

Wheat leaf rust resistance conferred by the terminal region of *Thinopyrum intermedium* chromosome 7J^sS

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Research Article

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Abstract

Partial amphiploid Xiaoyan 78829 contains 14 *Thinopyrum intermedium* chromosomes and exhibits excellent resistance to fungal disease. To transfer this resistance to common wheat, a translocation line WTT26 was developed by pollen irradiation. Disease evaluation showed that WTT26 was nearly immune to 14 common races of *Puccinia triticina*, highly resistant to *P. graminis* f. sp. *tritici* Ug99 race PTKST at the seedling stage as well as to *Blumeria graminis* f. sp. *tritici* under field conditions at the adult plant stage. Cytogenetic identification, single nucleotide polymorphism array analysis and *Th. intermedium*-specific marker amplification, revealed that WTT26 carries the T4BS·7J^SS translocation. Novel translocation lines with desirable yield characters and excellent leaf rust resistance was developed using irradiation. A total of 124 specific markers were developed using specific-locus amplified fragment sequencing to further enrich the marker information of chromosome 7J^SS. Furthermore, a refined physical map of chromosome 7J^SS that contains 74 specific markers was constructed with its dissection into six physical regions. Combining data from specific marker amplification and resistance evaluation resulted in a new leaf rust resistance locus mapped in the 0–69.29 Mb region on chromosome 7J^SS. These new developed translocation lines and markers will contribute to wheat breeding for disease resistance.

Key message

A terminal segment (0-69.29 Mb) of *Thinopyrum intermedium* chromosome 7J^SS contains a novel leaf rust resistance locus, confers wheat excellent leaf rust resistance and will contribute to wheat breeding.

Introduction

Common wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD) is one of the three most important food crops worldwide, which provides at least 20% of the total calories consumed by humankind (Fu et al. 2009; Rahmatov et al. 2016). Three ancestors, *Triticum urartu* Thum. ex Gandilyan ($2n = 2x = 12$, AA), *Aegilops speltoides* Tausch ($2n = 2x = 12$, SS) and *Aegilops tauschii* Coss. ($2n = 2x = 12$, DD), sequentially hybridized to form common wheat (McFadden and Sears, 1946; Qi et al. 2007). Polyploidization, domestication, and natural and artificial selections have reduced genetic diversity in modern wheat (Tanksley and McCouch, 1997; Hao et al. 2020; Wang et al. 2021), and current wheat cultivars are therefore considered more vulnerable to biotic and abiotic stresses (Fatima et al. 2020). Diseases and pests, two major biotic stresses, have become major limitations in wheat production and reduce the projected harvest of the world by an average of 21% per year (Savary et al. 2019; Hafeez et al. 2021). The top four fungal diseases, including leaf rust, *Fusarium* head blight, septoria tritici blotch and stripe rust, account for over half of this loss (Hafeez et al. 2021).

Leaf rust, caused by *Puccinia triticina* Eriks. (*Pt*), is one of the most common diseases of wheat throughout the world due to its frequent and widespread occurrence in most of the wheat-growing regions around the world (Kolmer, 2013). Leaf rust occurs severely in areas where dew is frequent during the jointing through flowering stages. Suitable conditions for the epidemic development of leaf rust include

mild temperatures during daytime and adequate moisture at night (Xiao et al. 2022). *Pt* damages wheat foliage by nutrient depletion, cell death and by covering the leaf surface with a consequent disruption in photosynthetic activities, which in turn causes premature senescence and finally leads to decreased grain filling (Fatima et al. 2020). Yield losses may typically vary between trace to 30% and even exceed 50% in susceptible wheat cultivars (Lin et al. 2022). Developing germplasm with novel sources of resistance through gene mining and the transfer thereof into susceptible varieties can mitigate wheat yield reductions (Feuillet et al. 2003).

To date, more than 80 leaf rust (*Lr*) resistance genes have been identified and documented in wheat and its relatives (Kumar et al. 2021; Zhang et al. 2021). This include seven cloned race-specific *Lr* genes namely *Lr1* (Cloutier et al. 2007), *Lr10* (Feuillet et al. 2003), *Lr13* (Hewitt et al. 2021; Yan et al. 2021), *Lr21* (Huang et al. 2003), *Lr22a* (Thind et al. 2017), and *Lr42* (Lin et al. 2022) encoding nucleotide-binding and leucine-rich repeat (NLR) proteins as well as *Lr14a* (Kolodziej et al. 2021) encoding a membrane-localized protein containing twelve ankyrin repeats. Two cloned race-nonspecific *Lr* genes are *Lr34/Yr18/Pm38/Sr57* (Krattinger et al. 2009) encoding an adenosine triphosphate-binding cassette (ABC) transporter and *Lr67/Yr46/Pm46/Sr55* (Moore et al. 2015) encoding a predicted hexose transporter. Additionally, approximately half of the current designated *Lr* genes come from the secondary and tertiary gene pools of common wheat (McIntosh et al. 2016; Bansal et al. 2017; Jiang et al. 2018). In particular, *Lr25* (Singh et al. 2012), *Lr26* (Singh et al. 1990) and *Lr45* (Friebe et al. 1996) were transferred from *Secale cereale* L. ($2n = 2x = 14$, RR) into wheat. Resistance genes *Lr19* (Sarma and Knott, 1966), *Lr24* (Hart et al. 1976), and *Lr29* (McIntosh, 1988; Dyck et al. 1994) originates from *Thinopyrum ponticum* and *Lr38* (Friebe et al. 1992) from *Th. intermedium*.

As a perennial forage grass in the tribe Triticeae, *Th. intermedium* (Host) Barkworth and D.R. Dewey [syn. = *Agropyron intermedium* (Host) Beauvoir = *Elytrigia intermedia* (Host) Nevski] ($2n = 6x = 42$), is considered a valuable source for wheat improvement because of its good disease resistance, strong stress tolerance and high crossability with common wheat (Shannon, 1978; Sharma et al. 1989; Cox, 1991; McIntosh, 1991; Li and Wang, 2009; Zheng et al. 2014). The genomic composition of *Th. intermedium* has been investigated for many years and various hypotheses have been proposed. It is generally believed that *Th. elongatum* ($2n = 2x = 14$, E^eE^e or J^eJ^e), *Th. bessarabicum* ($2n = 2x = 14$, E^bE^b or J^bJ^b) and *Pseudoroegneria spicata* ($2n = 2x = 14$, StSt) are probably the donor species of *Th. intermedium* (Zhang et al. 1997; Chen et al. 1998; Liu et al. 2018; Qiao et al. 2021). To map important disease-resistance genes within *Th. intermedium*, Kantarski et al. (2017) constructed the first consensus genetic map containing all *Th. intermedium* linkage groups (*Thi*-LG) using genotyping-by-sequencing (GBS). In detail, this map contains 21 LGs and 10,029 markers. The number of markers on each LG ranges from 237 to 683, and the cumulative length covers 5061 cM with an average distance of 0.5 cM between each pair of markers. Referring to this genetic map and a set of 550,885 75mer markers, obtained scaffolds were oriented, ordered and joined together to form the 21 chromosomes (https://phytozome-next.jgi.doe.gov/info/Tintermedium_v2_1). Since the genome identity of every *Thi*-LG had been unknown, Wang et al. (2020) compared GBS sequences between *Ps. spicata* and *Th. intermedium*, and revealed

that the *Thi*-LG2, 4, 8, 11, 13, 17 and 21 belonged to St genome. Qiao et al. (2021) used annotated Core Data Services (CDS) of *Th. elongatum* to align with *Th. intermedium* GBS sequences and concluded that *Thi*-LG1, 6, 7, 10, 14, 18 and 20 could be assigned to J^e genome and the rest of the chromosomes to the J^s genome. Furthermore, 745 *Thi*-specific sequence-tagged site (STS) markers were developed and verified, which could rapidly and economically identify *Th. intermedium* chromosomes in wheat. Using HiFiAsm, RACON and JUICER pipeline, the resulting sequences were polished and contigs were then oriented, ordered and joined together to form the final assembly consisting of 21 chromosomes with 99.992% of the assembled sequences. These 21 chromosomes were numbered and oriented based on the subgenome group's J, S and V (https://phytozome-next.jgi.doe.gov/info/Tintermedium_v3_1).

