

Introgression of tetraploid *Thinopyrum elongatum* 6EL segments enhances the stripe rust resistance of adult wheat plants

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Abstract

Preventing the widespread occurrence of stripe rust in wheat largely depends on the identification of new stripe rust resistance genes and the breeding of cultivars with durable resistance. We obtained a wheat–tetraploid *Thinopyrum elongatum* 6E (6D) substitution line and determined that chromosome 6E contains genetic material conferring superior resistance to stripe rust at the adult stage. In this study, three novel wheat–tetraploid *Th. elongatum* translocation lines were generated from the offspring of a cross between common wheat and the 6E (6D) substitution line. Genomic *in situ* hybridization (GISH), fluorescence *in situ* hybridization chromosome painting (FISH painting), repetitive sequential FISH, and 55K SNP analyses indicated that K227-48, K242-82, and K246-6 contained 42 chromosomes and were 6ES·6DL, 2DL·6EL, and 6DS·6EL translocation lines, respectively. The assessment of stripe rust resistance revealed that K227-48 was susceptible to a mixture of *Pst* races, whereas the 6EL lines K242-82 and K246-6 exhibited adult plant resistance to stripe rust. Thus, this resistance was due to the 6EL chromosome. The overall good agronomic performance of K246-6 implies this line may be a useful germplasm resource for wheat breeding programs. Furthermore, 34 PCR-based markers for chromosome 6EL were developed using the whole-genome sequence of diploid *Th. elongatum*. This novel translocation line may be applicable for breeding wheat lines resistant to stripe rust. Additionally, the markers developed in this study will enable the accurate tracing of tetraploid *Th. elongatum* chromosome 6E and the mapping of additional favorable genes on 6EL.

Introduction

Stripe rust, which is caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is one of the most devastating airborne diseases of wheat worldwide, affecting more than 80% of cultivated wheat and seriously threatening food security and production (Wellings 2011; Chen et al. 2014; Zeng et al. 2022). Therefore, it is imperative that breeders develop wheat cultivars exhibiting durable resistance to stripe rust. Depending on the growth stages in which certain disease-resistance genes are expressed, stripe rust resistance can be classified as all-stage resistance (ASR) and adult-plant resistance (APR) (Chen 2005). Earlier research indicated ASR is generally a qualitative trait, mostly controlled by a single gene that is easily exploited during breeding, including *Yr1*, *Yr9*, *Yr15*, *Yr24*, and *Yr28* (Murphy et al. 2009; Chen 2020). The mechanism underlying stripe rust resistance is often easily overcome by new pathotypes of the causative phytopathogenic fungus, resulting in decreased resistance or even a complete loss of resistance in many new disease-resistant cultivars after 3–5 years of extensive commercial cultivation (Bariana et al. 2007; Ellis et al. 2014; Chen 2020). In contrast to ASR, APR is mostly a quantitatively inherited trait controlled by multiple genes, with many APR genes conferring race-nonspecific and durable resistance (Gebrewahid et al. 2020). For example, the APR genes *Yr18*, *Yr29*, *Yr30*, and *Yr46* are associated with durable stripe rust resistance in several regions worldwide, making them highly valuable and commonly used disease resistance genes (Singh et al. 2005; Szychala et al. 2023). However, the resistance conferred by these genes (except for *Yr30*) is accompanied by the development of dry leaf tips, which can adversely affect the wheat yield (Szychala et al. 2023). Therefore, it is important to

extend the effective-use period of major cultivars, while also rationally exploiting APR genes during the breeding of new cultivars with durable disease resistance.

Wild relatives of wheat possess many valuable genes for improving the disease resistance of wheat (Friebe et al. 1996). For example, diploid ($2n = 2x = 14$, EE genome), tetraploid ($2n = 4x = 28$, EEEE genome), and decaploid ($2n = 10x = 70$, EEEEEESTtStSt genome) *Thinopyrum elongatum* (Host) D. R. Dewey accessions are important sources of genes related to the resistance to salinity, drought, and disease (Fedak et al. 1999; Li et al. 2004; Fu et al. 2012). After intergeneric hybrids were first generated via the distant hybridization between common wheat and *Thinopyrum*, many disease resistance genes were identified and wheat–*Thinopyrum* derivative lines were produced over a period exceeding 40 years (Fedak et al. 1999; Wang et al. 2020). Several genes conferring resistance to various diseases, such as stripe rust (e.g., *Yr69*) (Hou et al. 2016), stem rust (*Sr24*, *Sr25*, *Sr26*, and *Sr43*) (Plotnikova et al. 2023; Yu et al. 2023), leaf rust (*Lr19*, *Lr24*, and *Lr29*) (Kloppers et al. 1995; Plotnikova et al. 2023), powdery mildew (*Pm51* and *PmSn0224*) (He et al. 2017), and Fusarium head blight (*Fhb7*) (Wang et al. 2020), were identified in or cloned from *Thinopyrum* species. Among these genes, *Yr69*, *Pm51*, and *PmSn0224* were derived from homologous group 2 of *Thinopyrum ponticum* (Hou et al. 2016; He et al. 2017); *Sr24*, *Sr25*, *Sr43*, *Lr19*, *Lr24*, *Lr29*, and *Fhb7* were derived from homologous groups 3 and 7 of *Th. elongatum* and *Th. ponticum* (Kloppers et al. 1995; Plotnikova et al. 2023; Yu et al. 2023); and *Sr26* was derived from homoeologous group 6 of *Th. ponticum* (Plotnikova et al. 2023). It is essential that new resistance genes from other *Th. elongatum* genotypes are identified and transferred into elite wheat germplasm resources to diversify and enhance wheat disease resistance.

