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Development and identification of a novel wheat-*Thinopyrum ponticum* disomic substitution line DS5Ag(5D) with new genes conferring resistance to powdery mildew and leaf rust

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Abstract

Background Powdery mildew (caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*)) and leaf rust (caused by *Puccinia triticina* (*Pt*)) are prevalent diseases in wheat (*Triticum aestivum* L.) production. *Thinopyrum ponticum* (2n = 10x = 70, $E^eE^eE^bE^bE^xE^xStStStSt$) contains genes that confer high levels of resistance to these diseases.

Results An elite wheat-*Th. ponticum* disomic substitution line, DS5Ag(5D), was developed in the Bainong Aikang 58 (AK58) background. The line was assessed using genomic in situ hybridization (GISH), oligo-nucleotide probe multiplex (ONPM) fluorescence in situ hybridization (FISH), and molecular markers. Twenty eight chromosome-specific molecular markers were identified for the alien chromosome, and 22 of them were co-dominant. Additionally, SNP markers from the wheat 660 K SNP chip were utilized to confirm chromosome identification and they provide molecular tools for tagging the chromosome in concern. The substitution line demonstrated high levels of resistance to powdery mildew throughout its growth period and to leaf rust at the adult stage. Based on the resistance evaluation of five F_5 populations between the substitution lines and wheat genotypes with different levels of sensitivity to the two diseases. Results showed that the resistance genes located on 5Ag confered stable resistance against both diseases across different backgrounds. Resistance spectrum analysis combined with diagnostic marker detection of known resistance genes of *Th. ponticum* revealed that 5Ag contained two novel genes, *Pm5Ag* and *Lr5Ag*, which conferred resistance to powdery mildew and leaf rust, respectively.

Conclusions In this study, a novel wheat-*Th. ponticum* disomic substitution line DS5Ag(5D) was successfully developed. The *Th. ponticum* chromosome 5Ag contain new resistance genes for powdery mildew and leaf rust. Chromosomic—specific molecular markers were generated and they can be used to track the 5Ag chromosome fragments.

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Consequently, this study provides new elite germplasm resources and molecular markers to facilitate the breeding of wheat varieties that is resistant to powdery mildew and leaf rust.

Keywords Disomic substitution line, Powdery mildew, Leaf rust, Fluorescence *in situ* hybridization, *Thinopyrum ponticum*

Background

Powdery mildew and leaf rust can reduce grain production by 5–50%, and in severely affected years, this reduction may exceed 62% in susceptible cultivars [1–3]. More than 100 powdery mildew and leaf rust resistance genes, respectively, have been discovered in wheat and related species, and 66 for powdery mildew (*Pm1–Pm69*) and 83 for leaf rust (*Lr1–Lr83*) resistance genes have been formally named [3–8]. However, the emergence of new virulent isolates has led to the loss of resistance to several genes. Therefore, digging novel resistance genes, especially those with broad-spectrum resistance are urgently required for wheat breeding programs. Notably, yield reduction can be mitigated by cultivating resistant cultivars, which is the most cost-effective and sustainable method of disease control.

Using distant hybridization and chromosome engineering to introgress exogenous resistance genes into common wheat effectively creates new germplasm resources for varieties and breeding high-yield, disease-resistant, and high-quality cultivars. Decaploid *Thinopyrum ponticum* (Podp.) Barkworth & D.R. Dewey. [syn. *Lophopyron ponticum* (Podp.) Á Löve, *Agropyron elongatum* (Host) P. Beauvois. *Elytrigia elongata* subsp. pontica (Podp.) Gamisans (2n = 10x = 70, $E^eE^eE^bE^bE^xE^xStStStSt$, or $JJJJJJJ^sJ^sJ^sJ^s$)], is rich in genes that can improve the resistance of wheat cultivars to diseases [1, 5], including powdery mildew, leaf rust, stem rust, stripe rust, *Fusarium* head blight, streak mosaic virus, and common root rot [9–13]. Among them, three leaf rust (*Lr19*, *Lr24,* and *Lr29*), five stem rust (*Sr24*, *Sr25*, *Sr26*, *Sr43,* and *SrB*), and one powdery mildew resistance gene, *Pm51,* have been formally documented [1, 5, 11–15]. And *Fhb7* has been successfully cloned from *Th. ponticum* [16].

The efficiency of generating wheat alien chromosome addition/substitution lines can be enhanced by marker assisted selection (MAS). Hence, it is essential to develop molecular markers linked to beneficial exogenous genes. For instance, 13 sequence-characterized amplified region (SCAR) markers, dispersed across chromosomes 1E–7E, can be utilized for identifying diploid *Th.elongatum* chromosomes within a wheat background [17]. Transcriptome sequencing of the common wheat variety Chinese Spring (CS) and CS-*Th. elongatum* amphidiploids facilitated the development of 35,193 SNP markers across seven E chromosomes [18]. Additionally, using 169 conserved orthologous set (COS) markers, derived from reported conserved orthologous genes of wheat and rice or *Brachypodium*, 60 molecular markers exhibited polymorphism on diploid genotypes of *Th.elongatum*. These COS markers were distributed among chromosomes of all seven homologous groups [19]. By comparing the specific-locus-amplified fragment (SLAF) sequences of CS and EA (a wheat-*Th. ponticum* translocation line TTh-4DS•4DL), 67 chromosome-specific SLAF markers have been developed for *Th. ponticum* [20]. All these markers can be used for MAS breeding in wheat.

Genomic in situ hybridization (GISH) can be used to accurately determine the presence of alien chromosomal segments in wheat. Many single-stranded oligonucleotide probes have been developed that can be synthesized by companies at high resolution and low cost. For example, the fluorescence in situ hybridization (FISH) karyotypes of CS and 373 Chinese wheat cultivars constructed using eight oligonucleotide probes did not only clearly distinguished the 21 pairs of wheat chromosomes but also identified 14 types of chromosome structural variations [21]. Alien disomic substitution (DS), addition, and translocation lines of *Th. ponticum* have been identified using GISH and FISH. For example, a wheat-*Th. ponticum* DS line DS1J^S(1D) is resistant to powdery mildew and stripe rust at the adult stage [10]. Another wheat-*Th. ponticum* DS line DS1J^S(1B), demonstrated resistance to powdery mildew and leaf rust throughout its growth period [4]. Moreover, a novel powdery mildew resistance gene was located on chromosome TTh-1DS•1DL in the wheat-*Th. ponticum* small alien segment translocation line WTT80. The resistance gene, in WTT80, was derived from a *Th. ponticum* chromosome segment [1]. Cytological identification of a wheat-*Th. ponticum* DS line Blue58 showed that the 4D chromosome of common wheat was substituted by chromosome 4Ag [5, 22, 23]. The 4Ag chromosome regions FL0.71–0.80 and FL0.75–0.89 contain blue grain genes [22, 23], while the powdery mildew resistance gene is located within the 3.79–97.12 Mb region of its short arm [5]. Decaploid *Th. ponticum* contains a large genome and rich genetic resources, highlighting its potential for discovering new resistance genes, which could be valuable for future wheat breeding. Notably, the combination of molecular

markers and cytological identification can improve the efficiency of transferring beneficial exogenous genes from the alien chromosomal lines to wheat plants.

In the study reported here, a novel wheat-*Th. ponticum* DS line was generated using distant hybridization. This line, Bainong Puyan 5814 (PY5814), is immune to powdery mildew and leaf rust. At the same time achieve the following goals: (1) characterize the chromosome composition of PY5814; (2) screen its exogenous chromosome-specific molecular markers; (3) evaluate its disease resistance; (4) analyze whether its disease resistance gene(s) is novel and derived from exogenous chromosomes, (5) and evaluate its agronomic performance. Thus, our findings provide a new germplasm and gene resource for breeding disease-resistant wheat varieties.