Our group have focused on the creation and application of wheat–*Th. intermedium* translocation lines with good disease resistance and excellent agronomic performance. In the present study, distant hybridization between common wheat and the wheat–*Th. intermedium* partial amphiploid Xiaoyan 78829 was conducted to develop disease resistant germplasm with applied value to wheat improvement. A new wheat–*Th. intermedium* translocation line with leaf rust, stem rust and powdery mildew resistance was produced. The origin of the alien chromosomal segment was characterized by cytogenetic identification, single nucleotide polymorphism (SNP) microarray analysis and *Thi*-specific marker amplification. To enrich the sequence information of chromosome 7J^sS, specific-locus amplified fragment sequencing (SLAF-seq) technology was also carried out to develop *Th. intermedium* specific markers. A relatively refined physical map of chromosome 7J^sS was constructed and a novel *Lr* locus was mapped using a series of smaller-fragment translocation lines. Moreover, this study might provide important insights into breeding elite germplasms with harmonious multi-disease resistance and high-yielding potential.

Materials and methods

Plant materials and rust races

WTT26 is a wheat–*Th. intermedium* BC₃F₉ translocation line with pedigree Xiaoyan81*4/3/Xiaoyan101//Misui/Xiaoyan78829 (Fig. S1). The pedigree of WTT11 was reported in a previous study (Yang et al. 2021). J26 was developed from the cross Jimai22*3/WTT26. Five novel translocation lines (RWTT26-1, RWTT26-3, RWTT26-6, RWTT26-10 and RWTT26-11) were obtained using pollen irradiation. Seed of *Th. intermedium* (PI401208), partial amphiploid Xiaoyan 78829, and wheat cultivars Misui, Xiaoyan 101, Xiaoyan 81, Jimai 22 and Chinese Spring (CS) are preserved in the laboratory of Zhensheng Li, Institute of Genetics and Developmental Biology (IGDB), Innovative Academy of Seed Design, Chinese Academy of Sciences (CAS), Beijing, China. A total of 35 near-isogenic lines (NILs) with Thatcher background, Thatcher, and 14 isolates of *Pt* races THTT, PHNT, PHTT, PGTH, FHKT, PHSQ, PHTQ, THKQ, PHTS, THST, THQS, THJS, NHTT and THKT, were provided by the College of Plant Protection, Hebei Agricultural University. The wheat line Federation*4/Kavkaz and the isolate of *Puccinia*

graminis f. sp. *tritici* Eriks. & E. Henn (*Pgt*) Ug99 race PTKST is maintained at the University of the Free State, Bloemfontein, South Africa.

Cytogenetic analyses

After the chromosome spreads were prepared, genomic *in situ* hybridization (GISH) and multi-color fluorescence *in situ* hybridization (mc-FISH) and mc-GISH were carried out following the procedures of Han et al. (2006, 2009) with some modification. The probe for GISH was made by means of *Th. intermedium* genomic DNA (gDNA) labelled with Alexa Fluor-488-dUTP (green), while the blocker was prepared with CS gDNA. Then, two repetitive sequences, pAs1 isolated from *Ae. tauschii* and pSc119.2 isolated from *S. cereale* were labeled with Texas-red-5-dCTP (red) and Alexa Fluor-488-dUTP (green) as probes in mc-FISH analysis respectively (Rayburn and Gill, 1986; McIntyre et al. 1990). Following mc-FISH analysis, the same cells were used to perform mc-GISH analysis. The *Th. intermedium* and *T. urartu* gDNA labeled with fluorescein-12-dUTP (green) and *Ae. tauschii* gDNA labeled with Texas-red-5-dCTP (red) were used as probes, whereas the *Ae. speltoides* gDNA was used as a block. After hybridization, slides were washed in 2× saline sodium citrate (SSC) buffer and then counterstained with 4, 6-diamidino-2-phenylindole. Cells with clear hybridization signals were photographed with a DP80 CCD camera attached to an Olympus BX53 microscope and analyzed using the program CellSens Standard 1.12 (Olympus, Tokyo, Japan).

Leaf rust resistance evaluation

Ten seedlings from the lines Xiaoyan 81 (susceptible control), WTT26, Xiaoyan 78829, Misui, Xiaoyan 101, J26, RWTT26-1, RWTT26-3, RWTT26-6, RWTT26-10, RWTT26-11 and Jimai 22 were grown in a growth chamber to assess their leaf rust response. At the two-leaf stage, inoculation was performed by spraying urediniospores of *Pt* race THTT onto the seedling leaves. Following the seedlings were immediately incubated in a chamber in darkness at 20°C and 100% relative humidity (RH) for 16 h. Subsequently the seedlings were transferred to a greenhouse under a 12 h light/12 h darkness cycle at 22 to 25°C and 70% RH. When Xiaoyan 81 showed severe disease symptoms on its leaves, infection types (ITs) were recorded according to the Stakman scale as modified by Roelfs et al. (1992). In details, “0” represents immunity, no visible uredinia and necrosis on leaves; “;” represents nearly immune, no visible uredinia with hypersensitive flecks on leaves; “1” represents highly resistant, small uredinia surrounded by necrosis; “2” represents moderate resistance, small to medium uredinia surrounded by necrosis; “3” represents moderate susceptible, medium uredinia without chlorosis or necrosis; and “4” represents highly susceptible, large uredinia without chlorosis or necrosis. Plants with IT scores 0–2 were considered resistant and 3–4 as susceptible.

To postulate *Lr* genes in WTT26, wheat line Thatcher was used as susceptible control. Isolates of 13 *Pt* races were used for rust assessment at the seedling stage. A set of 30 NILs carrying documented genes were used as controls. The inoculation and evaluation procedures followed were as described above.

Stem rust resistance evaluation

Assessment of the stem rust response of WTT26, its parents Xiaoyan 78829, Misui, Xiaoyan 101 and Xiaoyan 81 as well as the susceptible control Federation*4/Kavkaz was conducted with an isolate of *Pgt* Ug99 race PTKST at the University of the Free State, Bloemfontein, South Africa. Ten seedlings were assessed for each of the lines. Inoculation and evaluation were performed as described by Li et al. (2016). ITs were applied according to the system of Stakman et al. (1962), in which 2 or lower indicate resistance, 3 to 4 indicate susceptible, the plus (+) and minus (-) signs indicate larger or smaller than expected pustules, respectively.

Wheat660K SNP array analysis

The gDNAs of WTT26, three replicates of Xiaoyan 81 and *Th. intermedium* were extracted and genotyped using the 660K wheat Affymetrix Axiom SNP array in the CapitalBio Technology Company (Beijing, China). SNPs were classified into six categories orderly as follows: Poly High Resolution, No Minor Homozygote, Mono High Resolution, Call Rate Below Threshold, Off-Target Variant and Other depending on their performance metrics. All SNPs with certain physical positions that were absent from the WTT26 genome but present in the three replicates of the Xiaoyan 81 genome were collected. The deleted ratio of each chromosome was calculated in 3-Mb sliding windows, with 1-Mb steps. Regions in which the deletion ratios were statistically higher than the average value for a given chromosome except for marker-deficient regions, were inferred to have experienced SNP elimination events. Homozygous SNPs showing pleomorphism between Xiaoyan 81 and *Th. intermedium*, especially those belonging to Poly High Resolution and No Minor Homozygote categories, were used to analyze the homoeologous group of alien segments. The ratio of heterozygous SNPs of WTT26 originating from *Th. intermedium* and Xiaoyan 81 was counted in 50-Mb sliding windows, with 1-Mb steps. The alien segments of WTT26 were homoeologous to the proportion of wheat chromosomes with highest heterozygosity.

Specific molecular marker analysis

In a previous study, 745 *Thi*-specific STS markers were developed using sequence alignment and amplification verification, which were only amplified in the *Th. intermedium* genome but not in the eight tested wheat landraces (Qiao et al. 2021). Specific STS markers were selected and amplified in the genomes of Xiaoyan 78829 and translocation lines WTT26, WTT11, RWTT26-1, RWTT26-3, RWTT26-6, RWTT26-10 and RWTT26-11 to identify their involved *Th. intermedium* chromosomes and to physically map the *Lr* resistance gene. The PCR amplification and detection were carried out according to Qiao et al. (2021).

