Brief Communication

Functional analysis of the glutathione S-transferases from *Thinopyrum* and its derivatives on wheat Fusarium head blight resistance

Xianrui Guo1,‡, Qinghua Shi1,§, Mian Wang1,†, Jing Yuan1, Jing Zhang1, Jing Wang1, Yang Liu1, Handong Su1,§, Zhen Wang2, Jinbang Li3, Cheng Liu3, Xingguo Ye4,§ and Fangpu Han1,†

1State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Innovation Academy for Seed Design, Chinese Academy of Sciences, Beijing, China
2Nanyang Academy of Agricultural Sciences, Nanyang, China
3Crop Research Institute, Shandong Academy of Agricultural Sciences, Jinan, China
4Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China

Received 20 August 2022; revised 6 November 2022; accepted 30 January 2023.

* Correspondence (Tel ++86 10 6480 7926; fax ++86 10 6485 4467; email fhan@genetics.ac.cn [F.H.]; Tel ++86 10 8210 5171; fax ++86 10 8210 9765; email yexingguo@caas.cn [X.Y.])
† These authors contributed equally to this work.
‡ These authors contributed equally to this work.
§ These authors contributed equally to this work.

Keywords: *Thinopyrum*, Fusarium head blight, Translocation line, Fhb7, glutathione S-transferases.

Fusarium head blight (FHB) is one of the devastating diseases for wheat production worldwide, which causes significant yield losses and reduces grain quality because of mycotoxins contamination in wheat grains. As wheat relatives, *Thinopyrum elongatum* and *Th. ponticum* are important genetic resources that can be used to improve wheat FHB resistance. Using recombinant inbred lines derived from a cross between two Thacher-*Th. ponticum* substitution lines, K11463 (7E1/7D) and K2620 (7E2/7D), the major FHB resistance locus *Fhb7* was mapped to the very distal region of the long arm of chromosome 7E2 (Guo et al., 2015). Wang et al. (2020) sequenced the genome of *Th. elongatum* and cloned the glutathione S-transferase-encoding *Fhb7* by genetic mapping. Relying on the recombination between *Th. elongatum* chromosome 7E and *Th. ponticum* chromosome 7E1, a resistant gene *Fhb-7EL* for FHB resistance was located to the long arm of 7E (Ceoloni et al., 2017).

To transfer the resistant gene *Fhb-7EL* to common wheat, hundreds of wheat-*Th. elongatum* translocation lines were developed by irradiating the pollen of the wheat-*Th. elongatum* addition line Chinese Spring (CS)-7EL at anthesis, among which Zhongke 1878 proved to carry an approximately 100 Mb 7EL chromosome on chromosome 6DL (Figure 1a, Figure S1, Appendix S1). After backcrossing Zhongke 1878 with the highly susceptible variety Jimai 22 for six generations, FHB resistance evaluation showed that the translocated chromosome could significantly increase the FHB resistance of Jimai 22 to the level of Sumai 3 by decreasing the number of diseased spikelets from 13.43 to 1.43 (Figure 1b,c).

To explore the nature of the FHB resistance gene, we inoculated the spikes of the line Zhongke 1878 with *F. graminearum* and performed single-molecule real-time isofrom sequencing after 96 h. Removing the transcripts derived from wheat and *Fusarium* species by blasting wheat reference genome and nucleotide database on NCBI, 25 transcripts were identified derived from alien chromatin by PCR in Zhongke 1878 (Figure S2, Tables S1 and S2). To study the mechanisms of FHB resistance in the line Zhongke 1878, next-generation sequencing-based transcriptomic analysis was performed on these 25 transcripts. Annotated as a GST protein, the expression of the transcript T26102 was significantly increased 48 h after inoculation with *F. graminearum* (Figure 1d,e, Table S1).

To illustrate the association between T26102 and FHB resistance, the distribution of T26102 was checked in a series of wheat-*Thinopyrum* derivatives. The homologue of T26102 was not only detected in wheat-*Th. ponticum* amphiploid SNTE20 but also in the wheat-*Th. ponticum* translocation lines 4460 and 4462 (Figure 1f, Figures S3, S4). After sequencing the amplified product, two different T26102 homologues were discovered in lines CS-7EL, Zhongke 1878 and SNTE20 respectively (Figure 1f, Figure S4). Although SNTE20, 4460 and 4462 were proven to carry the GST-encoding *Fhb7* homologues, all three lines were identified as susceptible to FHB as well as the susceptible control Jimai 22 (Figure 1g,h). Expression analysis revealed that *Fhb7* homologues were induced in lines 4460, 4462 and SNTE20 after inoculating with *F. graminearum* (Figure 1i). Similar results were also reported in the wheat-*Th. ponticum* partial amphiploid SNTE122 and translocation line TNT-B (Guo et al., 2022). More puzzling was that the Fhb7 homologue and its promoter shared by 4460 and 4462 were identical to the one in the wheat-*Th. ponticum* substitution line 7E2/7D used as the resistant parent to map Fhb7 (Figure 1f, Figures S4, S5). All these results casted our doubt on the FHB-resistant function of the GSTs.