Results

Development and cytological identification of PY5814

PY5814 was developed from the BC_6F_2 progeny of *Th. ponticum*/Lankaoaizao 8 (LKAZ8)//Keyu 818 (KY818)/3/AK58*7 (Fig. S1). To detect the constitution of the chromosomes in PY5814, high-resolution GISH/FISH karyotypes of PY5814 and AK58 were constructed using a

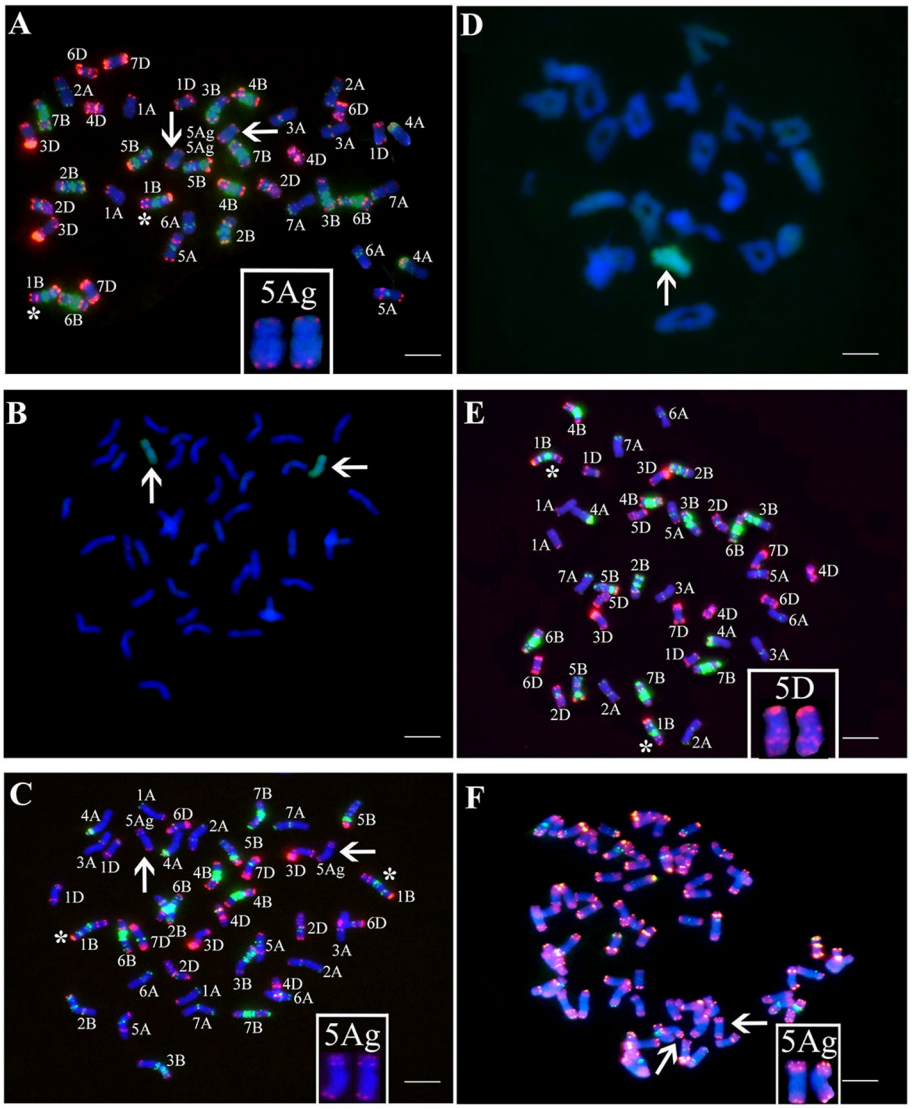

Fig. 1 FISH/GISH karyotype pattern of PY5814 and its parents. **A**, **E** FISH/GISH karyotypes of PY5814 (**A**) and AK58 (**E**) RTC with ONPM#4–1. **B**, **D** GISH analysis of PY5814 RTC (**B**) and PMC (**D**), the green signals are for the *Th. ponticum* gDNA probe labeled by fluorescein-12-dUTP (green). **C**, **F** FISH karyotypes of PY5814 (**C**) and *Th. ponticum* (**F**) with ONPM#7.Common wheat chromosomes were blue (DAPI); arrows and asterisks refer to 5Ag chromosome and T1RS•1BL translocation, respectively; bar = 10 μm

genomic DNA (gDNA) probe of *Th. ponticum* and oligonucleotide probes (Fig. 1). PY5814 comprised 42 chromosomes, with two from *Th. ponticum* replacing a pair of wheat chromosomes (Fig. 1A-C). Analysis of pollen mother cell meiotic metaphase I (PMC-MI) showed that the two exogenous chromosomes were paired as a ring bivalent, indicating that the two *Th. ponticum* chromosomes in PY5814 were homozygous (Fig. 1D). In contrast to the FISH karyotype of common wheat CS [21] and AK58 (Fig. 1E), PY5814 lacked a pair of 5D chromosomes and contained 20 pairs of common wheat chromosomes. Comparing the FISH karyotypes constructed by the probes of ONPM#4–1 and ONPM#7 (Fig. 1A, C), it was found that the sub-telomeric of the exogenous chromosome short arm of PY5814 contained Grass-5S-1 and Grass-5S-2 signals. 5S rDNA was located in the homologous groups 1 and 5 of wheat [24, 25]. Similarly, comparing the FISH karyotypes of PY5814 and *Th. ponticum* constructed using ONPM#7 probes (Fig. 1C, F), it was observed that the signal and arm ratio of a pair of chromosomes in *Th. ponticum* was consistent with the exogenous chromosomes contained in PY5814. Therefore, the disomic substitution line of PY5814 was confirmed to be DS5Ag(5D). In addition, PY5814 contained a pair of T1RS•1BL translocation chromosome inherited from AK58, and the majority of its genetic background was derived from AK58.

Molecular identification of PY5814 and its parents

The gDNA of CSN5BT5D, CSN5DT5B, PY5814 and its parents were amplified using 169 wheat COS markers, 423 SSR and 12 EST markers [9, 19, 26], respectively. In summary, 28 primer pairs amplified specific bands in PY5814 and *Th. ponticum,* with no amplification observed in AK58, KY818, LKAZ8, CSN5BT5D, or CSN5DT5B; and of which 22 were co-dominant markers of 5Ag and 5D (Fig. 2; Table S1). Furthermore, 82 molecular markers specific to the wheat 5D chromosome failed to produce specific bands in *Th. ponticum*, PY5814, and CSN5DT5B, yet generated specific bands in common wheat AK58, KY818, LKAZ8, and CSN5BT5D (Table S2). Notably, 78 of these specific markers were located within the fifth homologous group.

PY5814 and its parents AK58, KY818, LKAZ8, and decaploid *Th. ponticum*, underwent genotyping using a wheat 660 K SNP chip. Subsequently, 378,318 high-quality SNP markers with specific physical positions were obtained and used to analyze the chromosomal composition of PY5814 (Table S3). Among them, excluding SNPs undetected in its common wheat parents, AK58, KY818, and LKAZ8, 2,681 were not detected in PY5814. Notably, 1677 (62.55%) of these missing SNPs were on chromosome 5D, which had the highest ratio of all wheat chromosomes (Fig. 3A). The homozygous SNP percentage (66.85%) on chromosome 5D was the lowest, and the heterozygous SNP percentage (11.75%) was the highest. Correspondingly, the minimum percentage of the same SNP loci between PY5814 and AK58 was on the chromosome 5D (54.63%), which also showed the maximum percentage of the same SNP loci between PY5814 and *Th. ponticum* (Fig. 3B).

A total of 22,346 SNPs (5.91%, Table S4) identified as homozygous and pleomorphic between *Th. ponticum* and three common wheat parents (AK58, KY818, and LKAZ8) were utilized to determine the homologous relationship between the alien chromosomes of PY5814 and the wheat chromosomes. According to the pedigree of PY5814, the alien chromosome-specific SNP loci should be consistent with those of *Th. ponticum* chromosomes. Of the 628 specific homozygous SNP loci detected for

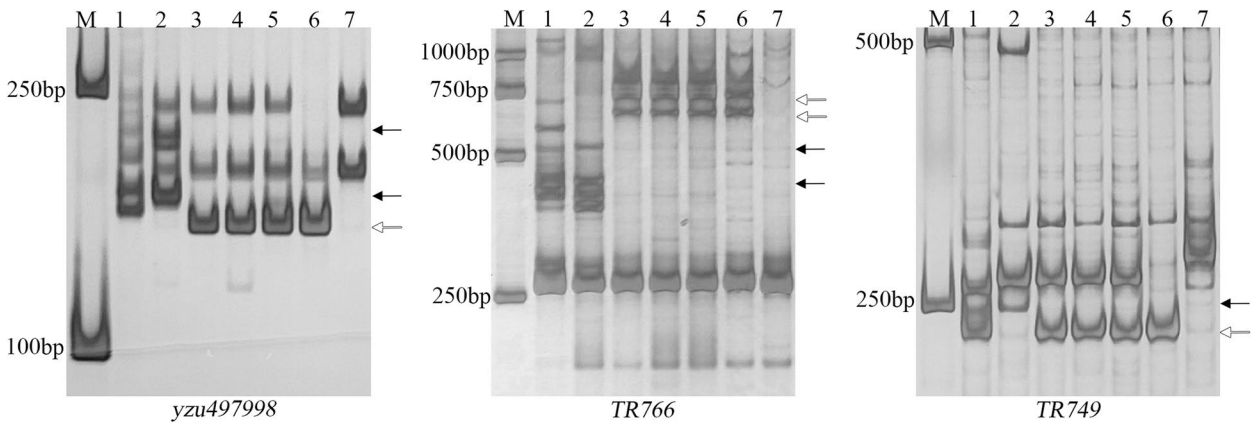

Fig. 2 Molecular marker analysis of PY5814. M: Marker DL2000; 1: *Th. ponticum*; 2: PY5814; 3: AK58; 4: KY818; 5: LKAZ8; 6: CSN5BT5D; 7: CSN5DT5B; Solid and hollow arrows indicate the specific fragment of chromosome 5Ag and 5D, respectively