Specific marker development and verification

The gDNAs of WTT26, Xiaoyan 78829 and *Th. intermedium* were extracted and sequenced using SLAF-seq. The SLAFs of WTT26, Xiaoyan 78829 and *Th. intermedium* were obtained after sequence identification, filtration, cluster, and correction (Chen et al. 2013a, b). To obtain the alien specific SLAFs, the SLAFs of WTT26 were firstly compared to the genomic sequences of CS (IWGSC RefSeq v1.0; IWGSC et al. 2018), A genome (Ling et al. 2018) and D genome (Zhao et al. 2017), and the low homoeologous (< 50%) ones were selected. Following comparison with the SLAFs of *Th. intermedium*, the high homoeologous (> 80%) ones were acquired. These SLAFs were then compared to the SLAFs of Xiaoyan 78829 and the high homoeologous (> 90%) ones were selected as the alien specific SLAFs of WTT26. Finally, based on their sequence information, primers were designed in <https://bioinfo.ut.ee/primer3-0.4.0/> and synthesized in Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). The PCR amplification system and separation of products were conducted according to Yang et al. (2021). Markers that amplified bands with the same sizes in WTT26, Xiaoyan 78829 and *Th. intermedium*, but not in wheat cultivars Misui, Xiaoyan 101, Xiaoyan 81 and CS, were identified as alien specific markers of WTT26.

In order to obtain physical positions, the PCR products of WTT26 were sequenced and compared to the *Th. intermedium* genome v3.1. These markers were further amplified in the gDNAs of RWTT26-1, RWTT26-3, RWTT26-6, RWTT26-10 and RWTT26-11 to determine the chromosomal regions of their alien segments. The amplification reaction system and procedure were the same as above.

Agronomic traits evaluation

During the growing season (2021–2022), two novel translocation lines RWTT26-3 and RWTT26-11 and their recurrent parent Jimai 22 were grown in triplicate in trial plots at the Changping Experiment Station, IGDB, Beijing, China (116.2°E, 40.6°N). Twenty seeds were sown per 2.0 m row with an inter-row spacing of 0.2 m. Five plants located in the center of the middle row of each plot were selected to determine agronomic traits at physiological maturity. These included plant height (PH), effective tiller number (ETN), kernel number per main spike (KNMS), total kernel number (TKN), thousand-kernel weight (TKW) and yield per plant (YPP). The mean value and standard deviation were calculated using SPSS version 19.0 software (IBM, New York, USA). Significant difference was determined by the Student's *t* test. One-way ANOVA analysis was conducted using data processing system (Tang and Zhang, 2013).

Results

Cytogenetic identification of WTT26

GISH analysis showed that WTT26 is a stable line with 44 chromosomes including 42 intact wheat chromosomes with blue counterstained coloration and two wheat–*Th. intermedium* whole-arm translocated chromosomes with green fluorescence signals on their long arms (Fig. 1a). The mc-FISH analysis revealed the pAs1- and pSc119.2-hybridization pattern on wheat chromosomes agreed in general with standard karyotype (Tang et al. 2014) except that the strong pSc119.2 band was missing

owing to the absence of the terminal of chromosome 4BS. Strong pSc119.2 and dispersive pAs1 signals were detected on the short and long arms of the translocated chromosome, respectively (Fig. 1b). Therefore, we speculated that chromosome 4B was broken in its short arm and the smaller segment was fused to the alien chromosome segment to produce the novel translocated chromosome. The mc-GISH showed that WTT26 carries 12 A genome, 14 B genome and 14 D genome chromosomes and two pairs of translocated chromosomes. One pair of translocated chromosomes involved the B and *Th. intermedium* genomes and the other involved A and B genomes as in CS (Fig. 1c).

Novel broad-spectrum *Lr* locus from the alien segment of WTT26

The seedling ITs for WTT26 and its parents were scored when susceptible control Xiaoyan 81 was fully infected (IT = 4). Xiaoyan 78829 was immune (IT = 0) and WTT26 nearly immune (IT = ;) to *Pt* race THTT. The wheat cultivars Misui and Xiaoyan 101 were both highly susceptible to THTT (ITs = 4) (Fig. 2a). Pedigree analysis suggested that the leaf rust resistance of WTT26 was derived from Xiaoyan 78829. Subsequently, the alien segment of WTT26 was transferred into another leaf rust-susceptible cultivar Jimai 22 to produce a novel translocation line J26 by conventional crossing, backcrossing and cytogenetic identification. Leaf rust assessment showed that the recurrent parent Jimai 22 was highly susceptible to *Pt* race THTT (IT = 4), whereas J26 was nearly immune (IT = ;) (Table 1), indicating that the *Lr* locus of WTT26 originated from its alien segment.

The seedling reactions of WTT26, 35 NILs carrying documented *Lr* genes and the susceptible control Thatcher to 13 *Pt* races were simultaneously recorded. Thatcher were highly susceptible to all *Pt* races tested (IT = 4). Three lines, including WTT26, TcLr19 and TcLr47, were all immune (IT = 0) or nearly immune (IT = ;). Specifically, WTT26 was nearly immune (IT = ;) to all 13 *Pt* races. TcLr19 was immune (IT = 0) to races PHTS and THQS and nearly immune (IT = ;) to isolates of the other 11 races. TcLr47 was immune (IT = 0) to races PGTH and FHKT and nearly immune (IT = ;) to the remaining 11 races. In addition, TcLr38 was immune (IT = 0) to race THKT, nearly immune (IT = ;) to PHTT, PHSQ, PHTQ, THKQ, PHTS, THST and NHTT, highly resistant (IT = 1) to PGTH and THJS, moderate resistant (IT = 2) to PHNT and FHKT and moderate susceptible (IT = 3) to THQS (Table S1). Thus, it is deduced that the *Lr* locus of WTT26 was broad-spectrum and different from *Lr38*.

The seedling response of WTT26 and its parents to *Pgt* race PTKST was recorded when the susceptible control Federation*4/Kavkaz was severely infected (IT = 3+). WTT26 was highly resistant to PTKST (IT = ;1). Xiaoyan 78829 was nearly immune to infection (IT = ;). Other wheat parents, i.e., Misui, Xiaoyan 101 and Xiaoyan 81, were susceptible (ITs ranging from 3 to 3+) (Fig. 2b, Table 1). Additionally, WTT26 was found resistant to powdery mildew at the adult plant stage under field conditions (Fig. 2c). However, J26 was susceptible to powdery mildew in the field, indicating that the *Pm* locus of WTT26 was not from its alien segment. Overall, WTT26 carried *Lr* and *Sr* loci conferring seedling resistance and a *Pm* locus conferring adult plant resistance. Among these loci, the *Lr* locus was proved to come from its alien segment and the *Pm* locus was proved to come from its wheat chromosomes.

The identification of alien chromosomal segments

Wheat660K SNP array was used for genotyping the genomes of WTT26, three replicates of Xiaoyan 81 and *Th. intermedium* following a standard procedure. A total of 645,395 out of 660,009 SNP markers with precise physical positions were used to analyze elimination events. Among them, 3,678 SNPs were absent in the WTT26 genome and present in three replicates of Xiaoyan 81. Sliding window showed that no obvious SNP elimination event occurred in the genome of WTT26, which was consistent with its mc-FISH result (Fig. S2).

In order to analyze the homoeologous relationship between the alien segments in WTT26 and wheat chromosomes, a total of 371 heterozygous SNPs consistent with the genotypes of *Th. intermedium* and Xiaoyan 81, were selected and counted in 50-Mb sliding windows with 1-Mb steps. The result showed that the peak appeared in the 0–165 Mb region of wheat chromosome 7B (Fig. 3), suggesting that the alien segment originated from group 7 of *Th. intermedium*.

The mc-FISH pattern of chromosome 1 in Xiaoyan 78829 showed that strong pAs1 signals were diffusely distributed on the top half of the short arm and clear pAs1 signals were distributed on the intercalary and bottom half of the long arm (Fig. 4a). Considering the pAs1 signal distributions of alien segments of WTT26 (Fig. 4b) and WTT11 (Fig. 4c), we deduced that chromosome 1 was broken at the centromere and its short arm and long arm were transferred into WTT26 and WTT11, respectively.

Based on homologous group of alien segments of WTT26 and WTT11, the specific STS markers of *Thi*-LG 19, 20 and 21 were selected to amplify the Xiaoyan 78829 genome to determine the identity of chromosome 1. PCR results showed that 9 of 54 (16.67%) 7J-specific markers, 38 of 67 (56.72%) 7J^s-specific markers, and 8 of 62 (12.90%) 7St-specific markers could amplify specific bands in Xiaoyan 78829 (Fig. 5), implying that chromosome 1 was chromosome 7J^s. These 38 7J^s-specific markers were further amplified in the genomes of translocation lines WTT26 and WTT11. Except for one marker that has not any amplification product, 19 and 18 markers amplified specific DNA bands in WTT26 and WTT11, respectively. As such, we confirmed that WTT26 and WTT11 separately carries the short arm and long arm of 7J^s. Thus, translocations T4BS·7J^sS and T7J^sL·2DL occurred in WTT26 and WTT11, respectively.

