on chromosome 7EL

To explore the nature of the FHB resistance gene in 7EL, we inoculated the spikes of translocation line Zhongke 1878 with *Fusarium graminearum* and performed full-length transcriptome sequencing after ninety-six hours. Among 34996 transcripts, 520 transcripts were filtered out as candidates expressing only from 7EL by blasting against the reference genome of Chinese Spring (CS) and the nucleotide database of *Fusarium graminearum* on the National Center for Biotechnology Information (NCBI). According to the identified sequences, one to three pairs of primers for each transcript were designed. In order to screen the transcripts specific to alien chromatin, polymerase chains reaction (PCR) was performed using genomic DNA of wheat-*Th. elongatum* addition line 7EL, CS, Zhongke 1878 and Jimai 22. Finally, 25 transcripts specific to the disease-resistant interval in line Zhongke 1878 were screened (fig. S5 and Data S1). Among them, 7 transcripts were annotated as resistant proteins containing the NB-ARC domain and 10 as unknown proteins (Table 2). Additionally, other proteins found included proteins such as a receptor kinase, ATPase subunit, dirigent-jacalin protein, GST family, nucleosome assembly protein, cold induced protein and retrotransposon protein (Table 2). Annotated as a GST protein, T26102 was chosen for further study because it was induced drastically 48h after inoculation with *Fusarium graminearum*, which was confirmed by qRT-PCR (Fig. 2A and fig. S6A). We also collected the RNA-seq data of CS-7EL line 4 d after water and *Fusarium graminearum* inoculation previously reported from NCBI (*15*), which further confirmed the induction of T26102 by *Fusarium*

131 *graminearum* (fig. S6B).

132

133 **Distribution of T26102 in Triticeae**

134 To explore the evolution of T26102, its homologs were checked in different wheat-*Thinopyrum*
135 derivates. The T26102 homologs were detected in the addition line 7EL, our developed wheat-*Th.*
136 *elongatum* translocation lines, such as Zhongke 1878, and the wheat-*Th.ponticum* translocation
137 lines 4460 and 4462 (Fig. 2B). The homologs of T26102 were also detected in wheat-*Thinopyrum*
138 partial amphiploids, such as octoploid SNTE20, XY693 and XY7631, and hexaploid 8802 and
139 8803 (Fig. 2B). Totally, 122 species belonging to Triticeae were collected and used for detecting
140 the T26102 homologs (Data S2). Except for *Thinopyrum*, the homologs of T26102 were detected
141 in four other genera, i.e., *Elymus*, *Leymus*, *Pseudoroegneria* and *Roegneria* (Fig. 2B).
142 By comparing sequences, we found that T26102 was homologous to the reported *Fhb7* with only
143 two amino acids difference between them. Furthermore, the protein sequences were at least 95%
144 identical across all the T26102 homologs in Triticeae plants (fig. S7). In some species, more than
145 one homolog was discovered. We detected two homologs of T26102 in our developed wheat-*Th.*
146 *elongatum* translocation lines, such as Zhongke 1878 and Zhongke 166 (fig. S7). Three homologs
147 of T26102 were detected in the *Th. intermedium* accession PI 440001 (fig. S7). Despite indel
148 variation and amino acid substitution across all the homologs of T26102, no premature termination
149 and code-shifting mutations occurred in the protein sequences. The main variation was the number
150 of Thr-Ser at the amino terminus of the protein sequence (fig. S7).

151

152 **Functional identification of T26102 homologs**

153 To identify the function of T26102, we evaluated the FHB resistance on wheat-*Thinopyrum*
154 derivatives carrying the homologs of T26102. Surprisingly, obvious differences of FHB resistance
155 were detected among different lines carrying the homolog of T26102. The wheat-*Th. ponticum*
156 translocation lines 4460, 4462 and wheat-*Th. elongatum* translocation line Zhongke 1878 all

157 carried the T26102 homolog (Fig. 2B and fig. S8). However, the translocation lines 4460 and 4462
158 were susceptible to FHB, whereas the Zhongke 1878 was resistant to FHB (Fig. 3A). Sequence
159 alignment analysis revealed that the protein sequences in 4460 and 4462 lines were identical to the
160 reported *Fhb7* from the 7E2/7D substitution line (fig. S7). Furthermore, some wheat-*Thinopyrum*
161 partial amphiploids carrying the homolog of T26102 were also identified as susceptible to FHB,
162 such as octaploid SNTE20 (Fig. 3A and fig. S8). Expression analysis revealed that the expression
163 of T26102 homologs was induced in 4460, 4462 and SNTE20 after inoculating with *Fusarium*
164 *graminearum* (Fig. 3B). We also discovered that lines 4460 and 4462 shared an identical promoter
165 with *Fhb7* from the 7E2/7D substitution (fig. S9). On the other hand, it was noticed that the partial
166 amphiploids 8802 and 8803 carried the same homolog of T26102 (fig. S7 and fig. S8). However,
167 they reacted differently to *Fusarium graminearum*, with 8802 showing high resistance and 8803
168 showing high susceptibility (Fig. 3C). Lines 8802 and 8803 also shared an identical promoter (fig.
169 S9). The expression of T26102 homolog in 8802 and 8803 was confirmed to be drastically induced
170 at 96h after inoculating with *Fusarium graminearum* (Fig. 3D). These results thus raised serious
171 questions about the ability of T26102 to confer resistance to FHB.
172 To verify the FHB resistance function of T26102, we took the overexpression vector pUbi::T26102
173 and transformed it into two wheat accessions Jimai 22 and 19AS161, both of which are highly
174 susceptible to FHB. The transgenic positive wheat plants overexpressing T26102 were used for
175 FHB resistance evaluation (Fig. 4A). Compared to the wild types, no T_0 transgenic lines showed
176 an improved FHB resistance regardless of any genetic background (Fig. 4B). This result further
177 suggests that T26102 is not the pivotal gene that confers wheat with resistance to FHB.
178

179 **Discussion**

180 Crop wild relatives are undeniably beneficial to modern agriculture because they provide breeders
181 with a broad pool of potentially useful genetic resources, especially with regard to the resistance
182 to disease and pest (*16*). Translocation lines between wheat and its wild relatives have been

183 successfully applied to wheat breeding, such as the wheat-rye 1BL/1RS translocation line and the
184 wheat-*Haynaldia villosa* 6AL/6VS translocation line (*13, 17*). The usefulness of the translocation
185 lines is dependent on whether the alien fragment could compensate for the replaced wheat
186 segments (*18*). In our study, translocation lines between wheat and *Th. elongatum* were
187 successfully applied to wheat FHB resistance breeding without yield penalty, and the translocation
188 for Zhongke 166 and Zhongke 545 occurred on chromosome 7DL. Their high yield potential in
189 the pilot experiment might be attributed to the compensation from the translocated 7EL segment
190 with the lost 7DL segment. Our practice revealed that Zhongke 1878, whose translocation occurred
191 on 6DL, also exhibited good yield potential. This result suggests that the application of
192 translocation lines should not be limited to the translocations between homeologous chromosomes.
193 Small segmental translocation lines with Fusarium head blight resistance can help narrow down
194 the region carrying the resistant gene. We applied this strategy to our work, and selected twenty-
195 five transcripts specific for disease-resistant regions on 7EL. We focused on T26102 because of
196 its dramatical induction after inoculation with *Fusarium graminearum* and its similarity to *Fhb7*.
197 Recently, *Fhb7*, also encoding the GST protein, was reported to confer wheat with a broad
198 resistance to *Fusarium* species and it was acquired by horizontal gene transfer (HGT) from
199 *Epichloë* to *Thinopyrum* (*12*). Interestingly, except for *Thinopyrum*, we were able to find homologs
200 of *Fhb7* in several species within the genera of *Elymus*, *Leymus*, *Pseudoroegeria* and *Roegeria*.
201 This was unsurprising, given that *Epichloë* often formed symbiotic associations with temperate
202 grasses of the subfamily *Pooideae* (*19*). Thus to us HGT did not appear to be an accidental
203 happening by chance only in *Thinopyrum*. It is possible that HGT happened before Triticeae
204 differentiation, or else *Fhb7* ought to be detected in the genus *Triticum* at large, which currently
205 does not appear to be the case.
206 Perhaps more interesting is the fact that T26102 is not causal in conferring FHB resistance. Out of
207 all the T26102 overexpression transgenic lines, none were able to improve FHB resistance.
208 Furthermore, some wheat-*Thinopyrum* translocation lines and partial amphiploids carrying the