Because of the advances in cytogenetic and molecular techniques applicable for identifying alien chromosomes, various wheat–*Thinopyrum* lines have been generated through chromosome engineering, including intergeneric amphidiploids (Guo et al. 2015; Hu et al. 2011) as well as addition (Liu et al. 2017; Jia et al. 2022), substitution (Wang et al. 2019), and translocation (Fu et al. 2012; Tanaka et al. 2017; Yang et al. 2022) lines. The partial amphiploid line Xiaoyan 7430 derived from wheat and *Th. ponticum* is highly resistant to stripe rust (Hu et al. 2011), whereas amphiploid lines 8801, 8802, and 8803 derived from tetraploid *Th. elongatum* and *Triticum durum* are highly resistant to stripe rust, powdery mildew, and Fusarium head blight (Guo et al. 2015). Moreover, wheat–*Thinopyrum* alien chromosome addition and substitution lines are valuable resources for identifying the genes of interest in *Th. elongatum* and the subsequent use of these genes in wheat breeding programs. A major gene for the stripe rust resistance at the seedling and grain-filling stages was detected on a *Th. ponticum* group 6 chromosome during the analysis of six wheat–*Th. ponticum* disomic addition lines derived from the partial amphiploid Xiaoyan 7430 (Jia et al. 2022). We previously developed a wheat–tetraploid *Th. elongatum* 1E (1D) substitution line resistant to stripe rust, a 3E (3D) substitution line tolerant to salt stress, a 4E (4D) substitution line resistant to stripe rust and powdery mildew, and 6E (6D) substitution lines resistant to stripe rust at the adult stage (Li et al. 2019; Gong et al. 2022; Gong et al. 2023; Zeng et al. 2023). However, the decreased fertility and genetic unpredictability of amphiploid, addition, and substitution lines make them inappropriate for commercial cultivation. Unlike addition and substitution lines, translocation lines are favored by wheat breeders because of their limited amounts of alien genetic

material, minor linkage drag, and regular meiotic behavior (Falke et al. 2009). In recent studies, the wheat–*Th. ponticum* translocation lines WTT34 and WTT80 carrying the T5DS·5DL-Th and TTh-1DS·1DL chromosomes were resistant to Ug99 and *Bgt* isolates and had excellent agronomic traits (Yang et al. 2021, 2022). Despite these successes, relatively few translocation lines from tetraploid *Th. elongatum* have been used by breeders to increase wheat disease resistance.

In this study, we developed three novel wheat–tetraploid *Th. elongatum* translocation lines from the BC₁F₅ and F₄ selfed progeny of the cross between 6E (6D) substitution line K17-1065-4 and common wheat. The chromosome composition of each translocation line was characterized using cytogenetic methods and the wheat 55K SNP array. Their stripe rust resistance and agronomic traits were also evaluated. Moreover, specific simple sequence repeat (SSR) molecular markers were developed for tetraploid *Th. elongatum* chromosome 6EL. These markers may be applicable for breeding novel wheat lines resistant to disease via the transfer of specific resistance-related genes.

Materials and methods

Plant materials

Tetraploid *Th. elongatum* (2n = 4x = 28, EEEE, PI 531750) is a homozygous tetraploid formed by natural doubling (Charpentier et al. 1988; Li et al. 2005). Line 8801 (2n = 6x = 42, AABBEE), which was obtained from the offspring of the hybridization between tetraploid *Th. elongatum* and *Triticum durum*, is immune to stripe rust, powdery mildew, and Fusarium head blight (kindly donated by Dr. George Fedak, Eastern Cereal and Oilseed Research Centre, Ottawa, Canada) (Guo et al. 2015). Both Shumai 482 (SM482) and Shumai 921 (SM921), which are susceptible to stripe rust, are wheat cultivars grown in Sichuan, China. The wheat–tetraploid *Th. elongatum* 6E (6D) substitution line K17-1065-4, which is highly resistant to stripe rust at the adult stage, was derived from the 8801/SM482//SM921 F₅ progeny (Gong et al. 2023). To decrease the size of alien fragments in the obtained translocation lines, (1) we crossed K17-1065-4 with the common wheat cultivar Chinese Spring (CS) *ph1b* mutant (hereafter CS*ph1b*), after which the F₁ population was backcrossed with CS*ph1b* and then K227-48 was selected from the BC₁F₅ progeny; (2) we obtained K242-82 and K246-6 from the hybrids resulting from the cross between K17-1065-4 and wheat cultivar SM482 and then they were self-crossed until the F₄ generation. All of these plant materials were stored at the Triticeae Research Institute, Sichuan Agricultural University, Chengdu, China.