Development, identification, and resistance evaluation of small fragment translocation lines

Firstly, the irradiated pollen of WTT26 were used to pollinate wheat cv. Jimai 22. The M₁ individuals with smaller alien fragments were then backcrossed twice with Jimai 22. Finally, homozygous translocation lines were picked out from self-bred progenies. The chromosome compositions of these stable lines with smaller translocated alien segments were characterized with GISH and mc-FISH analyses. RWTT26-1 was observed to have 21 pairs of intact wheat chromosomes and a pair of wheat–*Th. intermedium* intercalary translocated chromosomes (Fig. 6a). Strong pSc119.2 bands were detected in the terminal on

the short arm and intercalary regions on the long arm of the translocated chromosome, whereas blurry pAs1 signals were found in the alien chromosomal segment (Fig. 6b). Additionally, the same deletion event of chromosome 4BS was kept as with WTT26. These results suggested that the chromosome segment of 7J^SS in WTT26 was broken and an unknown wheat chromosome segment was connected to the breakpoint to form the translocated chromosome T4BS·7J^SS-W. RWTT26-3 was revealed to carry 40 wheat chromosomes and two wheat–*Th. intermedium* terminal translocated chromosomes (Fig. 6c). An obvious secondary constriction and the typical FISH pattern, i.e., clear pAs1 signals in the terminal and pericentromeric regions on the short arm and strong pSc119.2 signals in the intercalary region on the long arm, proved that the wheat fragment of this new translocation originated from chromosome 6B. The end of 7J^SS with dispersed pAs1 signals adhered to the main part of chromosome 6B to form the translocated chromosome T6BS·6BL·7J^SS (Fig. 6d). RWTT26-6 had 40 wheat chromosomes and a pair of terminal translocated chromosomes (Fig. 6e). Strong and clear pAs1 signals were in the terminal regions on the long and short arms of translocated chromosomes, respectively, indicating that the chromosome segment of 7J^SS connected to the terminal region on wheat chromosome 7DS to form the translocated chromosome T7J^SS·7DS·7DL (Fig. 6f). RWTT26-10 carries 42 wheat chromosomes and a pair of wheat–*Th. intermedium* translocated chromosomes (Fig. 6g). The pSc119.2 signals were observed in the terminal, intercalary and pericentromeric region on the long arm and subterminal region on the short arm and the pAs1 signals were observed in the intercalary region on the long arm and in the terminal region on the short arm, suggesting that the chromosome segment of 7J^SS connected to the terminal region of wheat chromosome 1BS to form the translocated chromosome T7J^SS·1BS·1BL (Fig. 6h). RWTT26-11 contains 42 wheat chromosomes plus two interspecific Robertsonian translocations (Fig. 6i). The obvious pAs1 bands detected on the short arm and strong pSc119.2 signals in the terminal and intercalary regions on the long arm (Fig. 6j), suggested that 7J^SS was translocated to the short arm of 5B to produce the new translocation T7J^SS·5BS.

At the seedling stage, the IT scores were recorded when the *Pt* pustules fully covered the leaves of the susceptible control Xiaoyan 81. Based on the disease symptoms of these five novel translocation lines, RWTT26-3, RWTT26-10 and RWTT26-11 were near immune to *Pt* race THTT (IT = ;), similar to their resistant parent WTT26, whereas RWTT26-1 and RWTT26-6 were highly susceptible (IT = 4) as its recurrent parent Jimai 22 (Fig. 7, Table 1).

Physical mapping of the novel Lr locus

Totally, 648,375, 887,297 and 718,912 effective SLAFs were obtained for WTT26, Xiaoyan 78829 and *Th. intermedium*, respectively. After sequence comparison, 4,750 SLAFs were selected as the specific SLAFs of the alien segment in WTT26. Then, the top 1,000 SLAFs most similar to those of *Th. intermedium* and Xiaoyan 78829 were selected to design primers. Finally, 124 PCR markers were developed, which could amplify specific bands in WTT26, Xiaoyan 78829 and *Th. intermedium* but absent in Misui, Xiaoyan 101, Xiaoyan 81 and CS. The success rate for specific SLAF markers developed was 12.40%.

These 124 amplified products were compared with the chromosome 7J^S (renamed as V07 in *Th. intermedium* genome v3.0) and 55 of them have highly credible physical positions in chromosome 7J^S (Table S2). We used these 55 new markers and 19 7J^S-specific STS markers to amplify the genomes of five translocation lines RWTT26-1, RWTT26-3, RWTT26-6, RWTT26-10 and RWTT26-11. These markers were assigned to the following six physical regions according to the amplification results: 7J^S-1 (0-69.29 Mb), 7J^S-2 (69.29-102.58 Mb), 7J^S-3 (102.58-123.35 Mb), 7J^S-4 (123.35–183.80 Mb), 7J^S-5 (183.80-192.25 Mb) and 7J^S-6 (192.25–505.20 Mb), which contained 16, 16, 6, 9, 4 and 23 markers, respectively (Fig. 7). The five translocation lines included different 7J^S chromosomal bins. Specifically, RWTT26-1 contains bin 7J^S-6 (192.25–505.20 Mb); RWTT26-3 covers bins 7J^S-1–7J^S-3 (0–123.35 Mb); RWTT26-6 contains bin 7J^S-2 (69.29-102.58 Mb); RWTT26-10 contains bin 7J^S-1 (0-69.29 Mb) and RWTT26-11 covers bins 7J^S-1–7J^S-4 (0–183.80 Mb). Combining the IT scores and the alien segments in these five translocation lines, the *Lr* locus was mapped to bin 7J^S-1 with the physical position 0–69.29 Mb on 7J^S. According to the gene annotation, a total of 419 genes (Thint.V07G000100-Thint.V07G041900) were deposited in this region. Among them, 11 genes were annotated as *NLR* genes (Table S3).

Agronomic performance of RWTT26-3 and RWTT26-11

The yield performance of translocation lines RWTT26-3, RWTT26-11 and their recurrent parent Jimai 22 was investigated during the 2021–2022 growing season (Table 2). The PH, ETN and TKN of RWTT26-3 and RWTT26-11 were significantly higher than those of Jimai 22. The KNMS did not produce significant differences. The TKW of RWTT26-11 and Jimai 22 were significantly higher than that of RWTT26-3. Comparing YPP, line RWTT26-11 was significantly higher yielding than RWTT26-3 and Jimai 22. In general, there was no obvious linkage drag associated with the alien segments of RWTT26-3 and RWTT26-11. Fortunately, RWTT26-11 exhibited more satisfactory key yield traits, making it potentially more useful as a donor parent for leaf rust resistance in wheat breeding.

Discussion

As one of the members in the wheat tertiary gene pool, *Th. intermedium* is mainly distributed in the Mediterranean and Eastern Europe regions (Tsvelev, 1983). Since 1936, many domestic and foreign scholars have been transferring valuable genes from *Th. intermedium* into common wheat (Armstrong, 1936; Peto, 1936; Group of remote hybridization, 1977; Chi et al. 1979; Sun, 1981; He et al. 1986; Dong, 2001). Thus, a series of essential resources, including wheat–*Th. intermedium* partial amphiploids [e.g. TAF46 (Cauderon et al. 1973), Xiaoyan 78829 (Group of remote hybridization, 1977), Zhong 1 to Zhong 5 (Chi et al. 1979; Sun, 1981), Otrastysuskaya (Fedak et al. 2000), TAI8335 (Chang et al. 2010), TE-3 (Yang et al. 2006), TE253-1, TE257, TE267 and TE346 (Bao et al. 2014), TE261-1, TE266-1 and TE346-1 (Cui et al. 2021)], addition lines [e.g. Z1 and Z3 (Larkin et al. 1995; Han et al. 2003), Hy37 (Yu et al. 2019) and TA3681 (Li et al. 2022)], substitution lines [e.g. W44 (Friebe et al. 1992), ES-23, ES-24, ES-25 and ES-26

(Wang et al. 2022)] and translocation lines [e.g. Z4 (Larkin et al. 1995; Lang et al. 2018), Yw642, Yw443 and Yw243 (Xin et al. 2001), CH13-21 (Zhan et al. 2015), CH4131 and CH4132 (Zheng et al. 2020), WTT11 (Yang et al. 2021), TA5566 and TA5567 (Li et al. 2022)] have been developed via distant hybridizations and chromosome engineering. Some commercial cultivars, such as Xiaobing 33, the Longmai series as well as the Xinshuguang series, have been developed from these germplasm (Dong, 2001).