209 homologs of T26102 were also identified susceptible to FHB. The homologs of *Fhb7* and their
210 promoter region were exactly the same in partial amphiploids 8802 and 8803, which exhibited a
211 contrasting reaction after inoculating with *Fasurium graminearum*. Above all, wheat-*Th. ponticum*
212 translocation lines 4460 and 4462, that carried an identical protein sequence to Fhb7 in the 7E2/7D
213 substitution line also failed to confer resistance. These results indicate to us that T26102, including
214 its homologs, are not responsible for FHB resistance.
215 Studies based on meiotic chromosome pairing revealed that *Th. elongatum* chromosome 7E paired
216 occasionally with *Th. ponticum* chromosomes 7E1 and 7E2 in hybrids, with frequencies of meiotic
217 pairing rates of 19.85 and 2.52, respectively (*20*). The genetic relationships based on molecular
218 markers also revealed that 7E was distant from 7E1 and 7E2 (*20*). It was reported that
219 resynthesized polyploids and natural polyploids have undergone many genetic changes, including
220 sequence deletion, rDNA loci changes, transposon activation and chromosomal rearrangement
221 (*21-27*). All these findings suggest that 7E and 7E2 have difference on the DNA level. *Fhb7* was
222 mapped to the distal end of 7E2 between the *XSdauK79* and *XSdauK80* markers based on
223 recombinations between 7E1 and 7E2 (*12, 20*). Although ~1.2 Mb region between the two markers
224 was identified on the 7E chromosome, the DNA components between the two mapping intervals
225 of 7E and 7E2 might be quite different. The fortuitous findings of FHB resistant candidate genes
226 from *Th. ponticum* are unlikely to be replicated using *Th. elongatum* as a reference genome.
227 So long as FHB remain as a major threat to worldwide agriculture, identifying and exploiting
228 resistance genes will always prove to be a useful endeavor. The lack of resistance conferred by the
229 *Fhb7* homologue T26102 in our work indicates that the bona fide resistance gene still lies
230 undiscovered, making it a suitable target for future work.
231
232 **References and Notes:**
233 1. R. Dean, J. A. Van Kan, Z. A. Pretorius, K. E. Hammond-Kosack, A. Di Pietro, P. D. Spanu,
234 J. J. Rudd, M. Dickman, R. Kahmann, J. Ellis, G. D. Foster, The top 10 fungal pathogens

in molecular plant pathology. *Mol. Plant Pathol.* **13**, 414-430 (2012).

2. M. McMullen, R. Jones, D. Gallenberg, Scab of wheat and barley: a re-emerging disease of devastating impact. *Plant Dis.* **81**, 1340-1348 (1997).

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

4. Z. Ma, Q. Xie, G. Li, H. Jia, J. Zhou, Z. Kong, N. Li, Y. Yuan, Germplasms, genetics and genomics for better control of disastrous wheat Fusarium head blight. *Theor. Appl. Genet.* **133**, 1541-1568 (2020).

5. S. Chakraborty, A. C. Newton, Climate change, plant diseases and food security: an overview. *Plant Pathol.* **60**, 2-14 (2011).

6. X. Zhang, J. Halder, R. P. White, D. J. Hughes, Z. Ye, C. Wang, R. Xu, B. Gan, B. D. L. Fitt, Climate change increases risk of Fusarium ear blight on wheat in central China. *Ann. Appl. Biol.* **164**, 384-395 (2014).

7. Z. Su, A. Bernardo, B. Tian, H. Chen, S. Wang, H. Ma, S. Cai, D. Liu, D. Zhang, T. Li, H. Trick, P. St Amand, J. Yu, Z. Zhang, G. Bai, A deletion mutation in *TaHRC* confers *Fhb1* resistance to Fusarium head blight in wheat. *Nat. Genet.* **51**, 1099-1105 (2019).

8. G. Li, J. Zhou, H. Jia, Z. Gao, M. Fan, Y. Luo, P. Zhao, S. Xue, N. Li, Y. Yuan, S. Ma, Z. Kong, L. Jia, X. An, G. Jiang, W. Liu, W. Cao, R. Zhang, J. Fan, X. Xu, Y. Liu, Q. Kong, S. Zheng, Y. Wang, B. Qin, S. Cao, Y. Ding, J. Shi, H. Yan, X. Wang, C. Ran, Z. Ma, Mutation of a histidine-rich calcium-binding-protein gene in wheat confers resistance to Fusarium head blight. *Nat. Genet.* **51**, 1106-1112 (2019).

9. Q. Chen, Detection of alien chromatin introgression from *Thinopyrum* into wheat using S genomic DNA as a probe-A landmark approach for *Thinopyrum* genome research. *Cytogenet. Genome Res.* **109**, 350-359 (2005).

10. S. Fu, Z. Lv, B. Qi, X. Guo, J. Li, B. Liu, F. Han, 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 (2012).

11. J. Dvořák, Meiotic pairing between single chromosomes of diploid *Agropyron elongatum* and decaploid *A. elongatum* in *Triticum aestivum*. *Can. J. Genet. Cytol.* **17**, 329-336 (1975).
12. H. Wang, S. Sun, W. Ge, L. Zhao, L. Kong, Horizontal gene transfer of *Fhb7* from fungus underlies Fusarium head blight resistance in wheat. *Science* **368**, eaba5435 (2020).
13. J. Wang, Y. Liu, H. Su, X. Guo, F. Han, Centromere structure and function analysis in wheat-rye translocation lines. *Plant J.* **91**, 199-207 (2017).
14. S. V. Rabinovich, Importance of wheat-rye translocations for breeding modern cultivar of *Triticum aestivum L*. *Euphytica* **100**, 323-340 (1998).
15. L. Gou, J. Hattori, G. Fedak, M. Balcerzak, A. Sharpe, P. Visendi, D. Edwards, N. Tinker, Y. Wei, G. Chen, T. Ouellet, Development and validation of expressed molecular markers specific for the long arm of chromosome 7E. *Crop Sci.* **56**, 354 (2016).
16. B. V. Ford-Lloyd, M. Schmidt, S. J. Armstrong, O. Barazani, J. Engels, R. Hadas, K. Hammer, S. P. Kell, D. Kang, K. Khoshbakht, Y. Li, C. Long, B. Lu, K. Ma, V. T. Nguyen, L. Qiu, S. Ge, W. Wei, Z. Zhang, N. Maxted, Crop wild relatives-undervalued, underutilized and under threat? *Bioscience* **61**, 559-565 (2011).
17. L. Xing, P. Hu, J. Liu, K. Witek, S. Zhou, J. Xu, W. Zhou, L. Gao, Z. Huang, R. Zhang, X. Wang, P. Chen, H. Wang, J. D. G. Jones, M. Karafiátová, J. Vrána, J. Bartoš, J. Doležel, Y. Tian, Y. Wu, A. Cao, *Pm21* from *Haynaldia villosa* encodes a CC-NBS-LRR protein conferring powdery mildew resistance in wheat. *Mol. Plant* **11**, 874-878 (2018).
18. J. D. Faris, S. S. Xu, X. Cai, T. L. Friesen, Y. Jin, Molecular and cytogenetic characterization of a durum wheat-*Aegilops speltoides* chromosome translocation conferring resistance to stem rust. *Chromosome Res.* **16**, 1097-1105 (2008).
19. A. Tanaka, D. Takemoto, T. Chujo, B. Scott, Fungal endophytes of grasses. *Curr. Opin. Plant Biol.* **15**, 462-468 (2012).
20. J. Guo, F. He, J. Cai, H. W. Wang, A. Li, H. Wang, L. Kong, Molecular and cytological

comparisons of chromosomes 7el$_1$, 7el$_2$, 7E^e, and 7E^i derived from *Thinopyrum*. *Cytogenet. Genome Res.* **145**, 68-74 (2015).

21. X. Ma, J. P. Gustafson, Allopolyploidization-accommodated genomic sequence changes in triticale. *Ann. Bot.* **101**, 825-832 (2008).

22. X. Guo, F. Han, Asymmetric epigenetic modification and elimination of rDNA sequences by polyploidization in wheat. *Plant Cell* **26**, 4311-4327 (2014).

23. H. Shaked, K. Kashkush, H. Ozkan, M. Feldman, A. A. Levy, Sequence elimination and cytosine methylation are rapid and reproducible responses of the genome to wide hybridization and allopolyploidy in wheat. *Plant Cell* **13**, 1749-1759 (2001).

24. C. Parisod, K. Alix, J. Just, M. Petit, V. Sarilar, C. Mhiri, M. Ainouche, B. Chalhoub, M. A. Grandbastien, Impact of transposable elements on the organization and function of allopolyploid genomes. *New Phytol.* **186**, 37-45 (2010).

25. R. T. Gaeta, J. C. Pires, F. Iniguez-Luy, E. Leon, T. C. Osborn, Genomic changes in resynthesized Brassica napus and their effect on gene expression and phenotype. *Plant Cell* **19**, 3403-3417 (2007).

26. J. A. Udall, P. A. Quijada, T. C. Osborn, Detection of chromosomal rearrangements derived from homologous recombination in four mapping populations of Brassica napus L. *Genetics* **169**, 967-979 (2005).

27. L. Comai, Genetic and epigenetic interactions in allopolyploid plants. *Plant Mol. Biol.* **43**, 387-399 (2000).

28. A. Kato, J. C. Lamb, J. A. Birchler, Chromosome painting using repetitive DNA sequences as probes for somatic chromosome identification in maize. *Proc. Natl. Acad. Sci. U. S. A.* **101**, 13554-13559 (2004).