To verify the FHB resistance function of T26102, we transformed the overexpression vector pUBiT26102 into three common wheat accessions 19AS161, Jimai 22 and Zhongmai 175. The transgenic positive wheat plants overexpressing T26102 were used for FHB resistance evaluation (Figure S6). A few bleached spikelets were observed on all spikes of both wild types and T0 transgenic plants 7 days after inoculation with *F. graminearum* (Figure 1j). Statistical analysis was performed between the wild type and the transgenic plants; no difference was discovered between them (Figure 1k). To rule out the effect of amino acid variation on the function of T26102, we also...
expressed the GST-encoding Fhb7 under the ubiquitin promoter and the same native promoter as reported by Wang et al. (2020) in common wheat varieties Zhengmai 7698 and Kenong 199. Regardless of the vector driven by the ubiquitin promoter or the native promoter, nearly half the inoculated spikes bleached in the Zhengmai 7698 transgenic plants expressing Fhb7 (Figure 1l). Except for the FHB evaluation on the T₀ generation, the T₁ transgenic plants on Kenong 199 background were chosen to verify the function of Fhb7. With obvious bleached spikelets on the inoculated spikes, no statistical difference in FHB resistance was discovered between the T₁ transgenic plants and the control Kenong 199 (Figure 1m,n). These results suggested the GST-
Figure 1  Functional analysis of the glutathione S-transferases on FHB resistance. (a) Cytological analysis on the line Zhongke 1878. Alien chromatin was detected by using the green probe 7EL-1. (b, c) FHB resistance evaluation on Zhongke 1878. The diseased spikes were photographed (b) and the number of diseased spikelets was calculated (c) at 21 days after inoculation with *Fusarium* species. (d, e) Expression pattern of T26102 in Zhongke 1878 measured by transcriptomic data (d) and qRT-PCR (e). (f) Sequence comparisons of *Fhb7* homologues among wheat- *Thinopyrum* derivatives. '1' and '2' indicate two different homologues in lines CS-7EL and SNTE20. (g, h) FHB resistance evaluation on wheat-*Thinopyrum* derivatives. The diseased spikes were photographed (g) and the number of diseased spikelets was calculated (h) at 21 days after inoculation with *Fusarium* species. (i) Expression analysis of T26102 96 h after inoculation with *Fusarium* species in wheat-*Thinopyrum* derivatives. (j-n) FHB resistance evaluation on the transgenic lines with *Fhb7* homologues. The diseased spikes were photographed and the number of diseased spikelets was calculated at 7 days after inoculation with *Fusarium* species. *pubi:* *Fhb7* indicated that *Fhb7* was driven by the *ubiquitin* promoter. *pHa:* *Fhb7* indicated that *Fhb7* was driven by the native promoter. (j, k) FHB resistance evaluation on the control (CK) and T0 transgenic lines overexpressing T26102. (l) FHB resistance evaluation on the T1 transgenic lines expressing *Fhb7* on the background Zhengmai 7698. (m, n) FHB resistance evaluation on the T1 transgenic lines expressing *Fhb7* on the background Kenong 199. ***P < 0.001, ns, P > 0.05.

encoding *Fhb7* also failed to confer wheat FHB resistance. All these results suggested that GSTs from *Thinopyrum*, including the GST-encoding *Fhb7* and its homologues, were not decisive for FHB resistance.

**Accession number**

All transcriptomic raw reads for the translocation line Zhongke 1878 are available from the NCBI BioProject under accession number PRJNA720120.

**Acknowledgements**

This work was supported by the National Key Research and Development Program of China (2016YFD0102001).

**Conflict of interest**

The authors declare no conflict of interest.

**Author contribution**

F. H. and X. Y. conceived the study. X. G., Q. S. and M. W. conducted the experiment. J. Y., Y. L., J. Z. and J. W. participated in vector construction. H. S. contributed bioinformatics analysis. Z. W., J. L. and C. L. helped FHB resistance evaluation. X. Y. contributed the transgene experiment. X. G. and F. H wrote the manuscript.

**References**


**Supporting information**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Appendix S1 Methods.**

**Figure S1** Coverage analysis on the Zhongke.

**Figure S2** Twenty-five specific alien transcripts identified by PCR in Zhongke 1878.

**Figure S3** Cytological analysis on wheat-*Thinopyrum* derivatives carrying *Fhb7* homologs.

**Figure S4** Protein sequence alignments of *Fhb7* homologs in wheat-*Thinopyrum*, derivatives.

**Figure S5** Promoter sequence alignment of *Fhb7* homologs.

**Figure S6** Expression analysis of *Fhb7* homolog in transgenic wheat plants.

**Table S1** The sequences and function annotation of twenty-five specific transcripts in line Zhongke 1878.

**Table S2** Primers used in this study.