Molecular cytogenetic characterization of a new wheat-Thinopyrum intermedium homoeologous group-6 chromosome disomic substitution line with resistance to leaf rust and stripe rust

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Thinopyrum intermedium (JJJJSStSt, 2n = 6x = 42), a member of tertiary gene pool of hexaploid wheat (Triticum aestivum L., AABBDD, 2n = 6x = 42), provides several beneficial genes for wheat improvement. In this study, line CH51 was developed from the BC1F8 progeny of a partial wheat-Th. intermedium amphiploid TAI8335 (2n = 56) and wheat cultivar (cv.) Jintai 170. Somatic metaphase chromosome counting showed that CH51 had stable 42 chromosomes. Genomic in situ hybridization (GISH) analysis showed that CH51 had 40 wheat chromosomes and two Th. intermedium chromosomes involving translocation between J-genome and St-genome chromosomes. Non-denaturing fluorescence in situ hybridization (ND-FISH) analysis revealed that CH51 lacked a pair of wheat chromosome 6B. Wheat 55K SNP array analysis verified that chromosome 6B had the highest percentage of missing SNP loci in both CH51 and Chinese Spring (CS) nullisomic 6B-tetrasomic 6D (CS-N6BT6D) and had the highest percentage of polymorphic SNP loci between CH51 and cv. Jintai 170. We identified that CH51 was a wheat-Th. intermedium T6StS.6J¶L (6B) disomic substitution line. Disease resistance assessment showed that CH51 exhibited high levels of resistance to the prevalent Chinese leaf rust and stripe rust races in the field. Therefore, the newly developed line CH51 can be utilized as a potential germplasm in wheat disease resistance breeding.

KEYWORDS
Thinopyrum intermedium, GISH, FISH, wheat 55K SNP array, disease resistance
Introduction

Hexaploid wheat (Triticum aestivum L., AABBDD, 2n = 6x = 42) is one of the most essential cereal crops around the world and provides the major food source for 30% of the global population [International Wheat Genome Sequencing Consortium (IWGSC), 2014]. Wheat diseases such as rusts, powdery mildew, and Fusarium head blight (FHB), however, have always been major threats to wheat production in almost all the wheat growing countries. Stripe rust, caused by Puccinia striiformis Westend. f. sp. tritici (Pst), may cause losses up to 70% and even higher (Rolfs et al., 1992; Wellings, 2011). Leaf rust, caused by P. triticina Eriks (Pt), is another devastating foliar disease, and can also cause severe yield reduction (McIntosh et al., 1995). Developing resistant cultivars is regarded as the most economical and effective means to control diseases. Nevertheless, because of a limited number of effective resistance genes in cultivated wheat and constantly evolving new virulent pathotypes capable of overcoming existing resistance genes in the pathogens, there is an urgent requirement to explore and utilize new resistant resources.

Thinopyrum intermedium (Host) Barkworth and D.R. Dewey (4n = 6x = 42), a perennial wild relative of hexaploid wheat, possesses many resistance genes that are similar to those that are resistant to stem rust, stripe rust, leaf rust, and powdery mildew pathogens (Li and Wang, 2009). Due to its high crossability with hexaploid wheat, several resistance genes have been incorporated into wheat (Li et al., 2019a). Up to now, a total of 81 leaf rust (Xu et al., 2022) and 83 stripe rust resistance genes (Li et al., 2020) have been officially named in wheat, respectively, but only Lr38 (Friebe et al., 1993) and Yr50 (Li et al., 2013) were reported from Th. intermedium. Therefore, it is of great value to explore new Th. intermedium genetic resources for broadening its application in wheat biotic resistance breeding.

Substitution lines between wheat and wild relatives are regarded as the optimal bridging materials for transferring beneficial genes from wild species to cultivated wheat (Liu and Wang, 2005). Compared with addition lines, substitution lines are cytogenetically more stable (Li et al., 2019b) and preferable to produce wheat-alien translocation lines by crossing with the high pairing ph1b mutant (Zhang et al., 2017). Chang et al. (2010) reported that wheat-Th. intermedium partial amphiploid TAI8335 was highly resistant to leaf rust, stem rust, stripe rust, and powdery mildew. Later, a wheat-Th. intermedium diosomic substitution line CH51 was selected from the BC1F8 progeny of TAI8335 and common wheat cultivar (cv.) Jintai 170. In this study, we newly developed line CH51 to stripe rust, leaf rust, FHB, and powdery mildew.