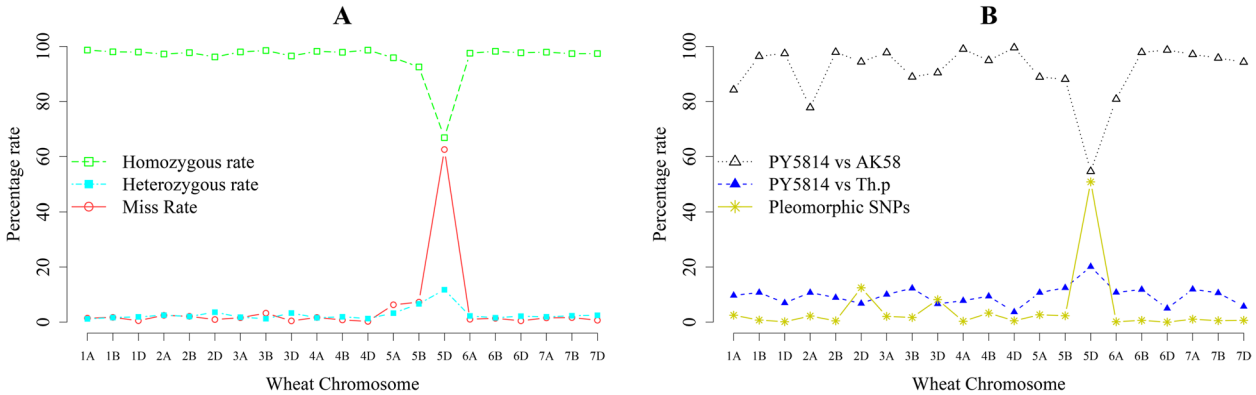

Fig. 3 Wheat 660 K SNP array analysis of PY5814 and its parents. **A** Lowest homozygous, highest heterozygous and miss rate on the 5D chromosome. **B** The difference of SNPs on 5D chromosome between PY5814 and its two parents was the most obvious

PY5814, which were consistent with *Th. ponticum*, 223 SNPs (50.80%, Fig. 3B) were mainly distributed on chromosome 5D. Thus, PY5814 deleted the 5D chromosome of common wheat, and the alien chromosome was homologous to the wheat chromosome 5D.

Resistance evaluation of PY5814

Powdery mildew resistance of PY5814 and its parents was identified at the three-leaf stage in a glass greenhouse. The infection types (ITs) scores at the seedling stage showed that AK58, LKAZ8, and CS were grade 4, and KY818 was grade 3. By contrast, PY5814 and *Th. ponticum* showed immune responses against powdery mildew (IT = 0) (Fig. 4A). The resistance spectrum of PY5814 to 24 single-pustule-derived Powdery mildew isolates was identified at the seedling stage. The results showed that PY5814 was highly resistant or immune to 22 pathotypes, except for E18 and 23-(2) (Table S5). The resistance spectrum of PY5814 differed from that of *Pm51* and the other resistance genes. Under field conditions, the resistance of PY5814 and its parents to Powdery mildew at the adult stage was identified over the four growing seasons (Fig. S2). The common wheat parents of PY5814 were highly susceptible with an IT score of 4, and control genotype CS was type 3 (Fig. 4B). *Th. ponticum* (IT = 0) and PY5814 (IT = 0) also showed immunity at the adult stages.

The responses of PY5814 seedlings to the six *Pt* pathotypes were evaluated and showed high resistance to all of them (Table S6). Lr19 from *Th. ponticum* was similar to the PY5814 resistance spectrum, and further molecular marker identification is required. Under field conditions in Xinxiang City, China, the resistance identification of PY5814 to leaf rust during the adult stage revealed its immunity to this disease. In contrast, AK58, KY818, LKAZ8, and Mingxian 169 (MX169) exhibited high

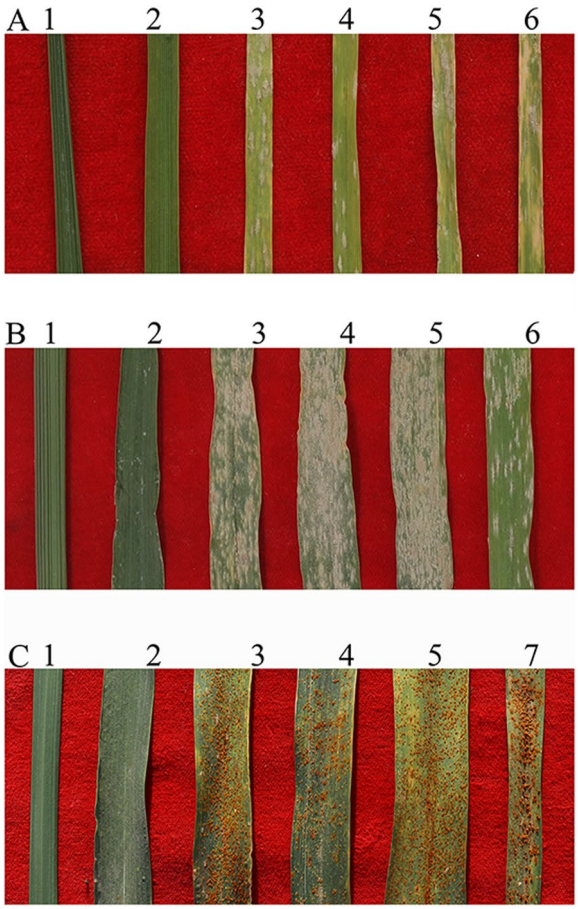

Fig. 4 Resistance evaluation of PY5814 and its parents. **A**-**C** Reactions to powdery mildew at seedling stage (**A**) and adult stage (**B**) and leaf rust at adult stage (**C**). 1: *Th. ponticum*; 2: DS5Ag(5D); 3: AK58; 4: KY18; 5: LKAZ8; 6: CS; 7: MX169

susceptibility to leaf rust, each receiving IT scores of 4, 3, 4, and 4, respectively (Figs. 4C, S2).

Chromosome 5Ag contained novel genes for powdery mildew and leaf rust resistance

We further verified that the resistance gene in PY5814 originated from the *Th. ponticum* chromosome rather than from interference with the common wheat genetic background. Using MAS and GISH, we screened 120 individual lines from five F_5 populations, consisting of 12 lines with the 5Ag chromosome and 12 lines without it in each population (Figs. 5, S3). Resistance to powdery mildew and leaf rust in these lines was identified at the adult stage. The results showed that each line with the 5Ag chromosome was resistant to disease, and each line without it was susceptible to disease. Simultaneously, three DS5Ag(5D) substitution lines were identified from the populations of PY5814/CS, PY5814/WM6, and PY5814/Q03073, all of which lacked 1RS•1BL (Fig. 5). The resistance identification showed that these lines were also immune to powdery mildew and leaf rust at the adult stage. Thus, these results confirmed that the powdery mildew and leaf rust resistance genes of PY5814 were derived from the 5Ag chromosome of *Th. ponticum*.

To ascertain the novelty of powdery mildew and leaf rust resistance genes present in PY5814, we utilized molecular markers (Table S7) linked to *Pm51*, *Lr19*, *Lr24,* and *Lr29* to amplify the gDNA of PY5814 and its parental lines via Polymerase chain reaction (PCR). The target band was not amplified in five pairs of coupling markers associated with the target gene. Correspondingly, the target band of 750 bp was successfully amplified with the *Lr19* repulsion marker SCS253 (Fig. S4). Thus, it can be concluded that the powdery mildew and leaf rust resistance genes harbored within the 5Ag chromosome of PY5814 are indeed novel genes. These were designated as *Pm5Ag* and *Lr5Ag*, respectively.