In this study, molecular marker amplification confirmed that Xiaoyan 78829 contains the 7J^S chromosome, which was consistent with the finding of Yu et al. (2022). Further marker amplification suggested that WTT26 carries the 7J^SS and WTT11 the 7J^SL chromosomes, respectively. The homoeologous analysis of WTT26 was carried out with no obvious SNP elimination regions present in the WTT26 genome. However, from the results an obvious peak was observed in wheat chromosome 7BS, indicating that the alien segment of WTT26 originated from a short arm of a group 7 chromosome. The results from wheat SNP microarray analysis was consistent with that of *Th. intermedium* specific marker amplification, suggesting the method of genomic analysis of wild relatives such as *Th. intermedium* based on wheat SNP microarray was feasible. However, analysis of WTT11 revealed that its alien segments were homoeologous to wheat group 6 and 7 chromosomes (Yang et al. 2021). The SNP elimination event, from the results of a Wheat660K SNP array analysis, in the WTT11 genome and sequence homology between chromosome 7J^S and wheat group 6 and 7 chromosomes were in support of their findings. Besides Xiaoyan 78829, other partial amphiploids such as Zhong 2, Zhong 3, Zhong 4, Zhong 5, TAI7045, y70-1-4, 78784, TAI7047 and 8024 also contains chromosome 7J^S (Yu et al. 2022). The disease and insect resistance genes identified in chromosome 7J^S may account for its high occurrence rate in the identified amphiploids (Tang et al. 2000; Lang et al. 2018; Yu et al. 2022). According to the report of Li et al. (2022), wheat–*Th. intermedium* translocation lines TA5566 and TA5567 had high resistance to Australian *Pgt* pathotypes, which came from partial amphiploid Zhong 5 and both had chromosome 7J^S fragments. Thus, chromosome 7J^S was found to carry leaf rust and stem rust resistance loci. In view of the facts that a *Lr* gene is sometimes accompanied by a *Sr* gene, take *Lr19/Sr25* and *Lr24/Sr24* in *Th. ponticum* as examples (Qi et al. 2007), we speculate that this novel *Lr* locus could be linked with a novel *Sr* locus in WTT26. This need to be confirmed in follow-up stem rust assessment studies.

Since *Th. intermedium* is a hexaploid species with a large and complex genome, it is likely that more resistance genes will be identified and functionally annotated. However, only one leaf rust resistance gene *Lr38* has been discovered and documented so far, which was in the distal half of the long arm of chromosome 7Ai-2 (Friebe et al. 1992). In our study the leaf rust resistance locus of WTT26 was mapped in the 0–69.29 Mb region on the short arm of 7J^S. Besides, the seedling ITs of WTT26 were different from those of TcLr38 to 13 *Pt* races. Specifically, WTT26 was nearly immune to 13 *Pt* races, whereas TcLr38 was only immune or nearly immune to 8 of 13 *Pt* races. Therefore, we believe that not only the *Lr* locus of WTT26 is different from *Lr38* but also the *Lr* gene in WTT26 is more effective than *Lr38*.

The SLAF-seq technique enables the development of specific PCR-based markers for plant species efficiently and conveniently by pair-end sequencing and bioinformatics analyses. Up to now, specific molecular markers of many wild relatives of wheat, such as *S. cereale*, *Th. elongatum* and *Th. ponticum*, have been developed depending on this technology. Specifically, a total of 404 universal PCR-based markers explicit to 14 individual rye chromosomes were developed and validated, which could enrich the marker densities for gene mapping and tracing rye-derived genes in wheat backgrounds (Han et al. 2020). Chen et al. (2013a) developed 61 specific molecular markers for *Th. elongatum* chromosome 7E. In a previous study, 67 PCR-based markers and eight FISH probes specific for *Th. ponticum* were developed, which could efficiently detect small *Th. ponticum* segments in a wheat genomic background (Liu et al. 2018). To map the novel *Lr* gene of WTT26, a total of 124 specific markers were developed using SLAF-seq in this study. After sequence assignment, 55 of them had clear physical positions. We used these markers to construct a physical map of chromosome 7J^SS and then mapped the leaf rust resistance locus to the 7J^SS-1 (0-69.29 Mb) region. In addition, these markers will be helpful for tracing the alien segment in wheat breeding and supplementing the sequence information of the *Th. intermedium* genome.

As a vast reservoir of potentially valuable genes, wheat wild relatives have been enriching wheat genetic diversity through the development of chromosome engineered germplasm. To date many important translocation lines, whose alien segments not only carry many different desirable traits but also eliminate genetic linkage drag, have been widely used in wheat breeding. From these the wheat-rye 1RS·1BL translocation, the most successful example of distant hybridization, has contributed to producing approximately 30% of wheat varieties released after the year 2000 (Wang et al. 2017). Two important reasons for this success are that chromosome 1RS carries many disease-resistant genes without any accompanied genetic drag linked with grain yield (Zeller and Hsam, 1983). Similarly, the 2N^VS segment from *Ae. ventricosa* Tausch ($2n = 4x = 28$, NNDD) was translocated to the distal end of wheat chromosome 2AS, which has been deployed in many USA and CIMMYT breeding programs at high frequency because of its yield advantage and multi-disease resistance (Gao et al. 2021). The chromosome 6VS coming from *Haynaldia villosa* (L.) Schur [syn. *Dasypyrum villosum* (L.) Candargy, $2n = 2x = 14$, VV] also contains the powdery mildew resistance gene *Pm21* and other useful genes (He et al. 2018; Xing et al. 2018). To date more than 40 commercially available wheat cultivars, carrying the 6VS·6AL translocation, have been released and cultivated on a large scale in China (Xing et al. 2021). Considering the unsatisfactory agronomic traits of WTT26, its pollen was further irradiated and used for the pollination of the widely adaptable and highly productive variety Jimai 22, leading to the development of five translocation lines with diverse alien chromosomal segments. Small fragment translocation lines RWTT26-3 and RWTT26-11 were both near immune to *Pt* race THTT. Remarkably, the YPP of two newly developed translocation lines equaled or exceeded that of their recurrent parent Jimai 22. The balance between desirable yield characters and excellent disease resistance would promote the use of these lines in wheat breeding.

In summary, a novel wheat– *Th. intermedium* translocation line WTT26 was developed. The alien segment was identified as the short arm of chromosome 7J^S by cytogenetic, wheat SNP microarray and *Thi*-specific marker amplification analyses. Disease resistance evaluation showed that WTT26 was nearly immune to 14 *Pt* races. The alien chromosome segment 7J^S was proved to include a novel leaf rust resistance locus. To shorten the alien fragment with resistance to leaf rust, WTT26 was irradiated, crossed, and backcrossed with the widely adapted variety Jimai 22, and consequently five different translocation lines with smaller *Th. intermedium* fragments were obtained. A physical map of chromosome 7J^S was constructed using newly developed and previous 7J^S-specific markers and the *Lr* locus was mapped in the 0–69.29 Mb region. These translocation lines and molecular markers will contribute to utilize the new resistance locus of WTT26 in wheat breeding and enrich the sequence information of *Th. intermedium*.

Declarations

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Author contributions

ZSL and QZ conceived the research; GTY performed the experiments; NZ performed leaf rust resistance evaluation; WB performed stem rust resistance evaluation; GTY and QZ drafted the manuscript; HWL and BL provided substantial help in preparing materials. All authors read and approved the final manuscript.

Compliance with ethical statements

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The authors declare that the experiments comply with the current laws of the country in which they were performed.

Data availability statement

Phenotype data of agronomic traits are presented in the Table 2. The genotype data and materials reported in this study are available upon request.