Materials and methods

Plant materials

The materials used in this study included common wheat Chinese Spring (CS), Jinchun 5, Jinmai 33, Jintai 170, Mingxian 169, Nanda 2419, Taichung 29, Sumai 3, Alondra’s, CS nullisomic-tetrasomic lines (CS-N6AT6D, CS-N6BT6D, and CS-N6DT6B), Th. intermedium (unknown origin), a partial wheat-Th. intermedium amphiploid TAI8335 (2n = 8x = 56), and its derived line CH51. TAI8335 was developed from BC1F8 progenies of the cross of Jinchun 5/Th. intermedium/Jinmai 33 (Chang et al., 2010). CH51 was selected from BC1F8 progenies of the cross of Jintai 170/ThAI8335/Jintai 170. CS and Taichung 29 were kindly provided by Dr. Zujun Yang, University of Electronic Science and Technology of China, Chengdu, Sichuan, China. Sumai 3 and Alondra’s were kindly provided by Dr. Xiue Wang, Nanjing Agricultural University, Nanjing, Jiangsu, China. All materials are maintained at Shanxi Province Key Laboratory of Crop Genetics and Gene Improvement, College of Agronomy, Shanxi Agricultural University, Taiyuan, Shanxi, China.

Genomic in situ hybridization analysis

Mitotic metaphase chromosomes of CH51 were analyzed by GISH according to the protocols in Zhang et al. (2001). Mitotic metaphase chromosomes were obtained from root tips and were spread according to the procedures in Lang et al. (2018). Total genomic DNA from Pseudorogneria spicata was used as a probe and labeled with fluorescein-12-dUTP (yellow-green fluorescence) (Enzo Life Sciences Inc., Farmingdale, NY, United States) using nick translation method. Sheared genomic DNA from CS was used as blocking DNA. Chromosomes were counterstained with propidium iodide (PI), and fluoresced red. GISH images were captured with an epifluorescence Zeiss Axioplan 2 microscope equipped with a SPOT 2.1 CCD camera (Diagnostic Instruments, Sterling Heights, MI, United States).

Non-denaturing fluorescence in situ hybridization analysis

Mitotic metaphase chromosomes of CH51 were further analyzed by ND-FISH according to the procedure of Fu et al. (2015). The oligonucleotide probes Oligo-psc119.2 and Oligo-pTa535 were used to identify wheat chromosomes according to the description by Tang et al. (2014). Probe
Oligo-pSc119.2 was 5′-end labeled with 6-carboxyfluorescein (6-FAM) generating green signals, and probe Oligo-pTa535 were labeled with 6-carboxytetramethylrhodamine (TAMRA) generating red signals (Shanghai Invitrogen Biotechnology Co., Ltd., Shanghai, China). Chromosomes were counterstained with 4,6-diamidino-2-phenylindole (DAPI) in Vectashield mounting medium (Vector Laboratories, Burlingame, CA, United States). FISH images were captured with an Olympus BX-51 microscope equipped with a DP-70 CCD camera (Shinjuku, Tokyo, Japan).

Wheat 55K SNP array analysis

Total genomic DNA of CH51, Jintai 170, CS-N6AT6D, CS-N6BT6D, and CS-N6DT6B were extracted using the CTAB method (Chen et al., 2004), and were genotyped on the wheat 55K SNP genotyping arrays (China Golden Marker Biotechnology Company, Beijing, China). There are 53,007 microchip probes per chip, including many diploid markers. Based on CS reference genome sequence IWGSC_RefSeq_v1.0, a total of 49,060 SNP marker loci had precise physical location information, evenly covering the entire wheat genome. Percentages of the same, polymorphic, or missing SNP loci in each chromosome in CH51, Jintai 170, CS-N6AT6D, CS-N6BT6D, and CS-N6DT6B were obtained by calculating the rate of the same, polymorphic, or missing SNP genotype loci number in total number of SNP loci. Microsoft Excel 2019 (Microsoft, Redmond, WA, United States) was used for data analysis and graphing.