In situ hybridization analyses

Slides with good metaphase spreads were prepared for analyses of the genomic constitution (GISH, FISH chromosome painting, and FISH analyses). For the GISH analysis, tetraploid *Th. elongatum* genomic DNA was labeled with dUTP-ATTO-550 (red) (Jena Bioscience, Jena, Germany) and used as a probe, whereas CS genomic DNA was used for blocking (1:150). The GISH analysis was performed as previously described (Han et al. 2006; Gong et al. 2019), with minor modifications. Specifically, a 14 µL hybridization solution comprising 1 µL tetraploid *Th. elongatum* genomic DNA probe, 3 µL blocking DNA,

and 10 μ L hybridization mixture (1 g dextran sulfate, 5 mL formamide, 1 mL 20 $\times$ saline sodium citrate (SSC), 1 mL salmon sperm, and 2 mL ddH₂O) was added to each slide. After a 5-min denaturation at 85°C, the slides were incubated for 6 h at 50°C. The GISH signals were detected using a fluorescence microscope (Olympus BX-63, Tokyo, Japan) with images captured using the DP-70 CCD camera. After the GISH analysis, the photographed slides were washed sequentially with 2 $\times$ SSC at 60°C for 10 min and then 75% and 100% (v/v) ethanol for 2 min. They were subsequently placed under bright light to eliminate the GISH signals.

The FISH chromosome painting analysis was conducted using the bulk oligonucleotide libraries Chr1–Chr7 to determine the homoeologous group relationships of the exogenous chromosomes in K227-48, K242-82, and K246-6, which represent the entire genome sequence of diploid *Th. elongatum* (Chen et al. 2023). The FISH chromosome painting method was previously described by Bi et al. (2020) and Chen et al. (2023). Chromosomes were counterstained and the microscopy slides were prepared and analyzed according to published methods (Tan et al. 2022). Images were captured as described in the GISH protocol. After the FISH chromosome painting analysis, the photographed slides were washed sequentially with 1 $\times$ PBS for 10 min, 2 $\times$ SSC at 56°C for 15 min, and 75%, 95%, and 100% (v/v) ethanol for 2 min. They were then placed under bright light to eliminate the signals.

The synthetic oligonucleotide probes Oligo-pSc119.2 and Oligo-pTa535 were used to clarify the genomic constitution of K227-48, K242-82, and K246-6. The FISH analysis was performed using an established procedure (Han et al. 2006). Photomicrographs were obtained as described in the GISH protocol. The wheat chromosome identity was determined by referring to the common wheat CS standard FISH karyotype, whereas the alien chromosome identity was determined by referring to the tetraploid *Th. elongatum* E genome FISH karyotype (Tang et al. 2014; Li et al. 2018).

Wheat 55K SNP analysis

To confirm the composition and break position of the translocated chromosomes in K227-48, K242-82, and K246-6, genomic DNA was extracted and genotypes were determined using the 55K single nucleotide polymorphism (SNP) array following the standard procedure at the China Golden Biotechnology Company (Beijing, China). The SNP loci in this array were distributed across 21 pairs of wheat chromosomes. Different bases, such as AA, AT, AG, CC, GG, and TT, signified distinct genotypes at specific positions. In addition, “NA” was used to indicate the absence of a particular position on the chromosome. The NA ratio was calculated as the number of SNP markers with the NA genotype divided by the total number of SNP markers. The distribution of NA on chromosomes was mapped using an online program (http://www.bioinformatics.com.cn/plot_basic_SNP_density_by_CMplot_107). The deletion rate of each chromosome was calculated in 10 Mb sliding windows, with 1 Mb steps.

Stripe rust resistance evaluation

Adult plant stripe rust resistance was assessed in a field at the Triticeae Research Institute, Sichuan Agricultural University, Chengdu, China during the 2022–2023 wheat growing season. The K227-48, K242-82, and K246-6 lines and their parents were inoculated with a mixture of *Pst* races (CYR32, CYR33,

CYR34, Sull-4, Sull-5, Sull-7, and G22-14) at the tillering stage using the smear method, with mixed talcum powder at the ratio of 1:50 (m/V) (Carter et al. 2009). Avocet S was used as the susceptible control. Infection types (ITs) were determined using a 0–9 scale. More specifically, the IT scores used to evaluate stripe rust resistance were as follows: 0–1, immune; 2–6, resistant; 7–8, susceptible; 9, highly susceptible (Line and Qayoum 1992).

Development and verification of PCR-based markers

The SSRs in the chromosome 6E sequence of diploid *Th. elongatum* (NCBI BioProject ID PRJNA540081) were identified using the perl-based program MISA (<http://pgrc.ipk-gatersleben.de/misa/download/misa.pl>). The detection criteria were as follows: single-nucleotide repeats with a minimum length of 10, dinucleotide repeats with a minimum length of 6, three to six nucleotide repeats with a minimum length of 5, and two SSR loci separated by more than 100 base pairs. Primer 3 Plus (<http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi>) was used to design primers for all SSR sequences. An e-PCR mock amplification was performed to analyze the whole-genome sequences of CS and diploid *Th. elongatum*. The SSR primers specific for the 6E chromosome were obtained by selecting the primers that amplified the target sequence only in *Th. elongatum*. The primers that amplified the target sequence in CS were eliminated. The sequences that were incomplete matches were identified by comparing the SSR sequences corresponding to these primers with the sequences of the 1E–5E and 7E chromosomes in diploid *Th. elongatum*. The remaining sequences were aligned with the whole-genome sequence of CS; the sequences with $\geq 10\%$ homology were removed to obtain a unique and specific SSR sequence for each physical location on the diploid *Th. elongatum* 6E chromosome. The specific SSR primers for chromosome 6EL were selected and synthesized at Sangon Biotech (Chengdu, China). The validity of the primers was verified by PCR as described by Gong et al. (2022). Primers that amplified the target sequence in diploid *Th. elongatum* (6E chromosome), but not in the wheat parents were retained. The chromosomal physical map was drawn using TBtools- (Toolbox for Biologists) v1.120.