29. F. Han, J. C. Lamb, J. A. Birchler, High frequency of centromere inactivation resulting in stable dicentric chromosomes of maize. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 3238-3243 (2006).

30. M. Lulin, S. Yi, C. Aizhong, Q. Zengjun, X. Liping, C. Peidu, L. Dajun, E. W. Xiu, Molecular cloning and characterization of an up-regulated UDP-glucosyltransferase gene induced by DON from *Triticum aestivum L*. cv. Wangshuibai. *Mol. Biol. Rep.* **37**, 785-795 (2010).

31. M. Pertea, D. Kim, G. M. Pertea, J. T. Leek, S. L. Salzberg, Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. *Nat. Protoc.* **11**, 1650-1667 (2016).

32. K. Wang, H. Liu, L. Du, X. Ye, Generation of marker-free transgenic hexaploid wheat via an Agrobacterium-mediated co-transformation strategy in commercial Chinese wheat varieties. *Plant Biotechnol. J.* **15**, 614-623 (2017).

Acknowledgments: We thank X. Wang (Nanjing Agricultural University) and J.B. Li (Nanyang Academy of Agricultural Sciences) for providing the *Fusarium* strains, Y.G Bao and H.G. Wang (Shandong Agricultural University) for providing some partial amphiploid accessions, Y.W. Li (Institute of Genetics and Developmental Biology, Chinese Academy of Sciences) and Moshe Feldman (Weizmann Institute of Sciences) for providing *Thinopyrum* species, and M.C. Luo (UC Davis Plant Sciences) for providing the wheat-*Th. elonatum* addition line 7EL. **Funding:** This work was supported by the National Key Research and Development Program of China (2016YFD0102001). **Author contributions:** F.H. designed the project. X.G., M.W. and J.Y. conducted the experiment and performed data analysis. F.H., Q.S., C.W., L.W., J.W., J.Z., H.S., Y.L. and S.F. conducted the field work. K.W. performed the wheat genetic transformation. X.G., J.Y. and F.H. wrote the paper. **Competing interests:** The authors declare no competing interests. **Data and materials availability:** All data are available in the manuscript, the supplementary materials or at the publicly accessible repositories. These data in the public repositories include all transcriptomic raw reads for the translocation line Zhongke 1878 in NCBI under BioProjectID (under submission). All materials were available from Fangpu Han.

339 **Supplementary Materials:**

340 Materials and Methods

341 Figures S1-S9

342 Data S1-S2

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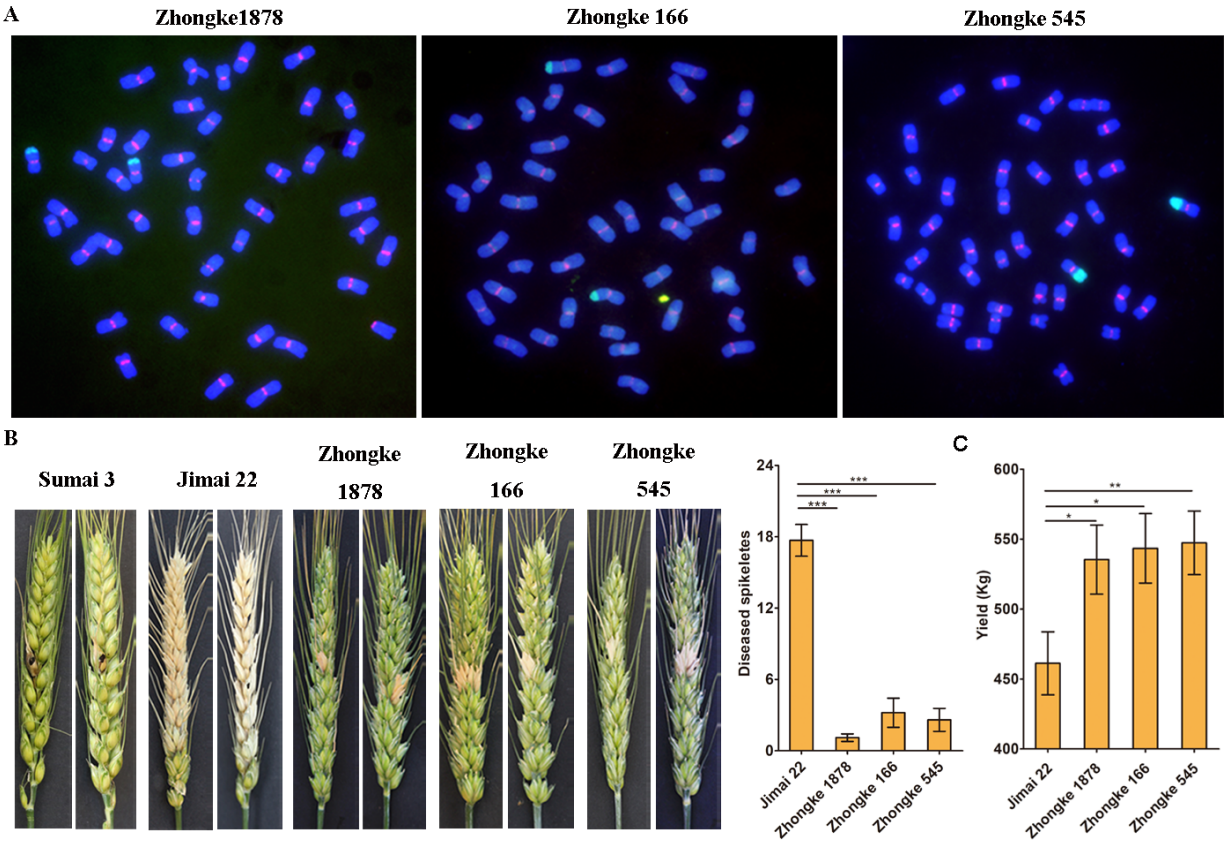

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347 **Fig. 1. Three wheat-*Th. elongatum* translocation lines with FHB resistance and yield**
348 **advantage.** (A) FISH analysis of the translocation lines Zhongke 1878, Zhongke 166 and Zhongke
349 545. (B) FHB resistance evaluation at 21 d after inoculation in field conditions. Sumai 3 was used
350 as FHB resistant control and Jimai 22 as susceptible control. (C) Grain yield comparisons between
351 translocation lines and the recurrent parent Jimai 22 after spraying *Fusarium graminearum*. The
352 grain yield was measured from a 13.3 m^2 plot in field.

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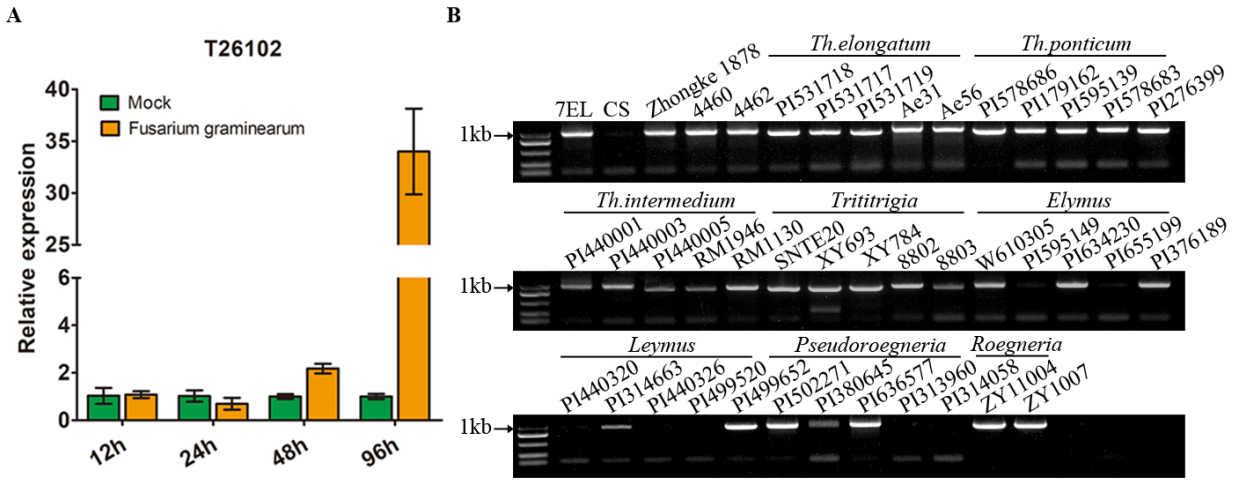

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355 **Fig. 2. Expression pattern and distribution of T26102.** (A) Expression pattern of T26102 after

356 inoculating with *Fusarium graminearum*. (B) Detection of T26102 among different species in