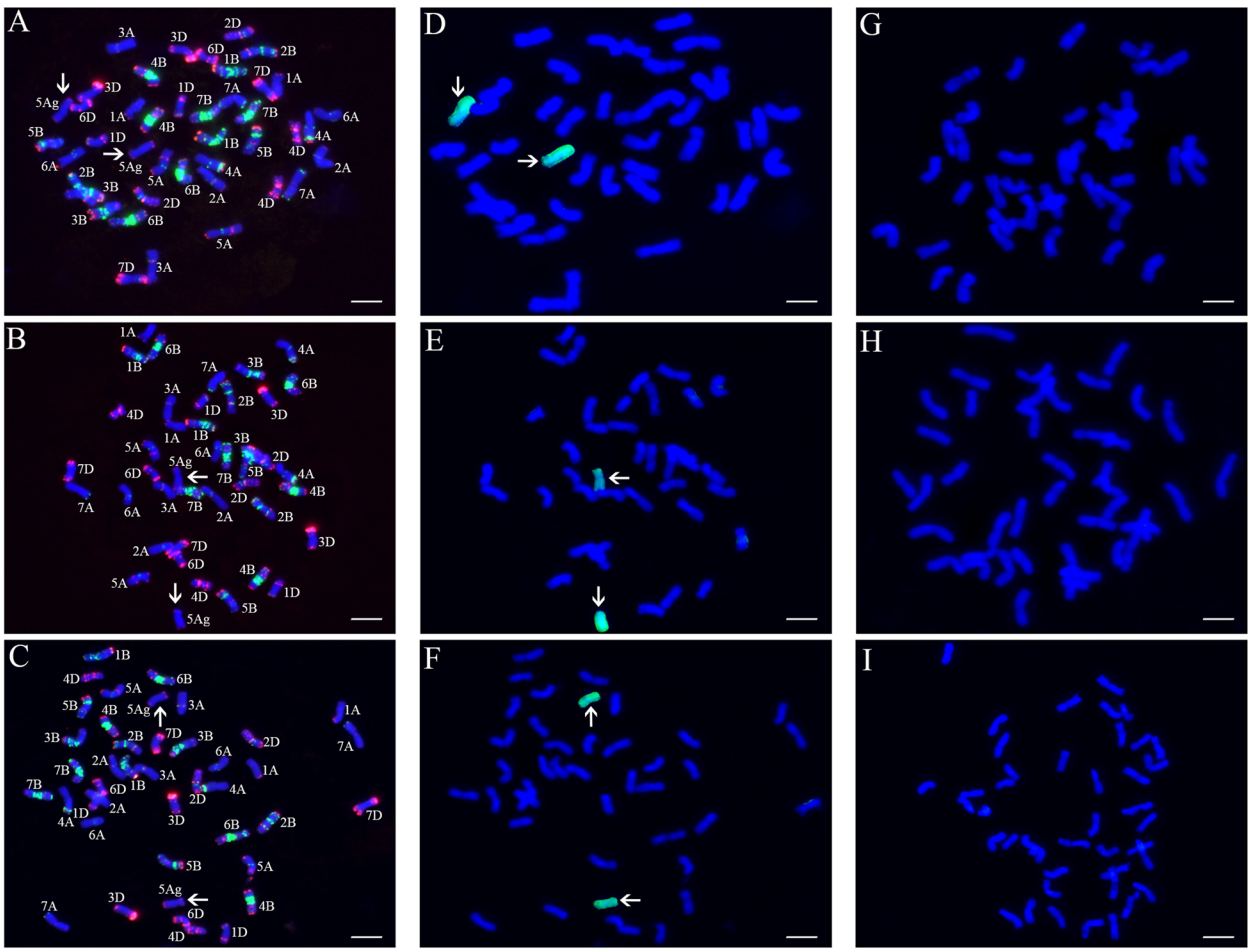

Fig. 5 FISH/GISH analysis was performed on the F_5 population using ONPM#4–1 probes. **A**–**F** FISH/GISH analysis of wheat-*Th. ponticum* DS5Ag(5D) resistant lines, which did not contain 1RS•1BL translocation lines, of PY5814/CS (**A**, **D**), PY5814/WM6 (**B**, **E**), and PY5814/Q03073(**C**, **F**) F_5 populations. **G**–**I** GISH analysis of F_5 susceptible lines of PY5814/CS (**G**), PY5814/WM6 (**H**), and PY5814/Q03073 (**I**). Arrows refer to 5Ag chromosome, bar = 10 μm

Fig. 6 Agronomic performance of PY5814 and its recurrent wheat parent AK58. **A** Plants of PY5814 (right) and AK58 (left). **B** Seeds of PY5814 (right) and AK58 (left)

Evaluation of PY5814 agronomic performance

The phenotypic characteristics and agronomic performance of PY5814 and its backcross parent AK58 were investigated over three consecutive years (Fig. 6A-B; Table 1). In 2019 and 2020, the mean plant height (PH) of PY5814 was significantly higher than that of AK58 ($P<0.05$). Additionally, in 2019, the mean thousand-kernel weight (TKW) of PY5814 was significantly higher than that of AK58 ($P<0.05$). This result may be due to planting inoculum spreaders in the experimental field and the serious incidence of AK58, leading to a decrease in TKW. The other agronomic indices analyzed were not significant for the three consecutive seasons. These results indicate that the agronomic performance of PY5814 is acceptable and that PY5814 can play a vital role in wheat disease resistance breeding.

Discussion

Chromosomal engineering is a notable method for mining new disease-resistant germplasm and broadening the genetic basis of common wheat. Alien chromosome translocation lines have been widely used in wheat breeding because they associate elite traits with valuable disease-resistance genes without genetic linkage drag. For instance, the wheat-rye translocation line T1RS•1BL, which contains the powdery mildew resistance gene *Pm8*, leaf rust resistance gene *Lr26*, stripe rust resistance gene *Yr9,* and stem rust resistance gene *Sr31*, has been widely used in wheat breeding worldwide [27]. In southern China, 35.8% of newly developed wheat cultivars contain the wheat-*Dasypyrum villosum* translocation line T6VS•6AL, which is highly resistant to all powdery mildew virulent isolates currently prevalent in China owing to it carrying the resistance gene *Pm21* [28].

In prior research, leveraging the resistance genetic reservoirs of decaploid *Th. ponticum*, or other polyploid species, was a pivotal intermediary step in the development of stable partial amphiploids (2n = 56). Subsequently, these partial amphiploids were subjected to backcrossing with wheat. Each successive generation of

Table 1 Agronomic index statistics for PY5814 and AK58

Year	Materials	Plant height	Spike length	Kernels number per main spike	Spikelets number per spike	Sterile spikelets number	Effective tiller number per plant	Thousand-kernel weight
2019	PY5814	77.58 ± 3.00*	9.64 ± 0.34	46.29 ± 5.47	19.77 ± 1.36	4.94 ± 0.98	7.29 ± 1.73	43.53 ± 1.55*
	AK58	70.69 ± 2.05	9.88 ± 0.12	42.55 ± 3.75	18.94 ± 1.42	4.33 ± 1.48	9.93 ± 2.14	36.24 ± 2.57
2020	PY5814	78.82 ± 1.92**	9.60 ± 0.02	47.78 ± 3.37	19.83 ± 0.60	3.33 ± 0.17	8.50 ± 1.76	43.99 ± 2.61
	AK58	72.57 ± 0.39	10.32 ± 0.76	46.28 ± 3.38	20.01 ± 1.38	3.11 ± 0.34	11.95 ± 1.25	44.85 ± 5.08
2021	PY5814	71.28 ± 0.46	9.02 ± 0.16	49.09 ± 1.44	18.57 ± 1.72	1.39 ± 0.38	13.06 ± 2.86	42.01 ± 1.78
	AK58	71.47 ± 2.67	9.69 ± 0.49	45.52 ± 1.06	18.22 ± 1.06	1.83 ± 0.34	13.94 ± 1.77	36.37 ± 3.10

* $P<0.05$; ** $P<0.01$

seeds underwent identification using GISH to meticulously select the alien substitution or addition lines. This process continued until a small fragment translocation line was spontaneously generated or induced through radiation exposure or utilizing the CS *ph1b* mutant. For example, He et al. [29] successfully cultivated the partial amphiploids SNTE20 (2n = 56) via the hybridization of *E. elongate* (2n = 10x = 70) with common wheat. Furthermore, a wheat-*Th. ponticum* disomic substitution line $DS1J^{S}(1B)$ and two introgression lines were developed through the crossbreeding of SNTE20 with common wheat [4, 11]. In this study, the direct backcrossing and phenotypic selection method, which does not require high-intensity GISH identification in the early stages, is a simple, low-cost, and effective method. Based on this method, a new wheat-*Th. ponticum* disomic substitution line, DS5Ag(5D), was obtained. The FISH karyotype of PY5814 matched that of AK58, as it was derived from the progeny of KY818//LKAZ8/*Th. ponticum*, subsequently backcrossed with AK58 seven times, and then self-pollinated twice. Its chromosome composition was $2n = 7A'' + 7B'' + 6D'' + 1''Ag$ (5Ag). This result is consistent with the results of the COS markers and the 660 K SNP chip.

Wheat SNP chip technology has been employed to characterize alien chromosomes or fragments introduced into the wheat background. Zhou et al. [30] successfully constructed the highest-density linkage maps of *Agropyron* using a wheat 660 K SNP array. The results showed that the 2P and 4P chromosomes of *Agropyron* were rearranged and provided evidence of synteny between the P *Agropyron* and the A, B, and D wheat genomes. Moreover, the homoeologous relationship between the P of 35 wheat-*A. cristatum* chromosome substitution/addition lines and the wheat genome was characterized. Wheat SNP arrays offer precise analytical capabilities to determine which chromosomes or fragments of common wheat have undergone replacement by alien chromosomes. They also aid in establishing the homologous relationship between the introduced alien chromosomes or fragments and the wheat genome, as well as identifying the breakpoint sites of the chromosome fragments. For example, when comparing the same SNP ratios between M862 and common wheat 7182, the lowest ratio was observed to be associated with the 4D chromosome (24.1%). Correspondingly, compared with *Leymus mollis*, the highest percentages of the same SNP loci were also 4D chromosomes (36.8%), indicating that M862 was a wheat-*L. mollis* DS4Ns(4D) [31]. The homoeology of WTA55 was a wheat-*Th. ponticum* disomic addition line derived from Xiaoyan 7430 and resistant to stripe rust, and the alien chromosome was determined using a wheat 660 K SNP array [32]. Similarly, the homoeology of the PY5814 alien chromosome was determined using the wheat 660 K SNP array in the present study. Similar to other studies, in our study, the results of the wheat SNP array were consistent with those of cytological methods. The wheat SNP array effectively detected elite genes from wild relatives in wheat and may significantly accelerate wheat chromosome engineering during breeding.