Evaluation of agronomic traits

During the 2022–2023 growing season, lines K227-48, K242-82, and K246-6 and their parents were grown in a field at the Triticeae Research Institute, Sichuan Agricultural University, Chengdu, China to evaluate the following six agronomic traits: plant height, tiller number, spike length, spikelets per spike, grains per spike, and thousand-grain weight. Specifically, 15 seeds were sown in a 1.5 m row with an inter-row spacing of 0.3 m. At physiological maturity, the agronomic traits of five plants in the center of the middle row were examined. Significant differences between the translocation lines and their wheat parents were analyzed using the IBM SPSS Statistics 24.0 software package.

Results

Cytogenetic composition of K227-48, K242-82, and K246-6

We used tetraploid *Th. elongatum* genomic DNA as a probe and CS genomic DNA for blocking to investigate the genomic constitution of K227-48, K242-82, and K246-6. According to the GISH analysis, the three lines contained 42 chromosomes, of which 40 were from common wheat. For the remaining two chromosomes, tetraploid *Th. elongatum* genome signals (red) were detected on the partial chromosome arm (Fig. 1a, d, g).

The FISH chromosome painting analysis was performed using E-genome-specific probes Chr1–Chr7 to confirm the genomic composition and homoeologous chromosome groups in K227-48, K242-82, and K246-6. For Chr6, a strong signal (green) was detected on both partial chromosome arms of a pair of chromosomes, indicating these chromosomes in lines K227-48, K242-82, and K246-6 were 6E chromosomes (Fig. 1b, e, h).

The repetitive FISH analysis using Oligo-pSc119.2 (green) and Oligo-pTa535 (red) was conducted to characterize the K227-48, K242-82, and K246-6 wheat chromosomes, which were compared with the FISH cytogenetic standard karyotypes of CS and the tetraploid *Th. elongatum* E genome. In K227-48, a pair of translocated chromosomes had a signal pattern that was similar to that of the 6E chromosome short arm and the common wheat 6D chromosome long arm, suggesting that K227-48 was a 6ES·6DL translocation line (Fig. 1c). In K242-82, a pair of translocated chromosomes had a signal pattern that was similar to that of the common wheat 2D chromosome long arm and the 6E chromosome long arm, indicating K242-82 was a 2DL·6EL translocation line (Fig. 1f). In K246-6, a bright red Oligo-pTa535 signal labeled the common wheat 6D chromosome short arm and the tetraploid *Th. elongatum* 6E chromosome long arm, implying K246-6 was a 6DS·6EL translocation line (Fig. 1i). In summary, K227-48, K242-82, and K246-6 were newly generated wheat–tetraploid *Th. elongatum* translocation lines with 6ES·6DL, 2DL·6EL, and 6DS·6EL, respectively.

Genotyping of the translocation lines using the wheat 55K SNP array

The SNPs of K227-48, K242-82, and K246-6 were identified using the wheat 55K SNP array. A total of 1,039, 710, and 603 SNPs were designated as NA (i.e., NA-SNPs) in K227-48, K242-82, and K246-6, respectively (Supplementary Table S1). The distribution of NA-SNPs on all chromosomes was statistically analyzed and visualized, which revealed that 299, 710, and 603 of these NA-SNPs were enriched in the 0–213.0 Mb region of the 6D chromosome in K227-48, in the 0–246.6 Mb region of the 2D chromosome in K242-82, and in the 217.2–473.4 Mb region of the 6D chromosome in K246-6 (Supplementary Table S1; Fig. 2a–c). On the basis of the percentage of NA-SNPs, the breakpoints on the translocated 6D (K227-48), 2D (K242-82), and 6D (K246-6) chromosomes were detected at 213.0, 246.6, and 217.2 Mb, respectively (Fig. 2d–f). A comparison with the physical positions of the 6D and 2D centromeres (IWGSC RefSeq v1.0) (i.e., 211.9–217.4 and 264.4–272.5 Mb, respectively) indicated that 6ES·6DL, 2DL·6EL, and 6DS·6EL in the three lines were whole-arm translocated chromosomes.

Assessment of the stripe rust resistance of the translocation lines at the adult stage

The stripe rust resistance of K227-48, K242-82, and K246-6 and the parents was evaluated at the adult plant stage in Sichuan province during the 2022–2023 cropping season using a mixture of *Pst* races (CYR32, CYR33, CYR34, Sull-4, Sull-5, Sull-7, and G22-14), with Avocet S chosen as the susceptible control (IT score of 9). Their common wheat parents SM482 and SM921 as well as *CSph1b* were susceptible to the *Pst* mixture (IT score of 8). Similarly, K227-48 carrying tetraploid *Th. elongatum* 6ES chromosomes was susceptible to the *Pst* races (IT score of 8). In contrast, lines K242-82 and K246-6 consisting of 6EL chromosomes had IT scores of 2 and 5, respectively. Their alien parent 8801 and 6E (6D) substitution line K17-1065-4 were immune and resistant to stripe rust, with IT scores of 0 and 2, respectively (Fig. 3). These results suggested the observed stripe rust resistance of the adult plants was conferred by a gene on 6EL.