357 Triticeae.

358

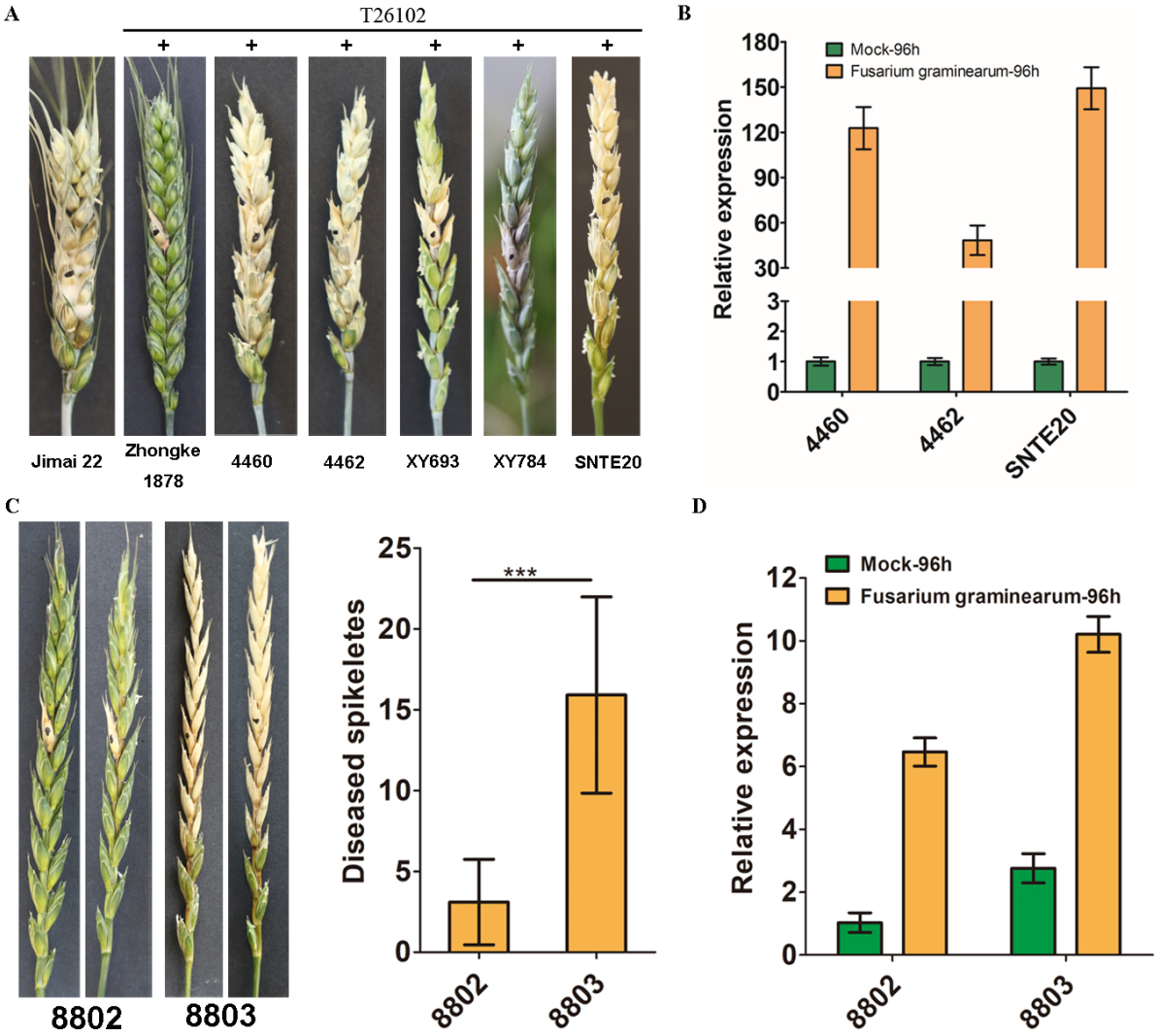
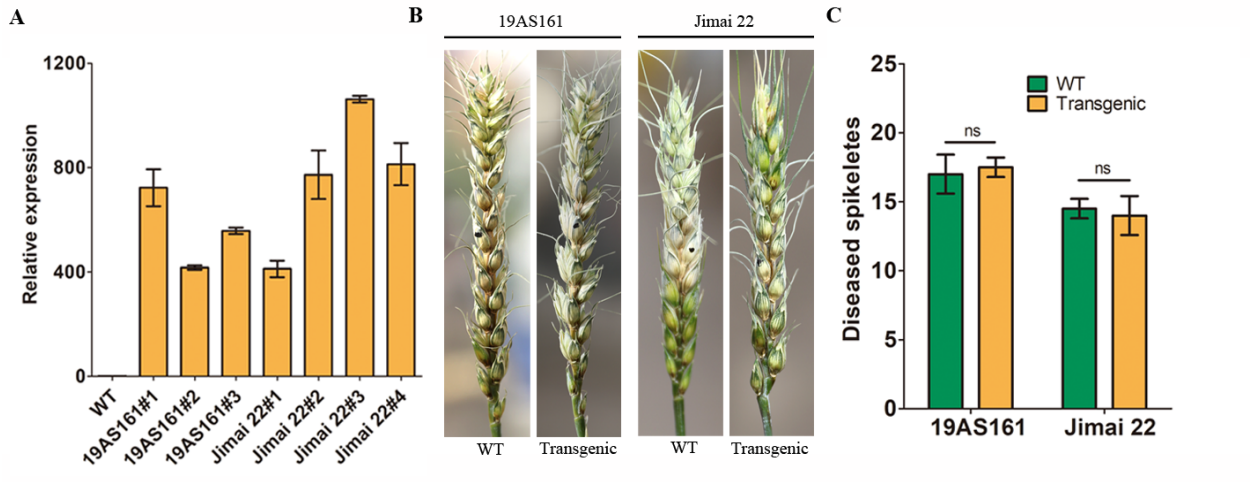


Fig. 3. FHB resistance evaluation and expression analysis of wheat-*Thinopyrum* derivative carrying T26102. (A) FHB resistance comparison among translocation lines and octaploid partial amphiploids. The resistance was evaluated at 10 d after inoculation with *Fusarium graminearum*. Zhongke 1878, wheat-*Th. elongatum* translocation line; 4460 and 4462, wheat-*Th. ponticum* translocation lines; XY693, XY784 and SNTE20, octaploid partial amphiploids derived from *Th. ponticum*. (B) Expression comparison of T26102 among 4460, 4462 and SNTE20. (C) FHB resistance comparison between hexaploid partial amphiploids 8802 and 8803. The resistance was evaluated at 21 d after inoculation with *Fusarium graminearum*. (D) Expression comparison of T26102 between 8802 and 8803.



369

370 **Fig. 4. Expression analysis and FHB resistance evaluation of T26102 transgenic lines.** (A)

371 Expression analysis of transgenic lines with T26102. (B) FHB resistance comparison between wild

372 type and T_0 transgenic lines.

373 **Table 1. Four types of translocation lines identified from 8400 seeds.**

Translocation type	Number	Percentage (%)
Short alien segmental translocation line	184	27.42
Medium alien segmental translocation line	141	21.01
Long alien segmental translocation line	247	36.81
Intercalary translocation line	99	14.76
In total	671	100

374

375 **Table 2. The transcripts specific for disease-resistant interval in line Zhongke 1878**

Transcripts	Length (bp)	Function annotation
T255	5439	CC-NBS-LRR protein
T1375	4353	CC-NBS-LRR protein
T2475	4015	unknown
T2921	3963	NB-ARC domain
T4619	3607	unknown
T6683	3480	unknown
T6981	3503	CC-NBS-LRR protein
T7667	3443	CC-NBS-LRR protein
T8538	3325	unknown
T9007	3358	CC-NBS-LRR protein
T10410	3204	unknown
T11006	3228	CC-NB-ARC domain
T14242	2493	Retrotransponson protein
T15795	2233	Lectin receptor kinase
T19432	1873	DNA repair exonuclease SbcCD ATPase
T19609	1861	unknown
T20934	1769	Retrotransponson protein
T26102	1394	GST protein
T26788	1389	Dirigent-Jacalin protein
T28749	1249	Nucleosome assembly protein
T30581	1098	unknown
T32029	996	unknown
T32111	993	unknown
T32749	934	Freezing-induced protein
T33754	844	unknown

376

Materials and Methods:

Induction and improvement of wheat-*Th. elongatum* translocation lines

At flowering stage, the spikes of wheat-*Th. elongatum* addition line 7EL were cut from the plant in the morning, and immediately radiated by γ rays derived from ^{60}Co with a dose of 18 Gy. Then the fresh pollens were pollinated to Jimai 22 with the stamens removed in advance. The hybrid seeds were harvested at maturity. The translocation lines were identified by utilizing fluorescence *in situ* hybridization (FISH). The translocation lines in which the ratio of the length of the alien fragment to the full length of 7EL ranged from 0 to 1/4 were considered to be short alien segmental types. The translocation lines in which the ratio ranged from 1/4 to 1/2 or from 1/2 to 1 were classified as medium or long alien segmental types. Using Jimai 22 as the recurrent parent, the integrated agronomic traits of the translocation lines were improved by continuous backcrossing.