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Figures

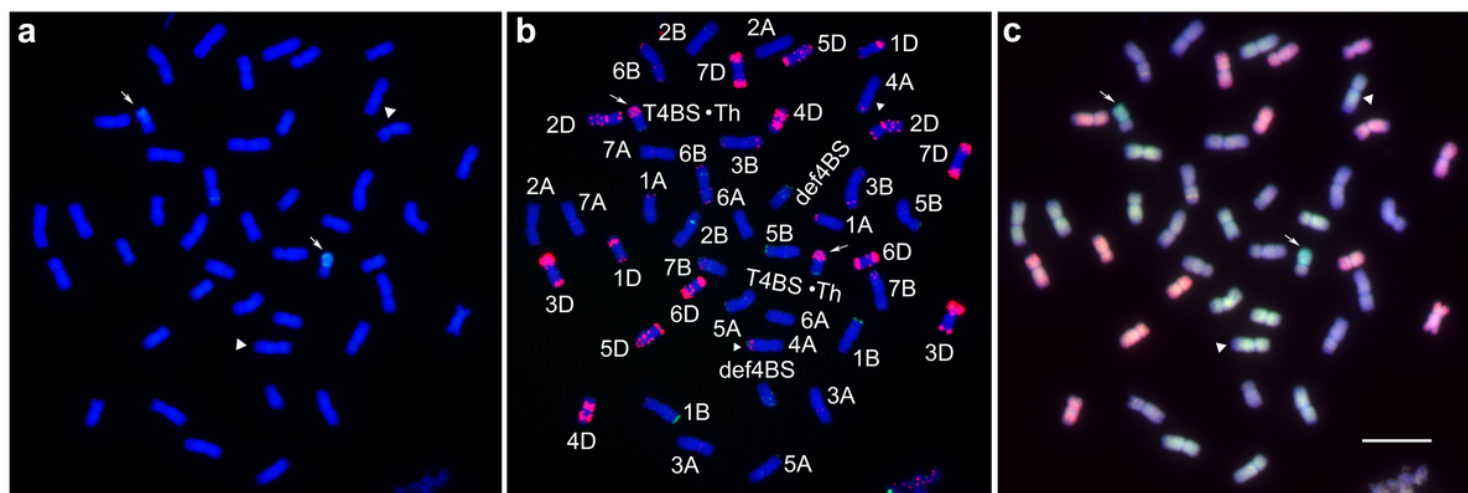


Figure 1

Cytogenetic analysis of WTT26. GISH analysis revealed 42 intact wheat chromosomes and two wheat– *Th. intermedium* translocated chromosomes with gDNA of *Th. intermedium* as a probe (green) (a). Mc-FISH pattern of WTT26 using two probes pAs1 (red) and pSc119.2 (green) (b). In mc-GISH, yellow, brown, red and green were A-genome chromosomes, B-genome chromosomes, D-genome chromosomes and *Th. intermedium* chromosome segments, respectively (c). The arrows note a pair of translocated

chromosomes, and triangles note one pair of chromosomes 4A carrying B-genome chromosome segments. Bar = 20 μ m.

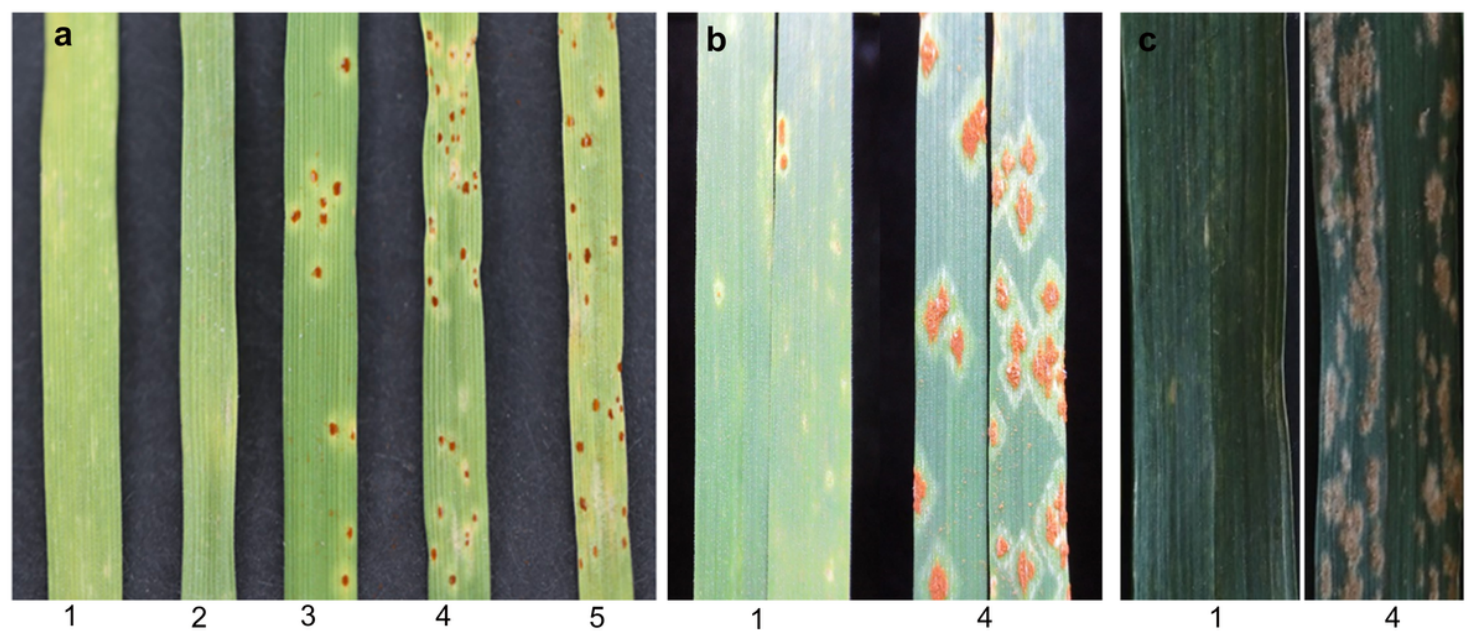


Figure 2

The disease resistance evaluation of WTT26 and its parents to *Pt* race THTT (a), *Pgt* race PTKST (b), and powdery mildew at the adult plant stage (c). 1: WTT26, 2: Xiaoyan 78829, 3: Xiaoyan 101, 4: Xiaoyan 81, 5: Misui.