Development of new SSR markers for detecting chromosome 6EL

A total of 83,685 SSR sequences were obtained for chromosome 6E using the diploid *Th. elongatum* reference genome. The Primer 3 Plus program was used to design primers for all SSR sequences. The e-PCR mock amplification was completed on the basis of the whole genomes of diploid *Th. elongatum* and CS, which indicated that 20,738 primer pairs uniquely amplified the target sequence from the diploid *Th. elongatum* 6E chromosome. After eliminating sequences that were $\geq 10\%$ homologous to 1-5E and 7E sequences in diploid *Th. elongatum* and the whole-genome sequence of CS, 3,382 unique SSR sequences were revealed to be specific to the 6E chromosome of diploid *Th. elongatum*.

We designed primers for 58 randomly selected SSR sequences located at different positions on 6E and then verified their specificity. In total, 34 primer pairs for markers were able to amplify the target sequences in K242-82, K246-6, 8801, and the 6E (6D) substitution line K17-1065-4, but not in K227-48, CS, *CSph1b*, SM482, and SM921. Hence, the identified SSR markers were specific to chromosome 6EL of tetraploid *Th. elongatum*. Thus, the PCR-based marker development success rate was 58.6%. The PCR amplification results were in accordance with the occurrence of a fracture–fusion event in the translocation lines. The primers for these markers were able to amplify the target sequence and map the chromosome according to the sequence location (Fig. 4; Supplementary Table S2).

Analysis of the agronomic performance of the translocation lines

The agronomic traits of the three translocation lines and their parents *CSph1b*, SM482, SM921, and 6E (6D) substitution line K17-1065-4 were evaluated in a research field at the Triticeae Research Institute, Sichuan Agricultural University, Chengdu, China. Line K227-48 was similar to *CSph1b* in terms of plant

height, spike length, and spikelets per spike, but it had significantly more tillers than *CSph1b* and K17-1065-4, while it had significantly fewer grains per spike and a lower thousand-grain weight than *CSph1b* and K17-1065-4 (Table 1; Fig. 5). The agronomic trait values (e.g., tiller number, spike length, spikelets per spike, and grains per spike) were significantly lower for K242-82 than for the common wheat parents SM482 and SM921 (Table 1). There was a distinct increase in the spike length of the K246-6 plants, but K246-6 did not differ significantly from SM482, SM921, and K17-1065-4 regarding plant height, tiller number, spikelets per spike, grains per spike, and thousand-grain weight (Table 1; Fig. 5).

Table 1
The agronomic traits of K227-48, K242-82, K246-6 and its parental lines

Materials	Tillers	Plant height(cm)	Spike length(cm)	Spikelet per spike	Grains per spike	1000-grain weight(g)
SM482	7.67 ± 0.52c	77.25 ± 4.26d	14.35 ± 0.36ab	22.83 ± 1.47ab	54.67 ± 3.98b	36.88 ± 1.25c
SM921	7.50 ± 0.55c	80.42 ± 2.25d	13.62 ± 0.71b	20.83 ± 2.04bc	50.17 ± 4.79bc	44.93 ± 1.31a
<i>CSph1b</i>	12.83 ± 2.64b	119.05 ± 2.67b	9.22 ± 0.82d	24.00 ± 3.03a	47.50 ± 5.79c	38.95 ± 1.42b
K17-1065-4	7.00 ± 0.89c	91.25 ± 4.17c	13.48 ± 0.70b	21.83 ± 1.17ab	65.83 ± 6.08a	38.45 ± 1.70b
K227-48	17.67 ± 4.50a	129.27 ± 3.13a	9.10 ± 0.55d	24.67 ± 0.82a	38.33 ± 2.34d	23.65 ± 0.43e
K242-82	3.40 ± 0.55d	63.06 ± 3.77e	11.04 ± 1.15c	18.80 ± 4.49c	27.80 ± 5.76e	23.03 ± 1.07e
K246-6	7.67 ± 1.03c	80.75 ± 2.42d	15.02 ± 0.92a	20.33 ± 1.37bc	47.00 ± 6.20c	35.23 ± 0.90d

Data in the columns indicate means ± standard errors. Different lowercase letters following the means indicatesignificant differences at the $p < 0.05$ levels

Discussion

The genetic diversity of wheat may be increased by transferring and using the genetic material associated with the desirable traits of wheat relatives. Distant hybridization, which involves a time-consuming genetic cross and selection, is optimized by embryo rescue, chromosome engineering, and molecular marker-assisted selection. The stripe rust-resistant partial amphidiploid lines 8801, 8802, and 8803 were obtained via the hybridization and continuous backcross involving tetraploid *Th. elongatum* and tetraploid durum wheat (Guo et al. 2015). The partial amphidiploid line Xiaoyan 784 was used as a bridge to generate two wheat–*Th. ponticum* disomic substitution lines resistant to stripe rust (Wang et al. 2019). Although these materials are highly resistant to stripe rust, intact alien chromosomes are