Disease response evaluation

During the two wheat-growing seasons in 2018–2020, all materials were sown in a randomized complete block design with three replicates for evaluating their responses to stripe rust, leaf rust, powdery mildew, and Fusarium head blight (FHB) at the heading stage. Fifteen seeds of each line were sown in 1.5 m rows, spaced 0.25 m apart. Stripe rust was tested at Xindu Experiment Station, Sichuan Academy of Sciences, Chengdu, Sichuan, China. Leaf rust, powdery mildew, and FHB were tested at the Experimental Farm of Shanxi Agricultural University, Jinzhong, Shanxi, China.

Stripe rust responses of Jinchun 5, Jinmai 33, Jintai 170, Th. intermedium, TAI8335, CH51, and Taichung 29 were inoculated with a mixture of Pst races CYR32, CYR33, and CYR34 (1:1:1 ratio) provided by the Institute of Plant Protection, Gansu Academy of Agricultural Sciences, Lanzhou, Gansu, China. Artificial inoculations were carried out by dusting spores onto the leaves. Wheat cv. Taichung 29 was used as the susceptible control. When spores were fully developed on Taichung 29, infection types (ITs) were recorded based on a 0–4 scale, where 0, 0, 1, 2, 3, and 4 indicated immune, highly resistant, resistant, moderately resistant, moderately susceptible, and susceptible, respectively (McIntosh et al., 1995).

Leaf rust reactions of all tested materials were recorded after being inoculated with a mixture of prevalent Pt races TRT, TRJ, and KHJ (1:1:1 ratio), which were collected from wheat-growing areas in northern China (Sheng et al., 2022). Inoculation method was according to Sheng et al. (2022). Wheat cv. Nanda 2419 was used as the susceptible control. ITs were recorded as 0–4 scale according to McIntosh et al. (1995).

Powdery mildew responses of all tested materials were evaluated after being inoculated with Blumeria graminis f. sp. tritici (Bgt) race E09 provided by the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China. Inoculations were carried out as described by Xiang et al. (1994). When conidia were spread across the susceptible control Mingxian 169, ITs were recorded on a 0–9 scale, where 0, 0–1, 2–3, 4–5, 6–7, and 7–9 indicated immune, near immune, highly resistant, moderately resistant, moderately susceptible, and highly susceptible, respectively (Sheng and Duan, 1991).

Fusarium head blight (FHB) reactions of all tested materials were recorded after being inoculated with Fusarium pathotype F0609 provided by Dr. Xiue Wang, Nanjing Agricultural University, Nanjing, Jiangsu, China. Plants were inoculated as described by Bai et al. (1999) and Zhang et al. (2020) when a spike was just beginning to flower. Sumai 3 was used as the resistant control, and Alondra's was used as the susceptible control. Disease severity was recorded 27 days post inoculation according to McIntosh et al. (1995).

Results

Cytological characterization of CH51 using genomic in situ hybridization and fluorescence in situ hybridization analyses

Wheat-Th. intermedium derived line CH51 was selected from the BC$_1$F$_8$ progeny of the cross of Jintai 170/TAI8335//Jintai 170. A total of 30 CH51 seeds were germinated for chromosome counting. The result showed that the somatic metaphase chromosome number of all 30 seeds are 2n = 42, confirming its cytogenetic stability.

Genomic in situ hybridization (GISH) analysis using Ps. spicata genomic DNA as a probe showed that CH51 had 40 wheat chromosomes and two Th. intermedium chromosomes displaying stronger hybridization signals along the entire short arm and at the telomeric region of the long arm.

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1. http://wheat-urgi.versailles.inra.fr/Seq-Repository/
According to Chen et al. (1999), GISH using the diploid progenitor *Ps. spicata* as a probe could label the entire length of St-genome chromosomes, the pericentromeric and telomeric regions of J*-genome chromosomes, and the telomeres of J*-genome chromosomes, indicating that the *Thinopyrum* chromosome in CH51 involved translocation between J*- and St-genome chromosomes. Sequential ND-FISH with probes Oligo-pSc119.2 and Oligo-pTa535 revealed that CH51 had 40 wheat chromosomes and a pair of unknown chromosomes, which substitute for the wheat chromosome 6B (Figure 1B). Therefore, we concluded that CH51 is a wheat-*Th. intermedium* T6StS.JsL (6B) disomic substitution line.