Although FISH and GISH can accurately identify alien chromosomes in distant hybridization derived wheat materials, these methods are time-consuming and labor-intensive. Molecular markers can complement these defects and play vital roles in tracking exogenous chromosomes or fragments in wheat. Currently, specific molecular markers have been developed and screened, including SSR [9], SLAF [14], STS [10], SNP [18], CAPS, and indels [11], which can be used to identify alien chromosomes or fragments in different wheat-*Th. ponticum* derived materials. For example, 20 and 61 SLAF markers have been developed specifically for chromosomes 1E and 7E of diploid *Th.elongatum*, respectively [33, 34]. Among the 223 SLAF-specific markers developed on the 4AgS chromosome of *Th. ponticum*, 16 were in the 3.79–97.12 Mb region of the 4AgS chromosome, closely linked to its powdery mildew gene [5]. In the present study, 28 chromosome 5Ag-specific molecular markers were identified; among these, 22 were co-dominant markers of chromosomes 5Ag and 5D. These markers can be used to identify alien chromosome fragments containing resistance genes and enable the determination of their homozygosity or heterozygosity. Moreover, they contribute to the enrichment of markers available for *Th. ponticum*.

Several wheat-*Th. ponticum* alien chromosome lines, which are resistant to powdery mildew and leaf rust, have been developed. The powdery mildew resistance gene *Pm51* and stripe rust resistance gene *Yr69* of the wheat-*Th. ponticum* introgression line CH7086 were in 2BL and 2AS, respectively [15, 35]. By irradiating the wheat-*Th. ponticum* disomic substitution line DS4Ag(4D), three small fragment translocation lines—WTT139, WTT146, and WTT323—resistant to powdery mildew were created [5]. However, only few studies have reported that chromosomes from the fifth homologous group of *Th. ponticum* contain genes that are resistant to both powdery mildew and leaf rust. Recently, two wheat-*Th. ponticum* distant hybridization materials—ES-7 and 11–20-1—were created, and their alien chromosomes belonged to the fifth homologous group [9, 36]. ES-7 is a disomic substitution line of DS5St(5A) with resistance to stripe rust at the adult stage but is not resistant to powdery mildew and leaf rust [36]. Similarly, 11–20-1, a wheat-*Agropyron. elongatum* translocation line T5ES·5DL carries a powdery mildew resistance gene; however, its resistance to

leaf rust and stripe rust remains unclear [9]. In the present study, we evaluated the resistance of wheat-*Th. ponticum* DS line DS5Ag(5D). Notably, DS5Ag(5D) exhibited immunity to powdery mildew during the seedling stage and sustained resistance to virulent powdery mildew and leaf rust isolates prevalent in China during adulthood.

The FISH karyotype of PY5814 was compared with that of CS constructed by Huang et al. [21], showing that PY5814 inherited the T1RS•1BL translocation line of the recurrent parent AK58. In northern China, 27.3% of wheat cultivars carry the T1RS•1BL translocation, although its usage has diminished as the resistance genes *Pm8*, *Yr9*, and *Lr26* have been overcome by new virulent isolates [27, 28]. This study also showed that AK58 is highly susceptible to powdery mildew and leaf rust, indicating that these pathogens have overcome the resistance genes contained on the 1RS chromosome. Although Xu et al. [37] found that AK58 is moderately resistant to E15 and E21, PY5814 is immune to these two powdery mildew pathotypes. Additionally, AK58 contains a QTL locus, which is speculated to be *PM4a* [37]. Our study revealed that the resistance spectrum to 24 *Bgt* isolates of PY5814 was different from *Pm4a* (Table S6). Specifically, at the seedling stage, PY5814 showed immunity against 22 out of 24 *Bgt* isolates. Simultaneously, three DS5Ag(5D) lines lacking the 1RS chromosome arm were also found to be resistant to powdery mildew and leaf rust. Analysis using molecular markers, FISH/GISH techniques, and resistance assessments across the five F_5 populations consistently indicated that plants containing the 5Ag chromosome exhibited resistance, while those lacking the 5Ag chromosome remained susceptible. Three wheat parents of PY5814 were highly susceptible to powdery mildew and leaf rust. Therefore, the powdery mildew and leaf rust resistance genes contained in PY5814 were derived from the alien chromosome 5Ag. In the field, we also identified the adult-stage stripe rust resistance of PY5814 by inoculating with a mixture of CYR32, CYR33, and CYR34 rust spores at the jointing stage. Although PY5814 exhibited immunity, its parent LKAZ8 was highly susceptible. In contrast, its recurrent parent AK58 showed high resistance to stripe rust (Fig. S5). Therefore, whether the stripe rust resistance of PY5814 is from *Th. ponticum* or its recurrent parent AK58 is unclear.

Molecular markers linked to resistance genes can be used to track MAS genes. Zhan et al. [15] developed 14 molecular markers linked to *Pm51*, of which the STS marker BQ246670 had a genetic distance of 1.5 cM from *Pm51* and a specific fragment length of approximately 500 bp. SCS253 is a repulsion phase marker linked to *Lr19*, and its specific fragment is approximately 737 bp. SCS265 is a coupling phase marker linked to *Lr19*, and its specific fragment is approximately 512 bp. The combination of the two SCAR markers can be used as a co-dominant marker to distinguish between homozygous and heterozygous loci [38]. Three SCAR markers, namely SCS1302, SCS1326, and SCOAB-1, produced specific bands of 607 bp, 613 bp, and 365 bp, respectively, in wheat samples carrying the *Lr24* gene. However, these markers failed to amplify the target band in samples lacking this gene [39]. SCAR markers OPY10 and UBC219 are the dominant markers linked to *Lr29*, producing specific bands of 950 bp and 1000 bp, respectively, in disease-resistant materials carrying this gene [40]. Among these markers, we used six molecular markers linked to the reported resistance genes *Pm51*, *Lr19*, *Lr24*, and *Lr29*, from *Th. ponticum* to analyze PY5814. PY5814 had no coupling phase marker bands but contained the *Lr19* repulsion phase marker SCS253 band. *Pm51*, *Lr19*, *Lr24*, and *Lr29* are not from the fifth homologous group. Further, results from this study showed that the resistance spectrum of *Pm5Ag*/*Lr5Ag* is different from that of *Pm51*, *Lr24*, and *Lr29* (Table S5-6). Combined with the molecular markers, FISH/GISH, resistance identification of the five F_5 populations, and multiple pathogen test analyses indicated that PY5814 contained new resistance genes for powdery mildew and leaf rust (designated as *Pm5Ag* and *Lr5Ag*). In conclusion, the wheat-*Th. ponticum* DS line PY5814, exhibiting multiple disease resistance and satisfactory agronomic performance, serves as a promising germplasm resource for wheat chromosome engineering during breeding. However, notably, the DS lines may lack stability in the breeding process due to the unpaired 5Ag and 5D chromosomes. To address this issue, we aim to induce small fragment translocation lines of 5Ag chromosome through radiation and CS *ph1b* in future research, to enhance the utilization of PY5814 resistance genes in wheat breeding.

Conclusion

In this study, a novel wheat-*Th. ponticum* disomic substitution line was successfully developed, designated as PY5814. Through ONPM-FISH/GISH and molecular marker analysis, it was identified as DS5Ag(5D). Among the screened 28 chromosome 5Ag specific molecular markers, 22 were co-dominant markers. Further disease resistance identification, ONPM-FISH/GISH, and diagnostic marker analysis revealed the presence of new resistance genes for powdery mildew and leaf rust, designated as *Pm5Ag* and *Lr5Ag*, respectively, on the *Th. ponticum* chromosome 5Ag. These findings offer valuable insights for leveraging *Th. ponticum* in wheat disease resistance breeding.