inevitably associated with unwanted genetic redundancy. Translocation lines carrying desirable alien chromosome fragments with low genetic linkage drag have been widely applied as chromosome engineering materials by wheat breeders. For example, one of the most successful cases of distant hybridization in wheat breeding is the wheat–rye 1RS·1BL translocation line, in which excellent disease resistance genes on the rye 1RS chromosome, such as *Yr9*, *Pm8*, *Lr26*, and *Sr31*, were introduced into common wheat (Rabinovich 1998; Lukaszewski 2000). Moreover, the wheat–*Haynaldia villosa* 6VS·6AL translocation line containing the powdery mildew resistance gene *Pm21* exhibits a strong and broad spectrum resistance to powdery mildew (Wu et al. 2021; Xing et al. 2021). It serves as a backbone parent in wheat breeding programs in China, with almost all of the disease-resistant varieties grown in the middle and lower reaches of the Yangtze River carrying *Pm21* (Wan et al. 2023). Furthermore, the wheat–*Aegilops ventricosa* 2N^S·2AL translocation line containing *Yr17*, *Lr37*, and *Sr38* has been widely used in the United States, Australia, and Europe (Bayles et al. 2000; Cruz et al. 2016). In the current study, we successfully developed and characterized three novel wheat–tetraploid *Th. elongatum* translocation lines (K227-48, K242-82, and K246-6) from the offspring of the hybridization between the wheat–tetraploid *Th. elongatum* 6E (6D) substitution line and CS*ph1b* or wheat cultivar SM482. The results of molecular cytogenetic analyses indicated K227-48, K242-82, and K246-6 possess 6ES·6DL, 2DL·6EL, and 6DS·6EL, respectively. Thus, K227-48, K242-82, and K246-6 are distinct genetically stable translocation lines. Moreover, the 6EL translocations resulted in high stripe rust resistance at the adult stage. Accordingly, these lines may be applicable for breeding novel wheat varieties resistant to disease. Notably, K246-6 may be a valuable germplasm resource for conferring stripe rust resistance to wheat with diverse genetic backgrounds without any yield penalties.

The mutating and evolving stripe rust pathogen physiological races potentially threaten the current sources of resistance used for breeding wheat. Hence, there is an urgent need for identifying new resistance genes. Adult plant stripe rust resistance is preferred by breeders because it is non-specific to races (Chen 2013; Zeng et al. 2019). To date, 29 of the 86 officially named stripe rust resistance genes confer APR, including *Yr18*, *Yr29*, *Yr30*, *Yr36*, *Yr46*, and *Yr56* (Klymiuk et al. 2022; Feng et al. 2023; Zhu et al. 2023), among which *Yr29*, *Yr36*, and *Yr46* are commonly used for breeding lines exhibiting durable disease resistance. In contrast, ASR, which is mediated by a single dominant resistance gene, may be ineffective following the widespread distribution of new highly virulent pathogen strains (Chen 2013; Chen 2020). Consequently, breeders should focus on the application of highly effective APR stripe rust resistance genes, while wheat growers should avoid cultivating highly susceptible varieties on which most of the inoculum is generated (Zheng et al. 2017; Hu et al. 2022). The wild relatives of wheat represent a natural genetic resource for improving wheat stripe rust resistance; approximately 25 resistance genes from wild relatives have been reported, including *Yr9* and *Yr83* in rye; *Yr8*, *Yr17*, *Yr19*, and *Yr28* in *Aegilops*; and *Yr50* and *Yr69* in *Thinopyrum*. In our previous studies, the 1E and 4E chromosomes of tetraploid *Th. elongatum* were revealed to harbor genes responsible for ASR to stripe rust, whereas the 6E chromosome was identified as a source of genes mediating APR to stripe rust (Li et al. 2019; Gong et al. 2023; Zeng et al. 2023). In the present study, we characterized two 6EL translocation lines (2DL·6EL and 6DS·6EL) exhibiting APR to stripe rust by inoculating adult plants with multiple *Pst*

ances. Therefore, the resistance genes carried by 6EL identified in this study likely differ from the above-mentioned resistance genes. In addition, we confirmed that the stripe rust resistance gene is located on 6EL, as evidenced by the susceptibility of the 6ES translocation line. Moreover, we transferred the APR genes of 6EL into a wheat cultivar. The resulting transgenic wheat plants will be evaluated to elucidate the functions of the transferred genes related to stripe rust resistance.

The wild species *Th. elongatum* has numerous favorable characteristics that may be exploited to improve wheat. Thus, molecular markers are continually being developed to efficiently track alien chromosome fragments. Hu et al. (2012) developed and characterized primer pairs for 258 expressed sequence tags and 46 PCR-based landmark unique genes that can distinguish *Th. elongatum* chromatin from wheat genomes, with 13 markers mapped on *Th. elongatum* 6E chromosomes. Furthermore, two RAPD markers and one ISSR marker for the *Th. elongatum* E genome were developed to produce SCAR markers; two of these markers were present in all seven *Th. elongatum* chromosomes, whereas one was useful for tracking *Th. elongatum* chromosomes 2E and 3E (Xu et al. 2012). Twelve primer pairs specific for 1–7E of *Th. elongatum* as well as one primer pair for 6E were obtained using ISSR/IRAP/REMAP technology (Chen et al. 2015). In another study, 169 markers (149 COS and 20 InDel) specific to wheat homoeologous groups 1–7 were identified, of which seven markers were detected on the *Th. elongatum* 6ES chromosome, whereas eight markers (amplicons with the same size) were detected on the 2EL and 6EL chromosomes (Gaál et al. 2018). However, the relatively low marker density and the unknown physical locations of these markers prevent the efficient identification of small structural variations and the induction of small translocations of stripe rust resistance-related fragments. We previously developed 33 pairs of SSR markers for 6E on the basis of the whole-genome sequence of diploid *Th. elongatum* (Gong et al. 2023). In the present study, we used the whole-genome sequence of diploid *Th. elongatum* to generate 34 SSR markers specific for important physical locations on the 6EL chromosome. These SSR markers may be useful for efficiently identifying small structural variations and for tracing tetraploid *Th. elongatum* 6EL chromosomes and chromosomal segments during the breeding for stripe rust resistance.