Fluorescence *in situ* hybridization (FISH)

The translocation lines were screened by FISH according to previously reported methods (*25*). The seeds harvested were germinated on moist filter paper in a petri dish at room temperature for 2-3 d. The roots were cut from the seedlings and then placed in nitrous oxide for 2h. Subsequently the roots were fixed in 90% acetic acid for 5 minutes and then washed three times by sterile water. Chromosome spreads preparation was performed as previously described (*28*). 7EL-1 was obtained by Dop-PCR from the 7EL library constructed by chromosome microdissection. It was specific for the genome of *Th. elongatum* and *Th. ponticum*. The probes were labeled using the nick translation method (*29*). Two repetitive sequences pAs1 and pSc119.2 were used to identify the whole set of wheat chromosome. 7EL-1 and pSc119.2 were labelled with Alexa Fluor-488-5-dUTP. The centromeric retrotransposon of wheat clone 6C6 and pAs1 were labeled with Texas-red-5-dCTP.

403 **Fusarium head blight resistance evaluation**

404 FHB evaluations were performed by using the single spikelet inoculation method (*30*). Equally
405 mixing three pathogenic *F. graminearum* strains (Fg16-2, Fg16-5 and Fg16-11) and one *Fusarium*
406 *asiaticum* strain (Fa301) in Mung bean broth produced fungal spores. For convenience, we referred
407 to the four mixed species as *Fusarium graminearum*. Approximately 20 μL of *F. graminearum*
408 fungal suspension (1×10^6 conidia/ml) was injected into the central spikelet at early flowering stage.
409 The inoculated spikes were covered with a plastic bag for 2 d to keep moist for fungal infection.
410 The percentage of diseased spikelets was calculated at 10 or 21 d after inoculation.
411
412 **RNA sequencing and screening transcripts induced by *Fusarium graminearum***
413 To explore the resistance gene for Fusarium head blight, the spikes of the translocation line
414 Zhongke 1878 were sampled for RNA sequencing after inoculating with *Fusarium graminearum*.
415 Three spikelets around the inoculated one from at least three spikes of different plants were
416 collected at 12, 24, 48, and 96h post inoculation and grounded in liquid nitrogen for total RNA
417 extraction using TRIzol® Reagent (Invitrogen). As lesions were observed on the glumes at 96h
418 post inoculation, the sample at 96h was selected for full-length transcriptome sequencing. Firstly,
419 we aligned the sequenced transcripts on the Chinese Spring reference genome by using the
420 software GMAP (with parameters: -min-trimmed-coverage 0.9 -min-identity 0.85). To remove the
421 transcripts derived from the inoculated *Fusarium graminearum* isolates, the unmapped transcripts
422 were blasted against the nucleotide database on the National Center for Biotechnology Information
423 (NCBI). In order to confirm their origin, one to three pairs of primers were designed for the
424 transcripts left. Polymerase chain reactions were carried out using the genome DNA of wheat-*Th.*
425 *elongatum* 7EL, Chinese Spring, Zhongke 1878 and Jimai 22. The functions of the transcripts
426 specific for Zhongke 1878 were annotated by using Blastx on the NCBI. In order to analysis their
427 expression patterns, RNA sequencing was conducted using samples collected at 12, 24, 48 and 96h
428 post inoculation. The data analysis was performed by employing HISAT2 and StringTie according

to the previously report (*31*). The FPKM value from StringTie was used to measure the expression level.

Distribution, expression analysis and genetic transformation of T26102

In order to detect the distribution of T26102 in different species in Triticeae, the fragment of T26102 was amplified by using the primer set of F-CGATAGAAGATAGCTTCAATCAACCCTTT and R- CTACTTCACCTCGGCATACTTGTC. The fragments amplified from different species were cloned onto the *pEASY*®-T1 simple cloning vector (TransGen Biotech Co, Beijing) for sequencing. The sequence comparison analysis was carried out using the software DNAMAN. First-strand cDNA synthesis from the total RNA was performed by using the FastKing RT kit (with gDNase) (TianGen Biotech Co, Beijing). The expression analyses were performed using the primer set (F-GGACTTCCCTTGGATCCTGC and R-ACCGACAATCATGTCCGCAT). The gene *actin* was used as an internal standard by the primer set of F-CAACGAGCTCCGTGTCGCA and R-GAGGAAGCGTGTATCCCTCATAG. The relative expression of T26102 was calculated by the $2^{-\Delta\Delta CT}$ method.

The 846 bp CDS of T26102 was amplified from the genomic DNA of the translocation line Zhongke1878. The CDS was cloned into the MCS of the modified pWMB110 vector under the *ubiquitin* promoter by using the EasyGeno Assembly Cloning kit (TianGen Biotech Co, Beijing). The recombinant plasmid was transformed into *Agrobacterium* strain C58C1 (Zoman Biotech Co, Beijing). The *Agrobacterium*-mediated wheat transformation using the immature embryos of 19AS161 and Jimai 22 was carried out as previously described (*32*). The positive T_0 plants expressing T26102 confirmed by RT-PCR were used for FHB resistance evaluation.

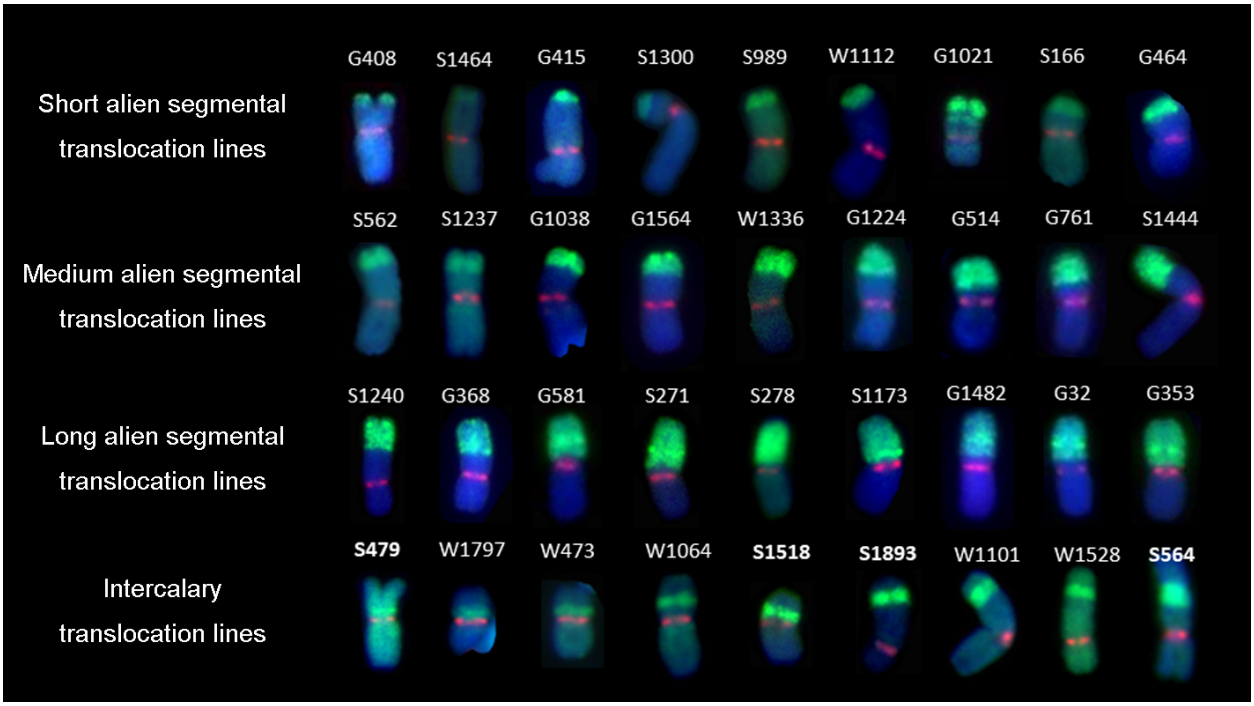


Fig. S1. Four types translocation lines identified by FISH. Each row represents one type, and the names of the translocation lines were placed at the top of the translocated chromosome.