### Wheat 55K SNP array analysis

Based on the reference genome sequence of CS, a total of 49,060 SNP loci having precise physical location information were used in the wheat SNP array analysis. Among them, a total of 46,380 and 48,288 valid SNP loci were identified in CH51 and Jintai 170, respectively (Table 1). A total of 41,186 SNP loci were common between CH51 and Jintai 170. As shown in Figure 2, chromosome 6B shared the minimum percentage of the same SNP loci (9.58%) between CH51 and Jintai 170, whereas other chromosomes shared much higher percentages of the same SNP loci ranging from 63.89% (on 6D) to 98.40% (on 4B). A total of 7,403 SNP loci were polymorphic between CH51 and Jintai 170. As shown in Figure 2, chromosome 6B had the highest percentage of polymorphic SNP loci (88.18%) between CH51 and Jintai 170, whereas other chromosomes had lower percentages of polymorphic SNP loci ranging from 1.05% (on 7B) to 35.94% (on 6D). In addition, a total of 471 SNP loci (0.96%) were simultaneously missing in both CH51 and Jintai 170, which could not be used in the statistical analysis (Table 1). The result indicated that wheat chromosome 6B in CH51 was substituted by a pair of homoeologous group-6 chromosome from *Th. intermedium*.

To verify whether wheat chromosome 6B was absent in CH51, CS-N6BT6D, CS-N6AT6D, and CS-N6DT6B were also included in genotyping with wheat 55K SNP genotyping arrays. As shown in Supplementary Table 1, chromosome 6B in CH51 had the highest percentage of missing SNP loci of 62.82%, whereas other chromosomes had much lower percentages, ranging from 0.86% (4B) to 6.77% (6D). For CS-N6BT6D, because it lacks the wheat chromosome 6B, we speculated that it should have a highest percentage of missing SNP loci on chromosome 6B, which was confirmed by SNP array analysis that chromosome 6B in CS-N6BT6D had the highest percentage (66.98%) of missing SNP loci (Supplementary Table 1). Combined with FISH-GISH results, it was demonstrated that CH51 was a wheat-*Th. intermedium* T6StS.JsL (6B) disomic substitution line.

### Assessment of responses to leaf rust, stripe rust, powdery mildew, and *Fusarium* head blight

At the heading stage, responses to stripe rust, leaf rust, powdery mildew, and FHB were recorded in Table 2. For stripe...
TABLE 1  SNP genotyping data obtained using wheat 55K SNP arrays for CH51 and wheat parent Jintai 170.