Conclusions

We developed K242-82 (2DL·6EL) and K246-6 (6DS·6EL) as two new wheat–tetraploid *Th. elongatum* translocation lines from a cross between the 6E (6D) substitution line K17-1065-4 and common wheat. These lines were subsequently characterized via GISH, FISH, FISH chromosome painting, and wheat 55K SNP array analyses. Moreover, K242-82 and K246-6, which harbor 6EL and exhibit APR, are potentially valuable germplasm resources for breeding new disease-resistant wheat cultivars. Additionally, 34 PCR-based markers specific for chromosome 6EL of tetraploid *Th. elongatum* were developed according to the whole-genome sequence of diploid *Th. elongatum*. All of these markers are expected to be applicable for tracing tetraploid *Th. elongatum* 6EL chromosomes and chromosomal segments during breeding. As part of our future research aimed at enhancing wheat breeding, we plan to use the *CSph1b* mutant and these translocation lines to generate lines carrying smaller translocated segments with the stripe rust resistance gene(s) without a genetic linkage drag.

Declarations

Competing Interests

The authors declare that they have no conflict of interest

Ethics approval and consent to participate

Not applicable.

Additional information

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Author Contributions

Chunyan Zeng, Liangxi Li, Dandan Wu, and Houyang Kang conducted the experiment, analyzed the data, and drafted the manuscript. Zaimei He, Wei Zhu, Lili Xu, Yiran Cheng, and Yi Wang developed substitution lines and evaluated disease resistance. Jian Zeng, Xing Fan, Lina Sha, Haiqin Zhang, Guoyue Chen, and Yonghong Zhou provided technique guidance. Dandan Wu and Houyang Kang designed the experiment and formulated the questions. All the authors read and approved the manuscript.

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Data Availability

Data are available within the article or its supporting information.

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Figures

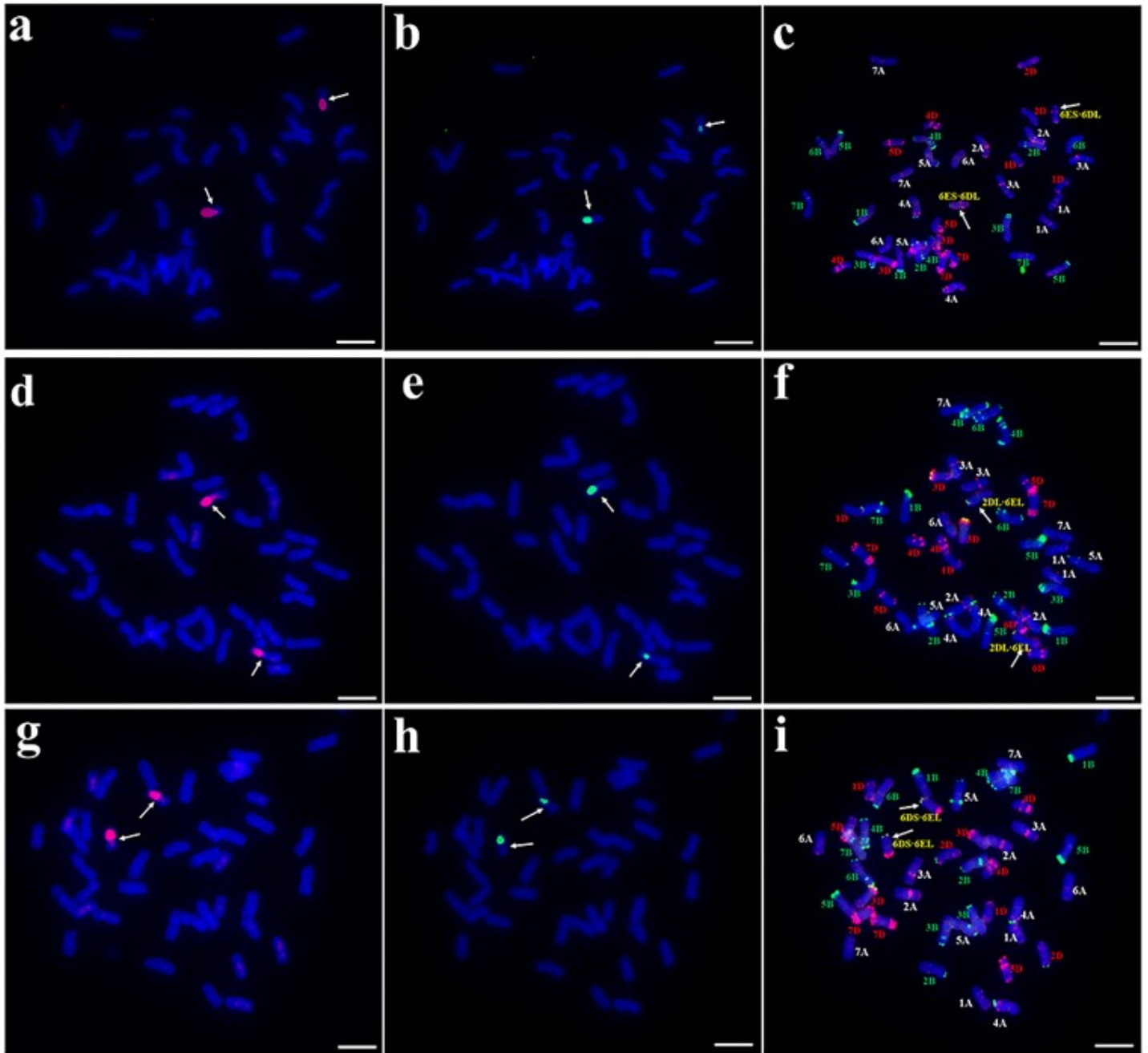


Figure 1

Cytogenetic analysis of wheat-tetraploid *Th. elongatum* translocation lines K227-48, K242-82 and K246-6. (a, d, g) GISH analysis using tetraploid *Th. elongatum* genomic DNA as a probe (red). (b, e, h) Chr6 (green) was used in FISH chromosome painting. (c, f, i) FISH analysis using probes Oligo-pTa535 (red) and Oligo-pSc119.2 (green). Arrows indicate translocated chromosomes. Scale bar: 10 μ m