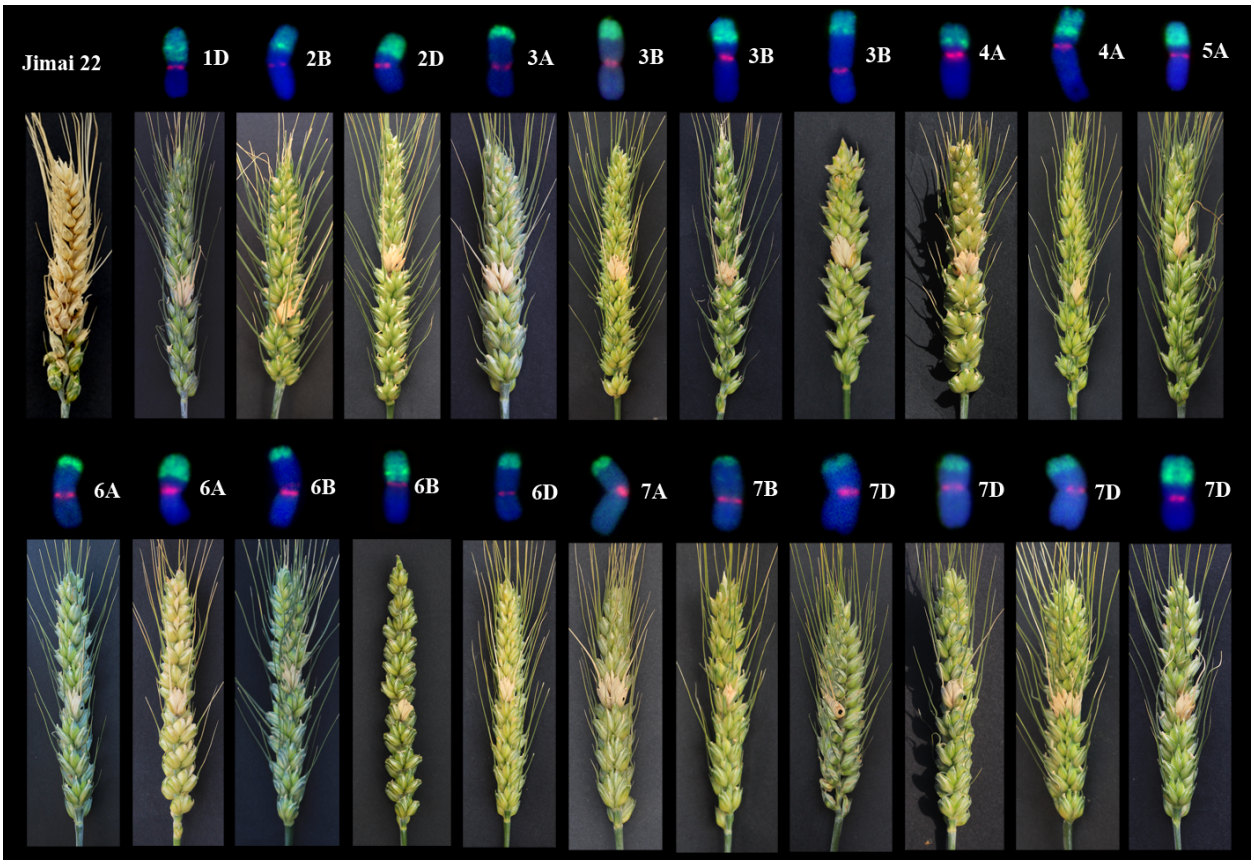
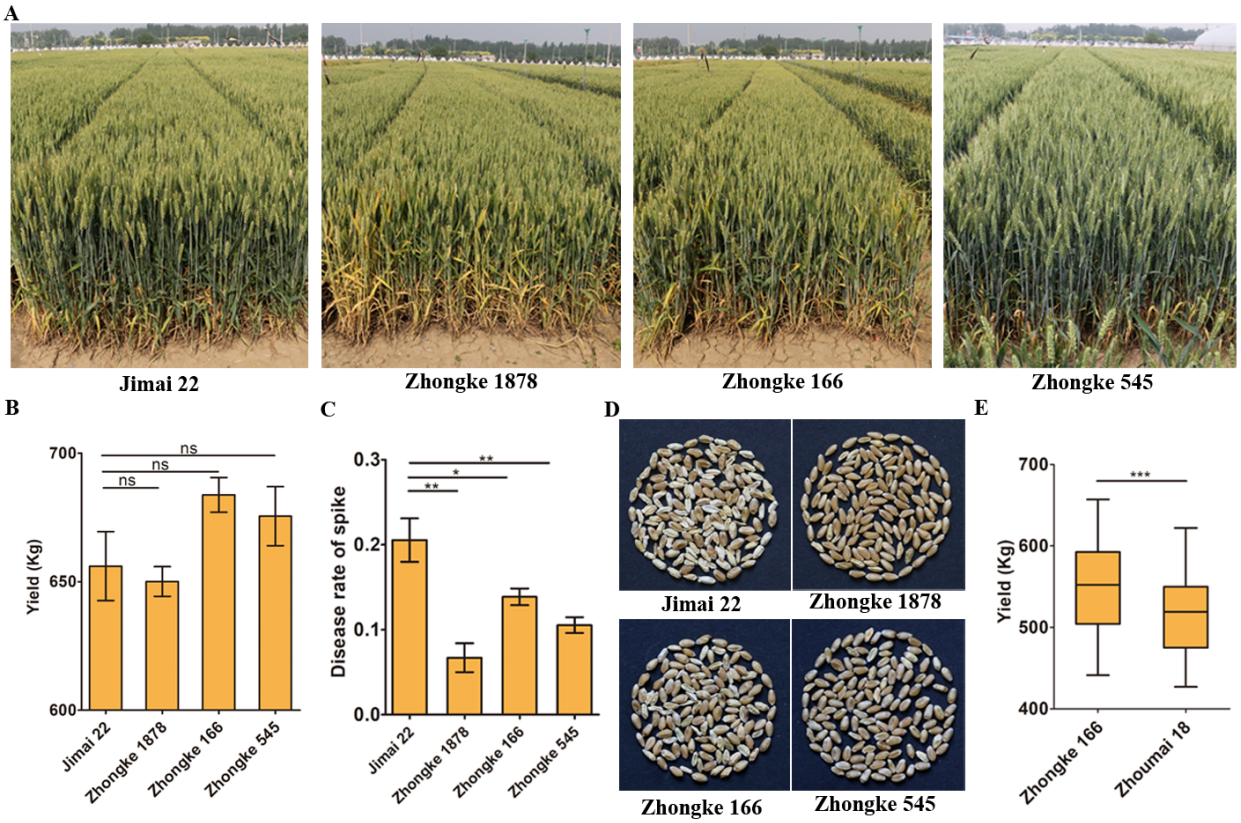


Fig. S2. FHB resistant translocation lines identified by single spikelet inoculation method. The FHB resistance was evaluated at 21 d after inoculation with *Fusarium graminearum* in field.



457

458 **Fig. S3. FHB resistant breeding application of wheat-*Th. elongatum* translocation lines.** (A)
459 Field plot performance of the translocation lines Zhongke 1878, Zhongke 166 and Zhongke 545.
460 (B) Grain yield of three translocation lines under natural conditions. The grain yield was measured
461 from a 13.3 m^2 plot. (C) The rate of diseased spikes under natural field conditions. (D) The seeds
462 of the translocation lines harvested under natural field conditions. (E) Yield comparison between
463 the translocation line Zhongke 166 and the national control Zhoumai 18. The yield data were
464 collected from the national wheat yield contest across 23 locations.

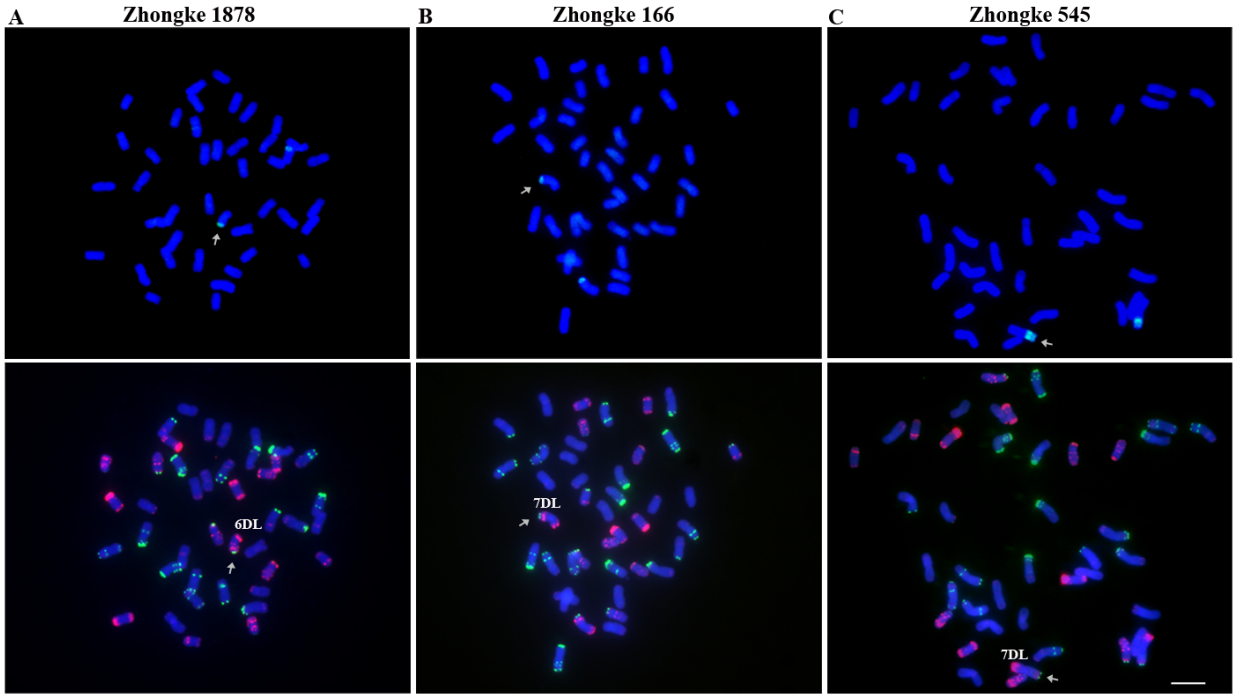


Fig. S4. Cytological analysis of the translocation lines. (A) Cytological analysis of Zhongke 1878. (B) Cytological analysis of Zhongke 166. (C) Cytological analysis of Zhongke 545. The translocation lines were identified by using the probe 7EL-1(three upper panels) and the translocated chromosomes were identified by using the probes pAs1 (red) and pSc119.2 (green) (three lower panel).