Methods

Plant materials

In this study, a diverse array of genetic resources was employed, including the wheat-*Th. ponticum* DS line PY5814, *Th. ponticum*, and six wheat cultivars (with a chromosome composition of 2n = 6x = 42, AABBDD): Bainong Aikang 58 (AK58), Keyu 818 (KY818), and Lankaoaizao 8 (LKAZ8), as well as CS, Sumai 3 (SM3), and Mingxian 169 (MX169). Additionally, two nullitetrasomic lines, CSN5BT5D and CSN5DT5B, were utilized. PY5814 was developed from a cross involving *Th. ponticum*/LKAZ8//KY818/3/AK58*7 (Fig. S1). Furthermore, interspecific hybrid F_1 seeds, resulting from the pollination of LKAZ8 with the female parent *Th. ponticum*, was obtained. Individuals carrying 56 chromosomes were selected as the female parent and further hybridized with the cultivar wheat KY818. For improving the general agronomic and quality traits of the alien chromosome lines, AK58 served as the recurrent parent. Powdery mildew-resistant individuals were selected for backcrossing with AK58 for seven generations, followed by self-crossing. Resistant plants from the BC_6F_2 generation underwent selection for FISH/GISH identification, ultimately leading to the acquisition of the DS line PY5814.

To determine whether the PY5814 resistance gene was derived from the chromosomes of *Th. ponticum* or was affected by the background wheat chromosomes, five F_5 populations were constructed with PY5814 as the female parent and susceptible wheat materials as the male parent: CS, AK58, LKAZ8, Wenmai 6 (WM6), and Q03073.

FISH and GISH analyse

The procedure for wheat seed rooting preparation followed Wang et al. [41] and Jia et al. [32]. The mitotic metaphase of the root tip cell metaphase (RTC-M) chromosomes preparation procedure was performed according to Komuro et al. [42] and Baker et al. [43]. Root tip meristem cells were digested in 2% cellulase Onozuka R-10 and 1% pectolyase Y23 (Yakult Pharmaceutical, Osaka, Japan) enzyme solution at 37 °C for 35 min. Following digestion, the suspensions were dropped onto the slides (8 μL per slide).

The ONPM#4–1 probes and sequences were based on those of Huang et al. [21] and Chen et al. [44] and comprised TAMRA (6-carboxytetramethylrhodamine, red)-modified AFA-3, AFA-4, pAs1-1, pAs1-3, pAs1-4, pAs1-6, and pSc119.2–1, and FAM (6-carboxyfluorescein, green)-modified $(GAA)_{10}$. The ONPM#7 probes and sequences reported by Chen et al. [44] were slightly modified (removed the BSCL242-1) and used to compare the 5Ag chromosomes of PY5814 and *Th. ponticum*. The modified ONPM#7 probes comprised TAMRA-modified Grass-5S-1 and Grass-5S-2, FAM-modified BSCL-135–1 and BSCL-135–2, along with eight oligonucleotide probes of ONPM#4–1. These oligonucleotide probes were synthesized and modified by TSINGKE Biotechnology Co., Ltd. (Nanjing, Jiangsu, China).

The RTC-M and PMC-MI chromosomes were utilized for GISH analysis. Chromosomes of the meiotic MI of PMC were prepared following Chen et al. [45]. Total gDNA from *Th. ponticum* was labeled with fluorescein-12-dUTP (Roche, Darmstadt, Germany) using nick translation and served as a probe. Non-labeled AK58 gDNA was employed as a block in the process. The probe/block ratio was 1:50–70. Details of the probe dosage and FISH/GISH procedure for each slide are in Huang et al. [21] and Chen et al. [44]. The chromosomes with high-definition hybridization signals were examined using a BX51 fluorescence microscope (Olympus, Tokyo, Japan). Images were captured using a Coolcube 1 CCD camera and analyzed using the Isis Karyotype Analysis Software System (Metasystems, Altlussheim, Germany).

Molecular marker analysis

gDNA was extracted from the young leaves of each wheat plant by using the cetyltrimethylammonium bromide (CTAB) method [46]. A total of 169 COS markers from wheat seven homologous groups, along with 423 SSR and 12 EST markers of wheat homologous group 5 [9, 19, 26] were selected to screen specific molecular markers of PY5814. Subsequently, we investigated whether PY5814 contains the powdery mildew resistance gene *Pm51* and the leaf rust resistance genes *Lr19*, *Lr24*, and *Lr29*, which have been reported in *Th. ponticum*. For this purpose, we selected 1 [15], 2 [38], 2 [39], and 1 [40] linkage markers (Table S7) to amplify gDNA from PY5814 and its parents. All primers were synthesized by TSINGKE Biotechnology Co., Ltd.

Each PCR comprised a total volume of 10 μL consisting of 5 μL of 2×Es Taq Master Mix (Dye, CoWin Biosciences, Beijing, China), 1 μL gDNA template (20–50 ng/μL), 0.2 μL forward primer (10 μmol/L), 0.2 μL reverse primer (10 μmol/L), and 3.6 μL ddH_2O. The amplification program consisted of one cycle at 94 °C for 3 min to pre-denaturation, followed by 36 cycles of 30 s at 94 °C, 30 s at 52–65 °C depending on specific primers, 1 min 10 s at 72 °C, and finally, a cycle of 72 °C for 10 min. PCR products were separated using 8% polyacrylamide gel electrophoresis and then silver stained, as described in the literature [47].

Wheat 660 K SNP array analysis

To investigate the PY5814 chromosome characterization and the homoeology of its alien chromosome, the gDNA of the tested materials was used, ensuring that

the detection concentration and purity met the standard criteria. The analysis was carried out on Axiom® wheat 660 K SNP genotyping arrays by China Golden Marker Biotechnology Co., Ltd. (Beijing, China). The threshold dish quality control (DQC) ≥ 0.82 && call rate (CR) ≥ 95 were set to obtain the original genotype data. Subsequently, high-quality genotype data were obtained by setting the threshold: BestandRecommended = 1 && BestProbeset = 1 && ConversionType = PolyHighResolution && call rate ≥ 97. SNPs present in AK58, KY818, and LKAZ8, but absent from PY5814, were screened to analyze chromosome deletion events. Theoretically, the chromosome of PY5814, with the highest miss rate and the lowest proportion of the same SNP between PY5814 and AK58, was considered to have experienced substitution events. Conversely, the chromosome of *Th. ponticum*, which had the highest proportion of the same SNP as PY5814, was regarded as homologous to the alien PY5814 chromosome.

Disease resistance assessment

CS, PY5814, and its parent materials were planted in a glass greenhouse in a 5 × 10 plastic cave dish with 10 plants per hole, with three replicates, and a mixture of *Bgt* was inoculated at the 2–3 leaf stage to identify the resistance to powdery mildew. In 2018–2022, plants were grown under field environmental conditions with a row length of 1.50 cm and row spacing of 0.24 cm. During the filling stage, the resistance of PY5814 and its parental materials to powdery mildew was evaluated. For this assessment, seeds of common wheat cultivars SM3 and MX169 were uniformly mixed, used as inoculum spreaders, and planted perpendicular to the experimental materials. Leaf rust was simultaneously identified in the field environment. At the jointing stage, the inoculum spreader plants were inoculated with a mixture of PHT and THT leaf rust spores using an atomizer. These pathogen types of leaf rust are prevalent in China. ITs were evaluated when the inoculum spreader plants exhibited the highest susceptibility.

The ITs of powdery mildew and leaf rust were scored following Li et al. [9] and Zhao et al. [48]: 0–2 represents resistance, while 3–4 indicates susceptibility. Seedlings of PY5814 were also stressed by using 24 single-colony cultures of *Bgt* pathotypes in the Prof. Yilin Zhou Laboratory, Institute of Plant Protection, Chinese Academy of Agricultural Sciences (Beijing, China). The names of the 24 *Bgt* pathotypes as well as infection methods and resulting ITs corresponded to those described by Yang et al. [1]. The reactions of PY5814 seedlings to the six *Pt* pathotypes currently prevalent in China were identified at the College of Plant Protection, Hebei Agriculture University (Baoding, Hebei, China). Infection methods were similar to those of Zhang et al. [49].

Evaluation of agronomic performance

During three consecutive years, from 2019 to 2021, PY5814 and its recurrent parent AK58 were cultivated in Xinxiang (113.5°E, 35.2°N, Henan, China). Each row measured 1.50 m in length, spaced 0.24 m apart, and sowed with 30 seeds. Three biological replicates were planted annually. At the physiological maturity stage, six whole plants of each genotype were randomly selected from the middle of the row to investigate agronomic traits, including plant height (PH), main spike length (SL), kernels number per main spike (KNPS), spikelets number per spike (SNPS), sterile spikelets number (SSN), effective tiller number per plant (ETN), and thousand-kernel weight (TKW). Significant differences between PY5814 and AK58 for all traits were compared using t-tests in SAS v9.3 (SAS Institute Inc., Cary, NC, USA).