| Chromosome | No. of markers | No. of valid markers in CH51 | No. of valid markers in Jintai 170 | No. of same markers | Percentage of same markers | No. of polymorphic markers | Percentage of polymorphic markers | No. of simultaneous missing markers | Percentage of simultaneous missing markers |
|------------|----------------|-----------------------------|-----------------------------------|---------------------|---------------------------|---------------------------|----------------------------------|-------------------------------------|----------------------------------------|
| 1A         | 2625           | 2581                        | 2587                              | 2543                | 96.88%                    | 57                        | 2.17%                            | 25                                  | 0.95%                                  |
| 1B         | 2595           | 2556                        | 2562                              | 1728                | 66.59%                    | 859                       | 33.10%                           | 8                                   | 0.31%                                  |
| 1D         | 2138           | 2107                        | 2108                              | 1999                | 93.50%                    | 129                       | 6.03%                            | 10                                  | 0.47%                                  |
| 2A         | 2622           | 2585                        | 2599                              | 2394                | 91.30%                    | 219                       | 8.35%                            | 9                                   | 0.35%                                  |
| 2B         | 2600           | 2486                        | 2499                              | 2467                | 94.88%                    | 42                        | 1.62%                            | 91                                  | 3.50%                                  |
| 2D         | 2247           | 2173                        | 2177                              | 2115                | 94.13%                    | 72                        | 3.20%                            | 60                                  | 2.67%                                  |
| 3A         | 2174           | 2130                        | 2140                              | 1878                | 86.38%                    | 284                       | 13.07%                           | 12                                  | 0.55%                                  |
| 3B         | 2595           | 2547                        | 2559                              | 1974                | 76.07%                    | 598                       | 23.04%                           | 23                                  | 0.89%                                  |
| 3D         | 1693           | 1600                        | 1679                              | 1495                | 88.30%                    | 194                       | 11.46%                           | 4                                   | 0.24%                                  |
| 4A         | 2592           | 2569                        | 2567                              | 2460                | 94.91%                    | 123                       | 4.75%                            | 9                                   | 0.34%                                  |
| 4B         | 2556           | 2534                        | 2541                              | 2515                | 98.40%                    | 32                        | 1.25%                            | 9                                   | 0.35%                                  |
| 4D         | 1420           | 1403                        | 1410                              | 1350                | 95.07%                    | 65                        | 4.58%                            | 5                                   | 0.35%                                  |
| 5A         | 2611           | 2580                        | 2587                              | 2193                | 83.99%                    | 409                       | 15.66%                           | 9                                   | 0.35%                                  |
| 5B         | 2586           | 2543                        | 2548                              | 2519                | 97.41%                    | 38                        | 1.47%                            | 29                                  | 1.12%                                  |
| 5D         | 1737           | 1716                        | 1717                              | 1483                | 85.38%                    | 242                       | 13.93%                           | 12                                  | 0.69%                                  |
| 6A         | 2623           | 2519                        | 2573                              | 2279                | 86.89%                    | 324                       | 12.35%                           | 20                                  | 0.76%                                  |
| 6B         | 2547           | 947                         | 2478                              | 244                 | 9.58%                     | 2246                      | 88.18%                           | 57                                  | 2.24%                                  |
| 6D         | 1728           | 1681                        | 1690                              | 1104                | 63.89%                    | 621                       | 35.94%                           | 3                                   | 0.17%                                  |
| 7A         | 2579           | 2530                        | 2533                              | 2280                | 88.41%                    | 266                       | 10.31%                           | 33                                  | 1.28%                                  |
| 7B         | 2487           | 2444                        | 2441                              | 2425                | 97.51%                    | 26                        | 1.05%                            | 36                                  | 1.44%                                  |
| 7D         | 2305           | 2149                        | 2293                              | 1741                | 75.53%                    | 557                       | 24.16%                           | 7                                   | 0.31%                                  |
| Total      | 49060          | 46380                       | 48288                             | 41186               | 83.95%                    | 7403                      | 15.09%                           | 471                                 | 0.96%                                  |
The homoeologous group-6 chromosomes of wild relatives of common wheat carry many desirable genes, such as higher micronutrient contents in grain and resistance to stripe rust, leaf rust, and powdery mildew. For example, Ardalani et al. (2016) reported that wheat-Th. bessarabicum substitution line DS66 (6D) and translocation line T6E3S.6DL had higher iron and zinc contents than the recipient wheat cv. "Roushan" and demonstrated that the gene(s) conferring high Fe and Zn contents was located on the short arm of Th. bessarabicum.
Recently, Zhang et al. (2021) isolated stem rust resistance genes from Secale cereale and 6R. Recently, Song et al. (2016) revealed that the bin of fraction length (FL) 0.81–1.00 of the long arm of Agropyron cristatum chromosome 6P carried leaf rust resistance gene(s). The powdery mildew resistance gene Pm21 derived from Hayauidia villosa is located on 6VS and encodes a CC-NBS-LRR (NLR) protein (He et al., 2018; Xing et al., 2018). Li et al. (2020) mapped a new stripe rust resistance gene Yr83 to the bin of FL 0.73–1.00 of the long arm of Secale cereale chromosome 6R. Recently, Zhang et al. (2021) isolated stem rust resistance genes Sr26 and Sr61 from Th. ponticum chromosomes 6Ae1 and 6Ae3, respectively, which encode unrelated NLR genes and remain effective against all known Pgt races, including the widely virulent Pgt race Ug99 (TTKSK). In the present study, we identified a wheat-Th. intermedium T6StS.6J-L (6B) disomic substitution line CH51, which exhibited high levels of resistance to the prevalent Chinese leaf rust and stripe rust races in the field (Figure 3).