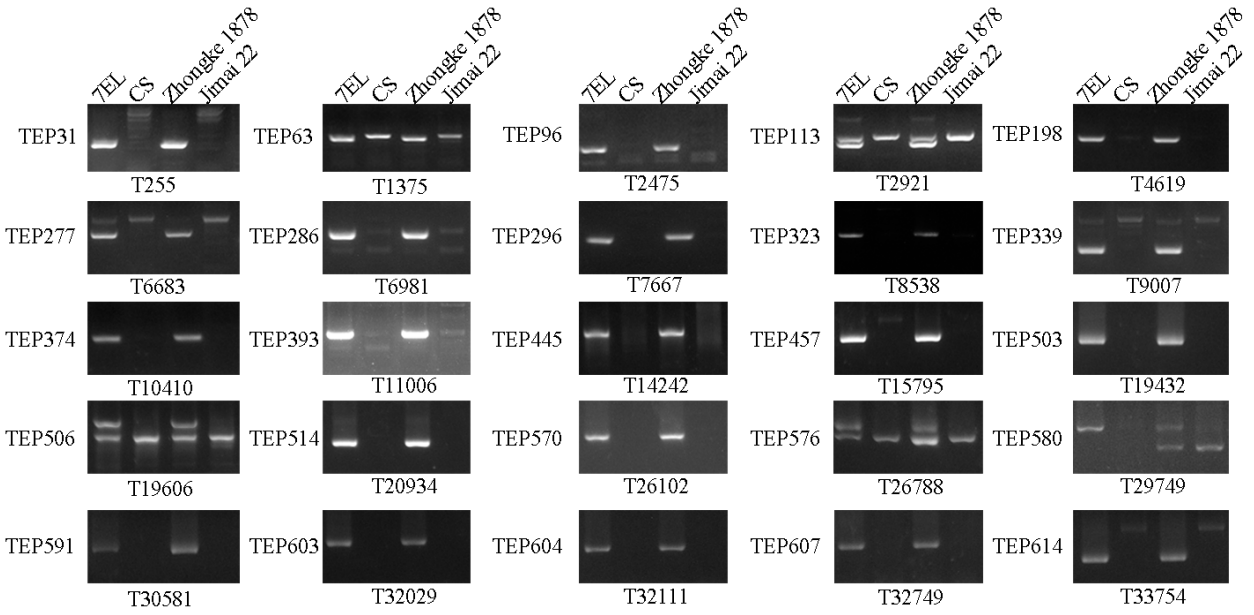


Fig. S5. The twenty-five specific alien transcripts identified by PCR. PCRs were performed by using the genomic DNA of the wheat-*Th. elongatum* addition line 7EL, Chinese Spring (CS), Zhongke 1878 and Jimai 22. The marker name was placed at the left of the electrophoretogram and the transcripts name placed at the bottom of the electrophoretogram.

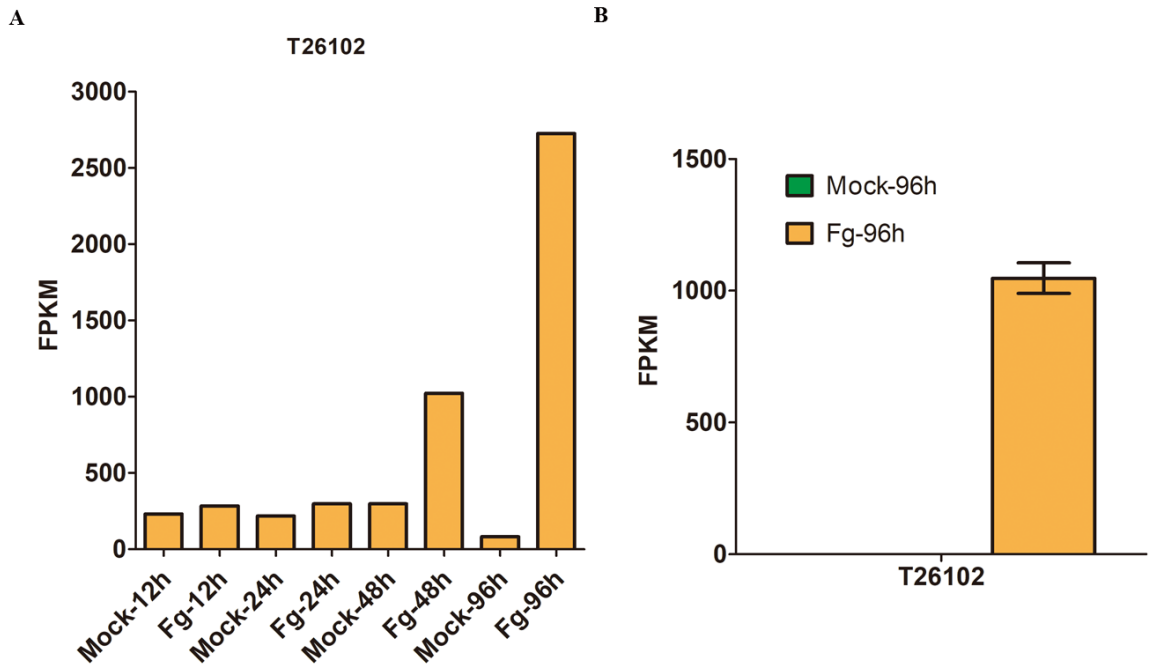


Fig. S6. Expression pattern of T26102 after inoculating with *Fusarium graminearum*. (A) Expression pattern of T26102 in Zhongke 1878 between Mock and *Fusarium graminearum* (Fg) treatment. (B) Expression analysis of T26102 in the addition line 7EL at 96h after Mock and Fg treatment.