Abbreviations

ONPM	Oligo-nucleotide probe multiplex
FISH	Fluorescence in situ hybridization
GISH	Genomic in situ hybridization
MAS	Marker assisted selection
PCR	Polymerase chain reaction
RTC-M	Root tip cell metaphase
PMC-MI	Pollen mother cell meiotic metaphase I
ITs	Infection types
DS	Disomic substitution line
PY5814	Bainong Puyan 5814
AK58	Bainong Aikang 58
KY818	Keyu 818
LKAZ8	Lankaoaizao 8
CS	Chinese Spring
WM6	Wenmai 6
MX169	Minxian 169
SM3	Sumai 3
PH	Plant height
SL	Main spike length
KNPS	Kernels number per main spike
SNPS	Spikelets number per spike
SSN	Sterile spikelets number
ETN	Effective tiller number per plant
TKW	Thousand-kernel weight

Supplementary Information

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Supplementary Material 1.
Supplementary Material 2.
Supplementary Material 3.
Supplementary Material 4.
Supplementary Material 5.
Supplementary Material 6.
Supplementary Material 7.
Supplementary Material 8.
Supplementary Material 9.

Supplementary Material 10.

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Authors' contributions
JLZ, ZGR, and ZJQ, conceived and designed the study; JLZ, YZJ, LJY, YLD, MMW, YFP, DYY, YZ, and CCR performed the experiments. PPZ was used to evaluate the resistance of single pustule-derived leaf rust pathotypes. JLZ, ZGR, and XJL created the addition lines; JLZ, YZJ, XDC, YLD, MMW, YFP, CCR, YZ, and JS repaired the experimental images and analyzed the data. ZGR, JLZ, and XDC provided reagents and tested consumables. JLZ drafted the manuscript, and all authors have read and approved the manuscript.

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Availability of data and materials
The datasets generated and analyzed during the present study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study did not involve any experiments involving human or animal participants that violated morality.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Yang G, Tong C, Li H, Li B, Li Z, Zheng Q. Cytogenetic identification and molecular marker development of a novel wheat-*Thinopyrum ponticum* translocation line with powdery mildew resistance. Theor Appl Genet. 2022;135(6):2041–57. https://doi.org/10.1007/s00122-022-04092-1.
2. Lin G, Chen H, Tian B, Sehgal SK, Singh L, Xie J, Rawat N, Juliana P, Singh N, Shrestha S, et al. Cloning of the broadly effective wheat leaf rust resistance gene *Lr42* transferred from *Aegilops tauschii*. Nat Commun. 2022;13(1):3044. https://doi.org/10.1038/s41467-022-30784-9.
3. Wang B, Meng T, Xiao B, Yu T, Yue T, Jin Y, Ma P. Fighting wheat powdery mildew: from genes to fields. Theor Appl Genet. 2023;136(9):196. https://doi.org/10.1007/s00122-023-04445-4.
4. Li M, Wang Y, Liu X, Li X, Wang H, Bao Y. Molecular cytogenetic identification of a novel wheat-*Thinopyrum ponticum* $1J^{s}$(1B) substitution line resistant to powdery mildew and leaf rust. Front Plant Sci. 2021;12:727734. https://doi.org/10.3389/fpls.2021.727734.
5. Yang G, Deng P, Ji W, Fu S, Li H, Li B, Li Z, Zheng Q. Physical mapping of a new powdery mildew resistance locus from *Thinopyrum ponticum* chromosome 4AgS. Front Plant Sci. 2023;14:1131205. https://doi.org/10.3389/fpls.2023.1131205.
6. Wu H, Kang Z, Li X, Li Y, Li Y, Wang S, Liu D. Identification of wheat leaf rust resistance genes in chinese wheat cultivars and the improved germplasms. Plant Dis. 2020;104(10):2669–80. https://doi.org/10.1094/PDIS-12-19-2619-RE.
7. Li Y, Wei ZZ, Sela H, Govta L, Klymiuk V, Roychowdhury R, Chawla HS, Ens J, Wiebe K, Bocharova V, et al. Dissection of a rapidly evolving wheat resistance gene cluster by long-read genome sequencing accelerated the cloning of *Pm69*. Plant commun. 2024;5(1):100646. https://doi.org/10.1016/j.xplc.2023.100646.
8. Kolmer JA, Bajgain P, Rouse MN, Li J, Zhang P. Mapping and characterization of the recessive leaf rust resistance gene *Lr83* on wheat chromosome arm 1DS. Theor Appl Genet. 2023;136(5):115. https://doi.org/10.1007/s00122-023-04361-7.
9. Li XJ, Jiang XL, Chen XD, Song J, Ren CC, Xiao YJ, Gao XH, Ru ZG. Molecular cytogenetic identification of a novel wheat-*Agropyron elongatum* chromosome translocation line with powdery mildew resistance. PLoS ONE. 2017;12(9):e0184462. https://doi.org/10.1371/journal.pone.0184462.
10. Wang YZ, Cao Q, Zhang JJ, Wang SW, Chen CH, Wang CY, Zhang H, Wang YJ, Ji WQ. Cytogenetic analysis and molecular marker development for a new wheat-*Thinopyrum ponticum* $1J^{s}$(1D) disomic substitution line with resistance to stripe rust and powdery mildew. Front Plant Sci. 2020;11:1282. https://doi.org/10.3389/fpls.2020.01282.
11. Li MZ, Yuan YY, Ni F, Li XF, Wang HG, Bao YG. Characterization of two wheat-*Thinopyrum ponticum* introgression lines with pyramiding resistance to powdery mildew. Front Plant Sci. 2022;13:943669. https://doi.org/10.3389/fpls.2022.943669.
12. Li HJ, Wang XM. *Thinopyrum ponticum* and *Th. intermedium*: the promising source of resistance to fungal and viral diseases of wheat. J Genet Genomics. 2009;36(9):557–65. https://doi.org/10.1016/S1673-8527(08)60147-2.
13. Martynov SP, Dobrotvorskaya TV, Krupnov VA. Genealogical analysis of the use of two wheatgrass (*Agropyron*) species in common wheat (*Triticum aestivum* L.) breeding for disease resistance. Russ J Genet. 2016;52(2):154–63. https://doi.org/10.1134/S1022795416020071.
14. Yang GT, Boshoff WHP, Li HW, Pretorius ZA, Luo QL, Li B, Li ZS, Zheng Q. Chromosomal composition analysis and molecular marker development for the novel Ug99-resistant wheat-*Thinopyrum ponticum* translocation line WTT34. Theor Appl Genet. 2021;134(5):1587–99. https://doi.org/10.1007/s00122-021-03796-0.
15. Zhan H, Li G, Zhang X, Li X, Guo H, Gong W, Jia J, Qiao L, Ren Y, Yang Z, et al. Chromosomal location and comparative genomics analysis of powdery mildew resistance gene *Pm51* in a putative wheat-*Thinopyrum ponticum* introgression line. PLoS ONE. 2014;9(11):e113455. https://doi.org/10.1371/journal.pone.0113455.
16. Wang H, Sun S, Ge W, Zhao L, Hou B, Wang K, Lyu Z, Chen L, Xu S, Guo J, et al. Horizontal gene transfer of *Fhb7* from fungus underlies *Fusarium* head blight resistance in wheat. Science. 2020;368(6493):eaba5435. https://doi.org/10.1126/science.aba5435.
17. Chen SQ, Gao Y, Zhu X, Zhang C, Cao WG, Fedak G, He ZT, Chen XL, Chen JM. Development of E-chromosome specific molecular markers for *Thinopyrum elongatum* in a wheat background. Crop Sci. 2015;55(6):2777–85. https://doi.org/10.2135/cropsci2014.08.0539.
18. Lou H, Dong L, Zhang K, Wang DW, Zhao M, Li Y, Rong C, Qin H, Zhang A, Dong Z, et al. High-throughput mining of E-genome-specific SNPs for characterizing *Thinopyrum elongatum* introgressions in common wheat. Mol Ecol Resour. 2017;17(6):1318–29. https://doi.org/10.1111/1755-0998.12659.