After transferring alien chromosomes into wheat, it is important to efficiently track alien chromosome(s) in wheat-alien introgression lines. GISH is regarded as a powerful and reliable technique for determining the genomic origin, size of introgressed fragments and breakpoint positions of the introgressions (Li et al., 2020). In this study, we used GISH analysis with Ps. spicata genomic DNA as a probe and showed that CH51 carried a pair of Th. intermedium 6Sts5/L -St-genome translocation chromosomes (Figure 1A). In addition, FISH is an efficient tool for the identification of wheat and alien chromosomes in wheat-alien introgression lines (Tang et al., 2014). We used FISH analysis to show that CH51 lacked a pair of wheat chromosome 6B but had a pair of Th. intermedium chromosomes (Figure 1B). A combination of GISH and FISH indicated that CH51 is a wheat-Th. intermedium T6StS.6J-L (6B) disomic substitution line.

With the rapid development of sequencing technologies, SNP array analysis is becoming increasingly popular in high-throughput genotyping wheat and wild relatives because of its high-density loci and reasonable cost (Winfield et al., 2016). Recently, SNP arrays also play a vital role in detecting the homoeologous relationships between wheat and alien chromosomes in wheat- alien introgression lines (Li et al., 2019b; Wang et al., 2020, 2022). In this study, results from the wheat 55K SNP array showed that chromosome 6B had the highest percentage of polymorphic SNP loci between CH51 and wheat parent Jintai 170 (Figure 2 and Table 1) and also had the highest percentage (62.82%) of missing SNP loci in CH51 (Supplementary Table 1). Combining with the cytology result, we concluded that CH51 is a wheat-Th. intermedium T6StS.6J-L (6B) disomic substitution line. In addition, SNP array results also verified that the tested materials, CS-N6BT6D, CS-N6AT6D, and CS-N6DT6B, used in the current study are correct, which correspond to the highest percentage of missing SNP loci of 62.82% (6B), 67.82% (6A), 76.50% (6D), respectively (Supplementary Table 1).

In this study, TAI8335 exhibited high levels of resistance to stripe rust, leaf rust, powdery mildew, and FHB in the field. Our results showed that the translocation chromosome T6StS.6J-L in CH51 carried resistance genes for stripe rust and leaf rust (Figure 3), but not for powdery mildew and FHB (Supplementary Figure 1). Therefore, the other six Th. intermedium chromosomes in TAI8335 should carry powdery mildew and FHB resistance genes and might also carry additional stripe rust and leaf rust resistance genes. For the future research, we will (1) backcross CH51 with the high pairing CS ph1b mutant to develop small segmental 6Sts or 6StL translocation lines for reducing the potential linkage drag and mapping the two genes; and (2) backcross TAI8335 with common wheat for transferring powdery mildew and FHB resistance genes and/or other stripe rust and leaf rust resistance genes.

**Conclusion**

A wheat-Th. intermedium T6StS.6J-L (6B) disomic substitution line CH51 was developed from the BGlF4 progeny of a partial wheat-Th. intermedium amphiploid TAI8335 and...
common wheat cv. Jintai 170. The chromosome composition of CH51 is 14A + 12B + 14D + 2T6StS.6J. CH51 exhibited high levels of resistance to the prevalent Chinese leaf rust and stripe rust races in the field. Therefore, the newly developed line CH51 can be utilized as a potential germplasm in wheat disease resistance breeding.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Author contributions

CL, JJ, and XZ conceived and designed the research. ZC and XZ contributed to the development of the materials. ZC performed the GISH experiment. GL performed the FISH experiment. HG, JL, and JJ carried out wheat 55K SNP analysis. ZC, XZ, and YG performed powdery mildew and FHB tests. SZ, XL, and JJ performed leaf rust test. CL and XW performed stripe rust test. JL wrote the manuscript. PZ, ZC, XZ, JJ, and CL helped with analysis and edited the manuscript. All authors contributed to the manuscript and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.1006281/full#supplementary-material

Supplementary Figure 1

Powdery mildew and Fusarium head blight (FHB) responses of tested materials at the heading stage. (A) Powdery mildew race E09 was inoculated on (from left to right): Th. intermedium, TAI8335, CH51, Jinchun 5, Jinmai 33, Jintai 170, Mingxian 169. (B) Fusarium pathogen F0609 was inoculated on (from left to right): Sumai 3, Jinchun 5, Jinmai 33, CH51, Alondra’s.

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