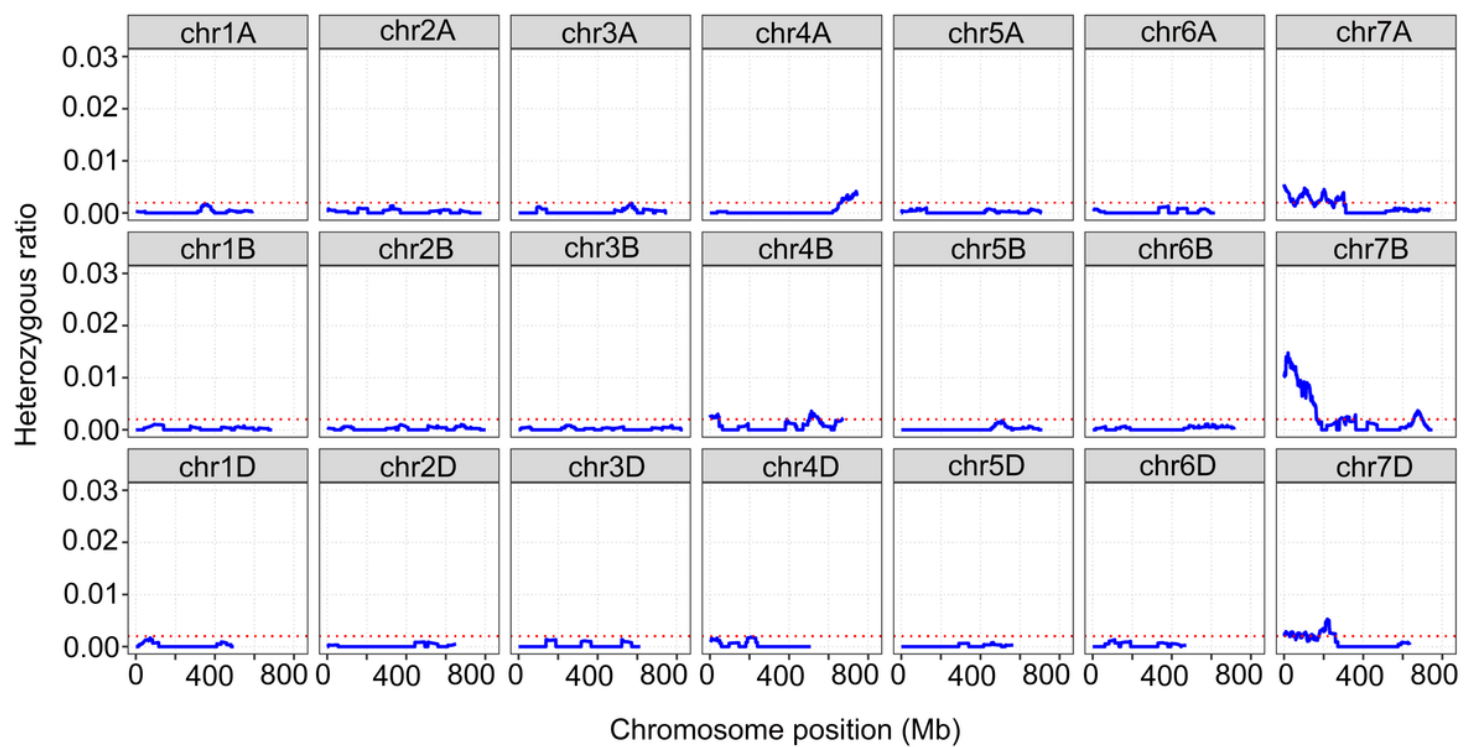


Figure 3

The homoeologous analysis of alien chromosome segment from WTT26. The heterozygous ratio was heterozygous SNPs in WTT26, in accordance with both *Th. intermedium* and Xiaoyan 81's genotypes, to all SNPs belonging to Poly High Resolution and No Minor Homozygote categories in each chromosome. It was counted in 50-Mb sliding windows at 1-Mb intervals and plotted along the chromosome. The alien segments were homoeologous to wheat chromosome fragment with the highest ratio.

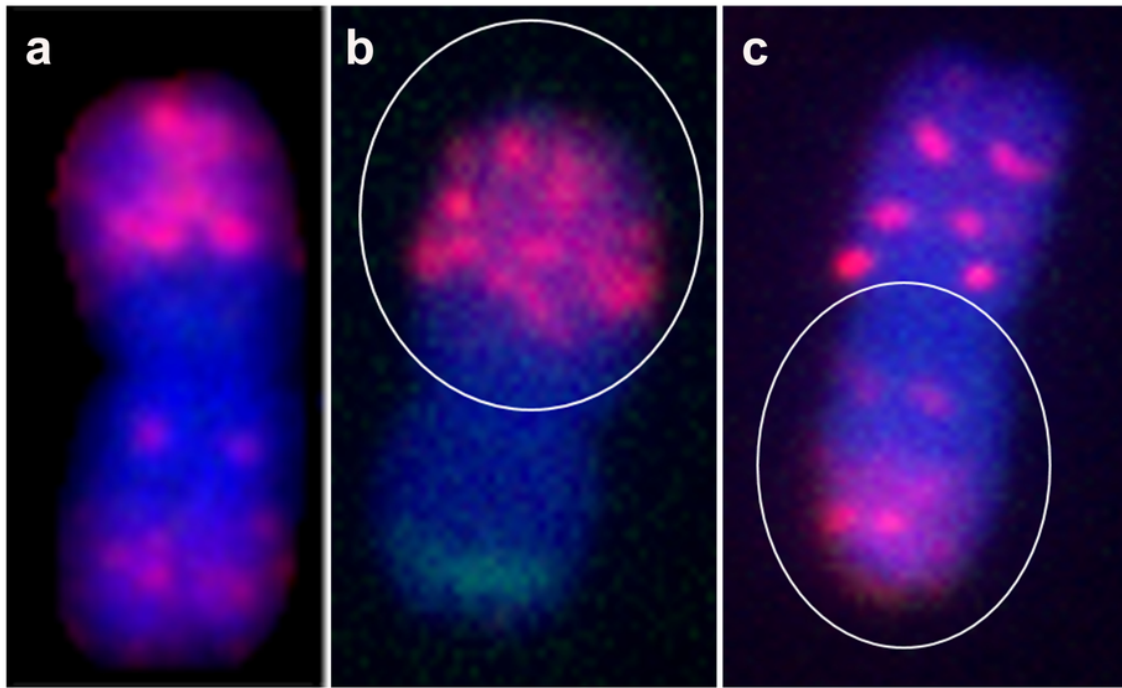


Figure 4

The mc-FISH patterns of Xiaoyan 78829 (a), WTT26 (b) and WTT11 (c) using pAs1 and pSc119.2.

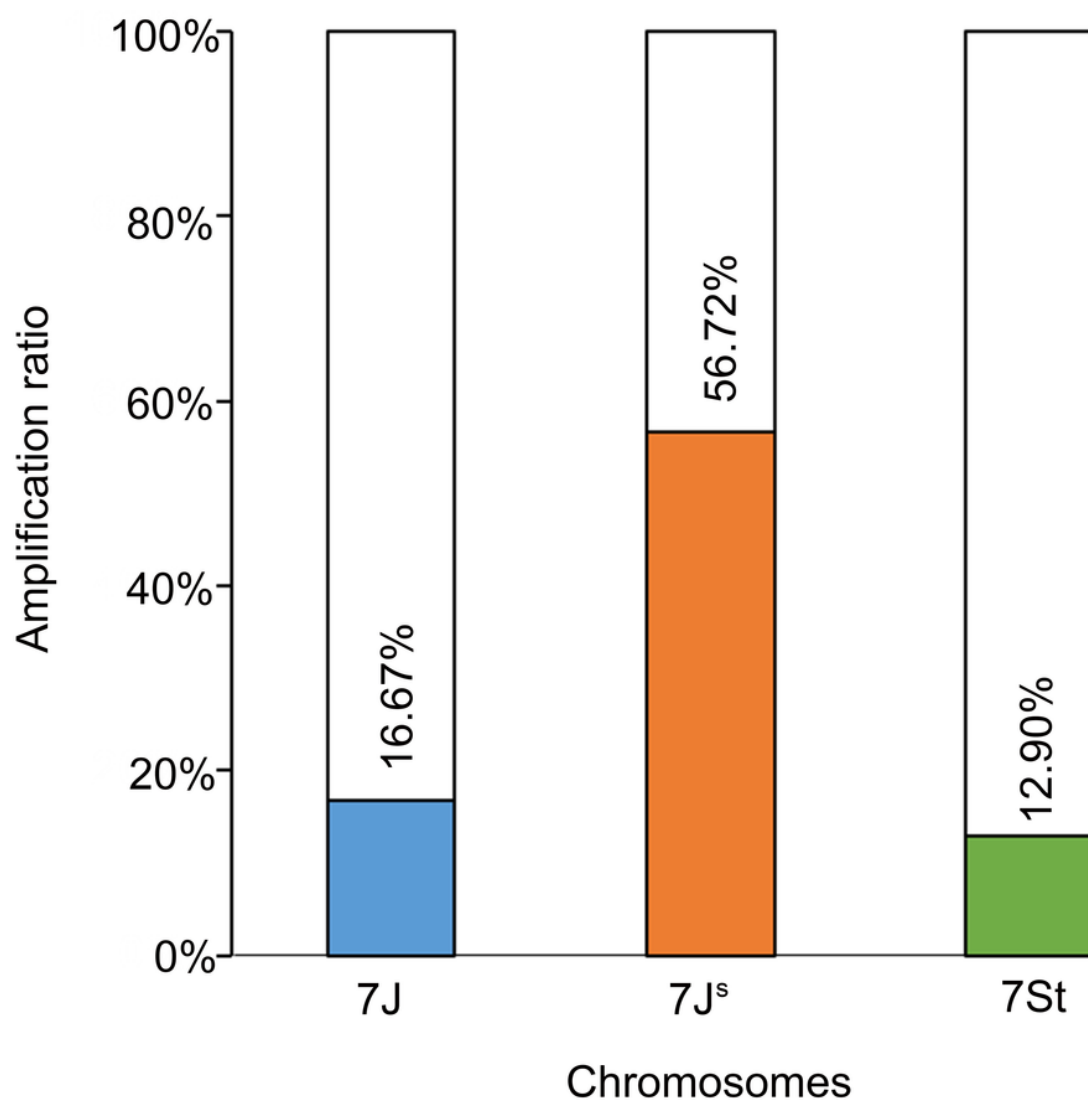


Figure 5

The amplification ratio of *Thi*-specific markers in Xiaoyan 78829.