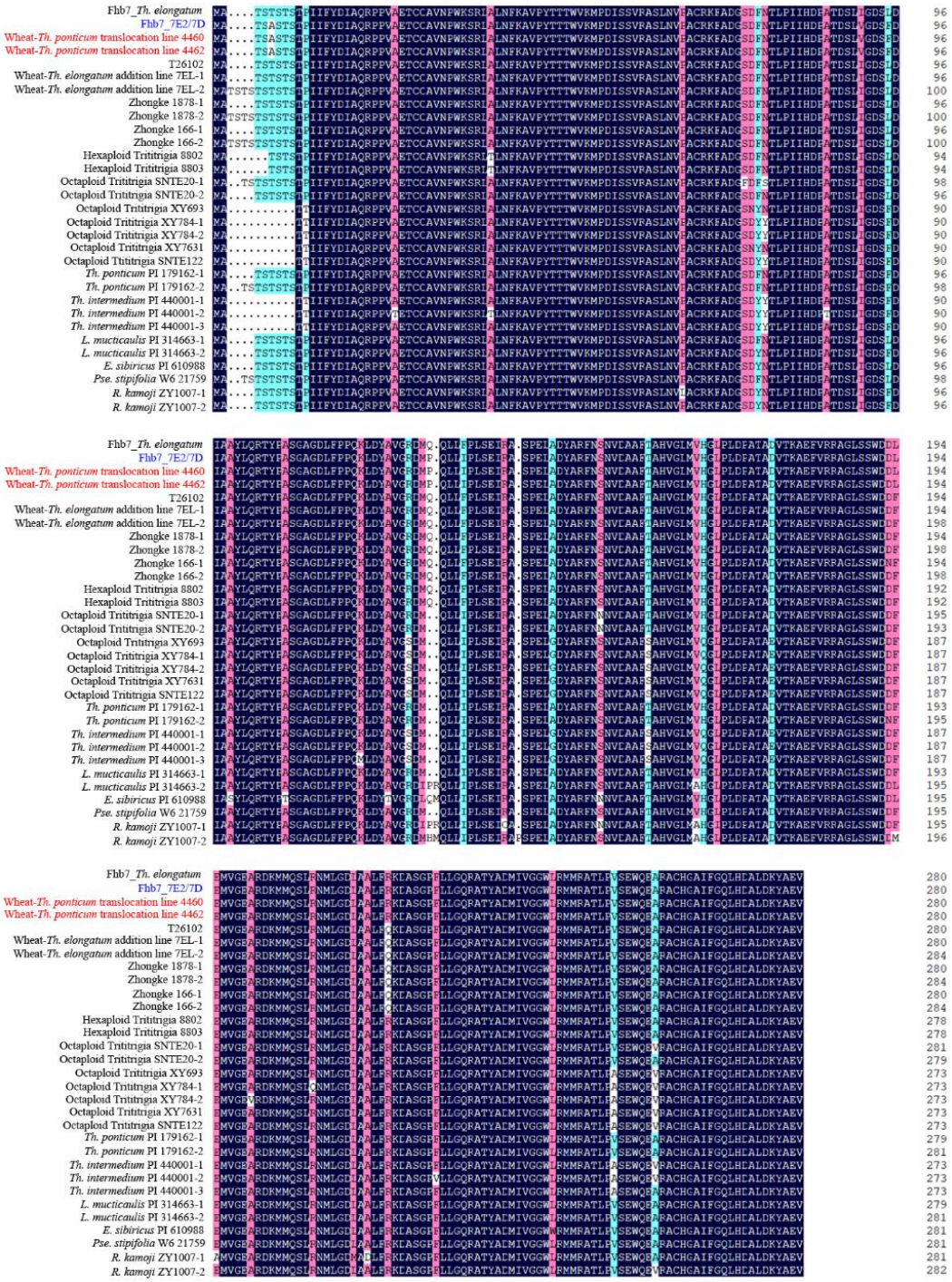


Fig. S7. Alignments of T26102 homologs in Triticeae. Protein sequences of T26102 homologs in genera *Thinopyrum*, *Elymus*, *Leymus*, *Pseudoroegneria* and *Roegneria* were aligned with the DNAMAN program. The protein sequences of T26102 homologs from wheat-*Th. ponticum* translocation lines 4460 and 4462 were identical to *Fhb7* in the 7E2/7D substitution line.

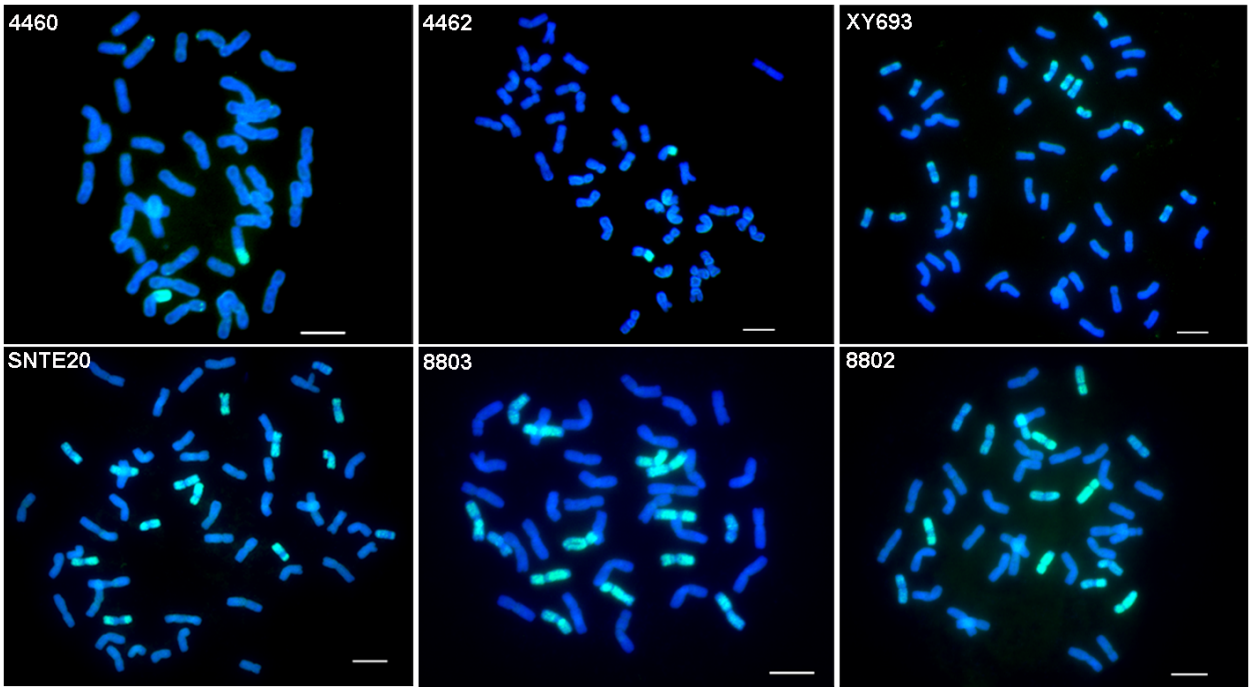


Fig. S8. Cytological analysis of wheat-*Thinopyrum* derivate carrying T26102. 4460 and 4462, wheat-*Th. ponticum* translocation lines; XY693 and SNTE20, octaploid partial amphiploids developed from *Th. ponticum*; 8802 and 8803, hexaploid partial amphiploids developed from *Th. elongatum* (2n=4x=28).

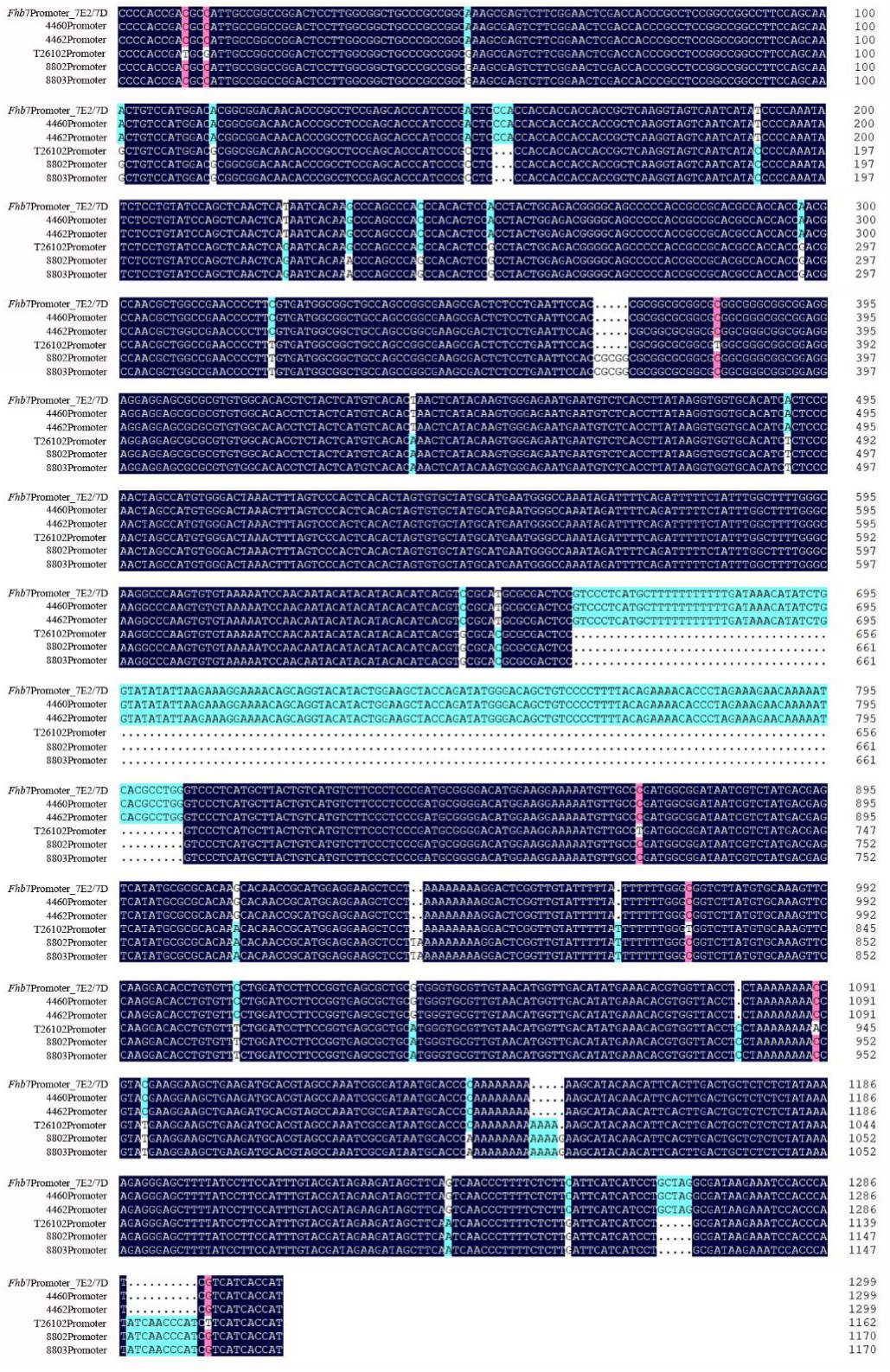


Fig. S9. Promoter sequence alignment of T26102 homologs.

Data S1. (separate file)

Distribution of the T26102 homologs in Triticeae.

Data S2. (separate file)

The sequences of twenty-five specific transcripts in line Zhongke 1878.