19. Gaal E, Valarik M, Molnar I, Farkas A, Linc G. Identification of COS markers specific for *Thinopyrum elongatum* chromosomes preliminary revealed high level of macrosyntenic relationship between the wheat and *Th. elongatum* genomes. Plos One. 2018;13(12):e0208840. https://doi.org/10.1371/journal.pone.0208840.
20. Liu L, Luo Q, Teng W, Li B, Li H, Li Y, Li Z, Zheng Q. Development of *Thinopyrum ponticum*-specific molecular markers and FISH probes based on SLAF-seq technology. Planta. 2018;247(5):1099–108. https://doi.org/10.1007/s00425-018-2845-6.
21. Huang X, Zhu M, Zhuang L, Zhang S, Wang J, Chen X, Wang D, Chen J, Bao Y, Guo J, et al. Structural chromosome rearrangements and polymorphisms identified in Chinese wheat cultivars by high-resolution multiplex oligonucleotide FISH. Theor Appl Genet. 2018;131(9):1967–86. https://doi.org/10.1007/s00122-018-3126-2.
22. Zheng Q, Li B, Mu S, Zhou H, Li Z. Physical mapping of the blue-grained gene(s) from *Thinopyrum ponticum* by GISH and FISH in a set of translocation lines with different seed colors in wheat. Genome. 2006;49(9):1109–14. https://doi.org/10.1139/g06-073.
23. Liu L, Luo Q, Li H, Li B, Li Z, Zheng Q. Physical mapping of the blue-grained gene from *Thinopyrum ponticum* chromosome 4Ag and development of blue-grain-related molecular markers and a FISH probe based on SLAF-seq technology. Theor Appl Genet. 2018;131(11):2359–70. https://doi.org/10.1007/s00122-018-3158-7.
24. Mukai Y, Endo TR, Gill BS. Physical mapping of the 5S rRNA multigene family in common wheat. J Hered. 1990;81(4):290–5. https://doi.org/10.1093/oxfordjournals.jhered.a110991.
25. Yu Z, Wang H, Xu Y, Li Y, Lang T, Yang Z, Li G. Characterization of chromosomal rearrangement in new wheat—*Thinopyrum intermedium* addition lines carrying *Thinopyrum*—specific grain hardness genes. Agronomy-Basel. 2019;9(1):18. https://doi.org/10.3390/agronomy9010018.
26. Li L, Sun F, Wu D, Zhen F, Bai G, Gao D, Li T. High-throughput development of genome-wide locus-specific informative SSR markers in wheat. Sci China Life Sci. 2017;60(6):671–3. https://doi.org/10.1007/s11427-016-0252-x.
27. An D, Ma P, Zheng Q, Fu S, Li L, Han F, Han G, Wang J, Xu Y, Jin Y, et al. Development and molecular cytogenetic identification of a new wheat-rye 4R chromosome disomic addition line with resistances to powdery mildew, stripe rust and sharp eyespot. Theor Appl Genet. 2019;132(1):257–72. https://doi.org/10.1007/s00122-018-3214-3.
28. Wu N, Lei YH, Pei D, Wu H, Liu X, Fang JX, Guo JT, Wang CL, Guo J, Zhang JL, et al. Predominant wheat-alien chromosome translocations in newly developed wheat of China. Mol Breed. 2021;41(4):30. https://doi.org/10.1007/s11032-021-01206-3.
29. He F, Xu JQ, Qi XL, Bao YG, Li XF, Zhao FT, Wang HG. Molecular cytogenetic characterization of two partial wheat amphiploids resistant to powdery mildew. Plant Breed. 2013;132(6):553–7. https://doi.org/10.1111/pbr.12104.
30. Zhou S, Zhang J, Che Y, Liu W, Lu Y, Yang X, Li X, Jia J, Liu X, Li L. Construction of *Agropyron* Gaertn. genetic linkage maps using a wheat 660K SNP array reveals a homoeologous relationship with the wheat genome. Plant Biotechnol J. 2018;16(3):818–27. https://doi.org/10.1111/pbi.12831.
31. Du X, Feng XB, Li RX, Jin YL, Shang LH, Zhao JX, Wang CY, Li TD, Chen CH, Tian ZR, et al. Cytogenetic identification and molecular marker development of a novel wheat-*Leymus mollis* 4Ns(4D) alien disomic substitution line with resistance to stripe rust and Fusarium head blight. Front Plant Sci. 2022;13:1012939. https://doi.org/10.3389/fpls.2022.1012939.
32. Jia H, Feng H, Yang G, Li H, Fu S, Li B, Li Z, Zheng Q. Establishment and identification of six wheat-*Thinopyrum ponticum* disomic addition lines derived from partial amphiploid Xiaoyan 7430. Theor Appl Genet. 2022;135(9):3277–91. https://doi.org/10.1007/s00122-022-04185-x.
33. Chen S, Huang Z, Dai Y, Qin S, Gao Y, Zhang L, Gao Y, Chen J. The development of 7E chromosome-specific molecular markers for *Thinopyrum elongatum* based on SLAF-seq technology. PLoS ONE. 2013;8(6):e65122. https://doi.org/10.1371/journal.pone.0065122.
34. Chen SQ, Qin SW, Huang ZF, Dai Y, Zhang LL, Gao YY, Gao Y, Chen JM. Development of specific molecular markers for *Thinopyrum elongatum* chromosome using SLAF-seq technique. Acta Agron Sin. 2013;39(4):727–34. (in Chinese with English abstract). http://www.chinacrops.org/zwxb/.
35. Hou L, Jia J, Zhang X, Li X, Yang Z, Ma J, Guo H, Zhan H, Qiao L, Chang Z. Molecular mapping of the stripe rust resistance gene *Yr69* on wheat chromosome 2AS. Plant Dis. 2016;100(8):1717–24. https://doi.org/10.1094/PDIS-05-15-0555-RE.
36. Mo Q, Wang CY, Chen CH, Wang YJ, Zhang H, Liu XL, Ji WQ. Molecular cytogenetic identification of a wheat-*Thinopyrum ponticum* substitution line with stripe rust resistance. Cereal Res Commun. 2017;45(4):564–73. https://doi.org/10.1556/0806.45.2017.037.
37. Xu X, Ni Z, Zou X, Zhang Y, Tong J, Xu X, Dong Y, Han B, Li S, Wang D, et al. QTL mapping reveals both all-stage and adult-plant resistance to powdery mildew in chinese elite wheat cultivars. Plant Dis. 2023;107(10):3230–7. https://doi.org/10.1094/PDIS-02-23-0399-RE.
38. Gupta SK, Charpe A, Prabhu KV, Haque QMR. Identification and validation of molecular markers linked to the leaf rust resistance gene *Lr19* in wheat. Theor Appl Genet. 2006;113(6):1027–36. https://doi.org/10.1007/s00122-006-0362-7.
39. Gupta SK, Charpe A, Koul S, Haque QMR, Prabhu KV. Development and validation of SCAR markers co-segregating with an *Agropyron Elongatum* derived leaf rust resistance gene *Lr24* in wheat. Euphytica. 2006;150(1–2):233–40. https://doi.org/10.1007/s10681-006-9113-8.
40. Tar M, Purnhauser L, Csosz L, Mesterházy, Gyulai G. Identification of molecular markers for an efficient leaf rust resistance gene (*Lr29*) in wheat. Acta Biol Szeged. 2002;46(3):133–4. http://www.sci.u-szeged.hu/ABS.
41. Wang DY, Du P, Pei ZY, Zhuang LF, Qi ZJ. Development and application of high resolution karyotypes of wheat "Chinese Spring" aneuploids. Acta Agron Sin. 2017;43(11):1575–87. (in Chinese with English abstract). http://zwxb.chinacrops.org/.
42. Komuro S, Endo R, Shikata K, Kato A. Genomic and chromosomal distribution patterns of various repeated DNA sequences in wheat revealed by a fluorescence *in situ* hybridization procedure. Genome. 2013;56(3):131–7. https://doi.org/10.1139/gen-2013-0003.
43. Baker L, Grewal S, Yang CY, Hubbart-Edwards S, Scholefield D, Ashling S, Burridge AJ, Przewieslik-Allen AM, Wilkinson PA, King IP, et al. Exploiting the genome of *Thinopyrum elongatum* to expand the gene pool of hexaploid wheat. Theor Appl Genet. 2020;133(7):2213–26. https://doi.org/10.1007/s00122-020-03591-3.
44. Chen JY, Tang YQ, Yao LS, Wu H, Tu XY, Zhuang LF, Qi ZJ. Cytological and molecular characterization of *Thinopyrum bessarabicum* chromosomes and structural rearrangements introgressed in wheat. Mol Breed. 2019;39(11):146. https://doi.org/10.1007/s11032-019-1054-8.
45. Chen PD, Qi LL, Zhou B, Zhang SZ, Liu DJ. Development and molecular cytogenetic analysis of wheat-*Haynaldia villosa* 6VS/6AL translocation lines specifying resistance to powdery mildew. Theor Appl Genet. 1995;91(6–7):1125–8. https://doi.org/10.1007/BF00223930.
46. Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW. Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA. 1984;81(24):8014–8. https://doi.org/10.1073/pnas.81.24.8014.
47. Pu J, Wang Q, Shen YF, Zhuang LF, Li CX, Tan MF, Bie TD, Chu CG, Qi ZJ. Physical mapping of chromosome 4J of *Thinopyrum bessarabicum* using gamma radiation-induced aberrations. Theor Appl Genet. 2015;128(7):1319–28. https://doi.org/10.1007/s00122-015-2508-y.
48. Zhao RH, Liu BL, Wan WT, Jiang ZN, Chen TT, Wang L, Bie TD. Mapping and characterization of a novel adult-plant leaf rust resistance gene *LrYang16G216* via bulked segregant analysis and conventional linkage method. Theor Appl Genet. 2023;136(1):1–13. https://doi.org/10.1007/s00122-023-04270-9.
49. Zhang PP, Yan XC, Gebrewahid TW, Zhou Y, Yang EN, Xia XC, He ZH, Li ZF, Liu DQ. Genome-wide association mapping of leaf rust and stripe rust resistance in wheat accessions using the 90K SNP array. Theor Appl Genet. 2021;134(4):1233–51. https://doi.org/10.1007/s00122-021-03769-3.

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