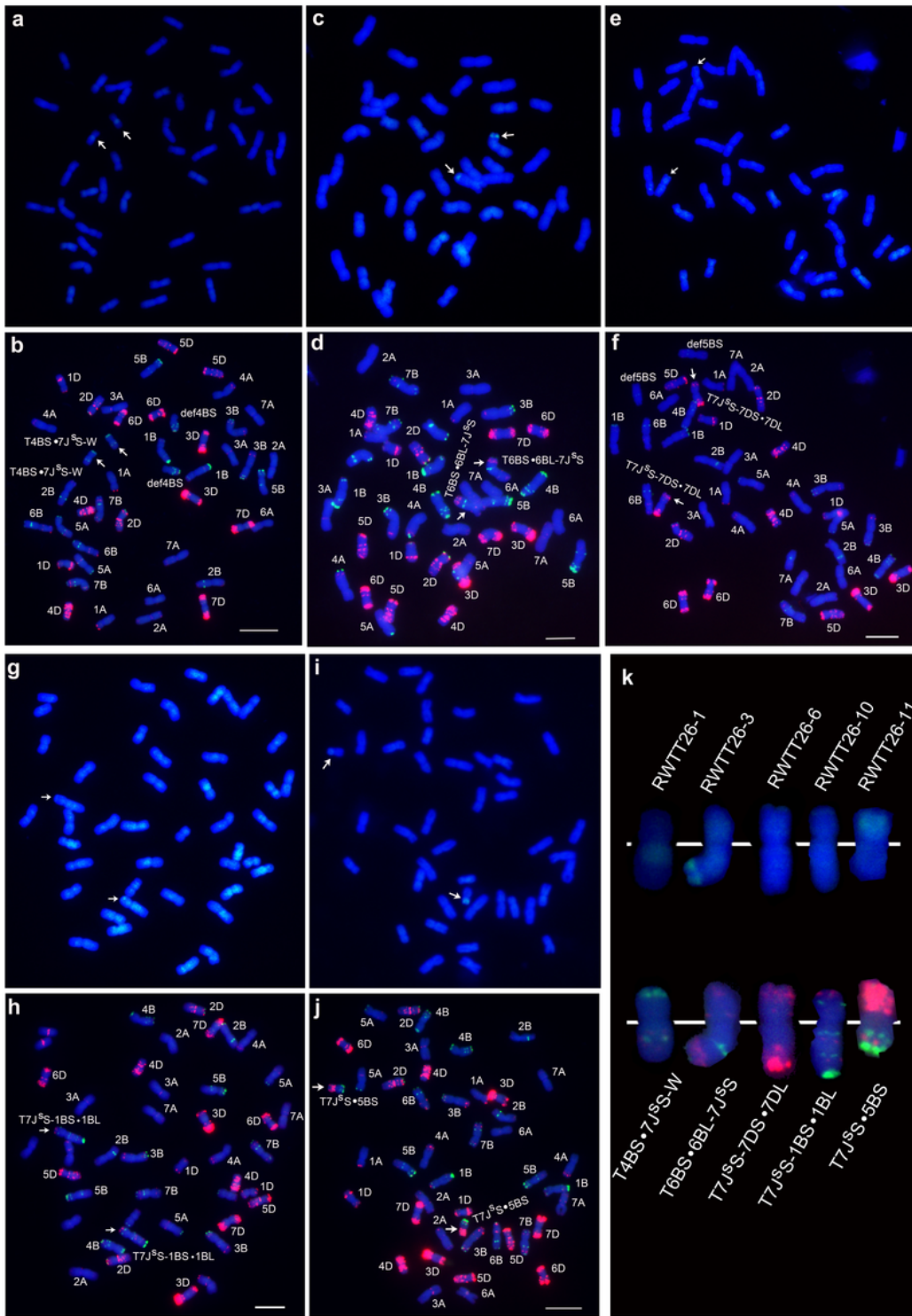


Figure 6

Cytogenetic analysis of five new translocation lines. The GISH pattern of RWTT26-1 (a), RWTT26-3 (c), RWTT26-6 (e), RWTT26-10 (g) and RWTT26-11 (i) with *Th. intermedium* gDNA as a probe. The mc-FISH pattern of RWTT26-1 (b), RWTT26-3 (d), RWTT26-6 (f), RWTT26-10 (h) and RWTT26-11 (j) using two probes pAs1 (red) and pSc119.2 (green). The arrows note a pair of translocated chromosomes. Bar = 20 μm.

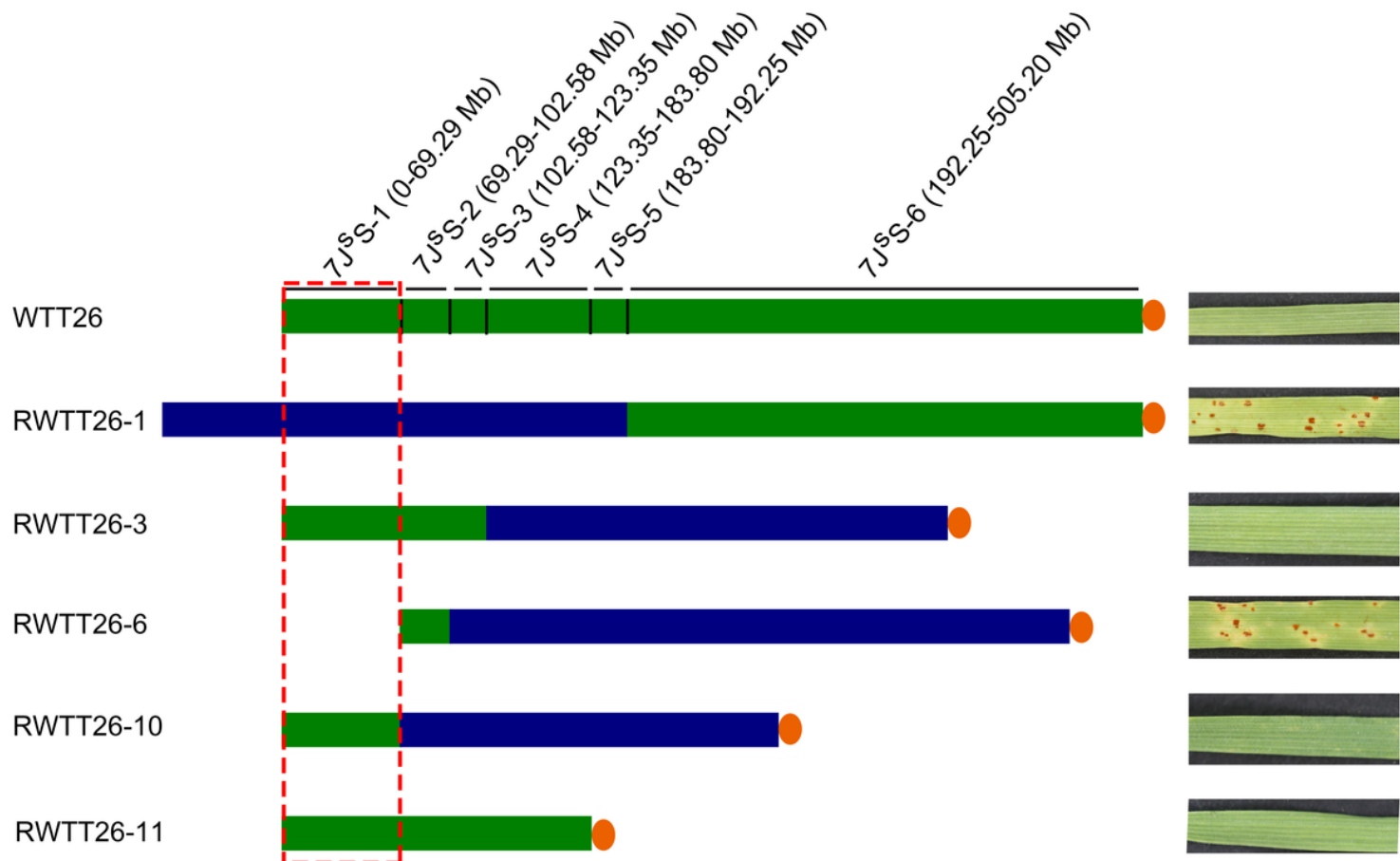


Figure 7

Physical mapping of *Lr* locus. Green and blue regions indicate chromosome segments of *Th. intermedium* and wheat, respectively. The dotted box indicates the physical region of the *Lr* locus.

Supplementary Files

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