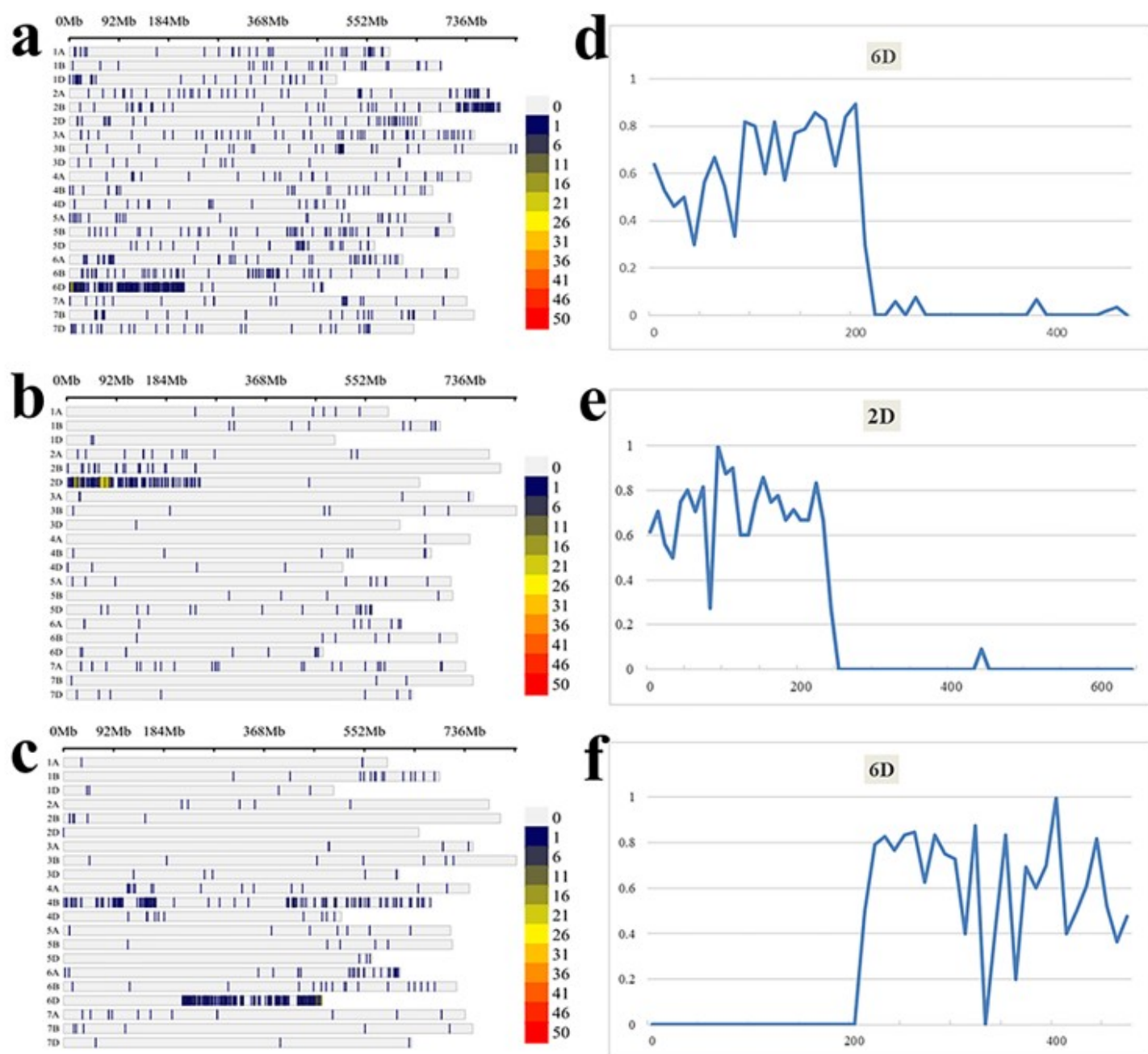


Figure 2

Analysis of the chromosomal composition of lines K227-48, K242-82, and K246-6 using a wheat 55K SNP array. (a, b, c) Distribution of deletion genotype SNP markers on wheat chromosome. (d, e, f) The SNP elimination analysis for chromosomes 6D, 2D, and 6D in the genome, the deleted ratio was calculated in 10 Mb sliding windows, with 1 Mb steps



Figure 3

The responses of lines K227-48, K242-82, K246-6 and their parents to mixture *Pst* race at the adult plant stage. 1, Avocet S; 2, SM482; 3, SM921; 4, CS*ph1b*; 5, 8801; 6, K17-1065-4; 7, K227-48; 8, K242-82; 9, K246-6

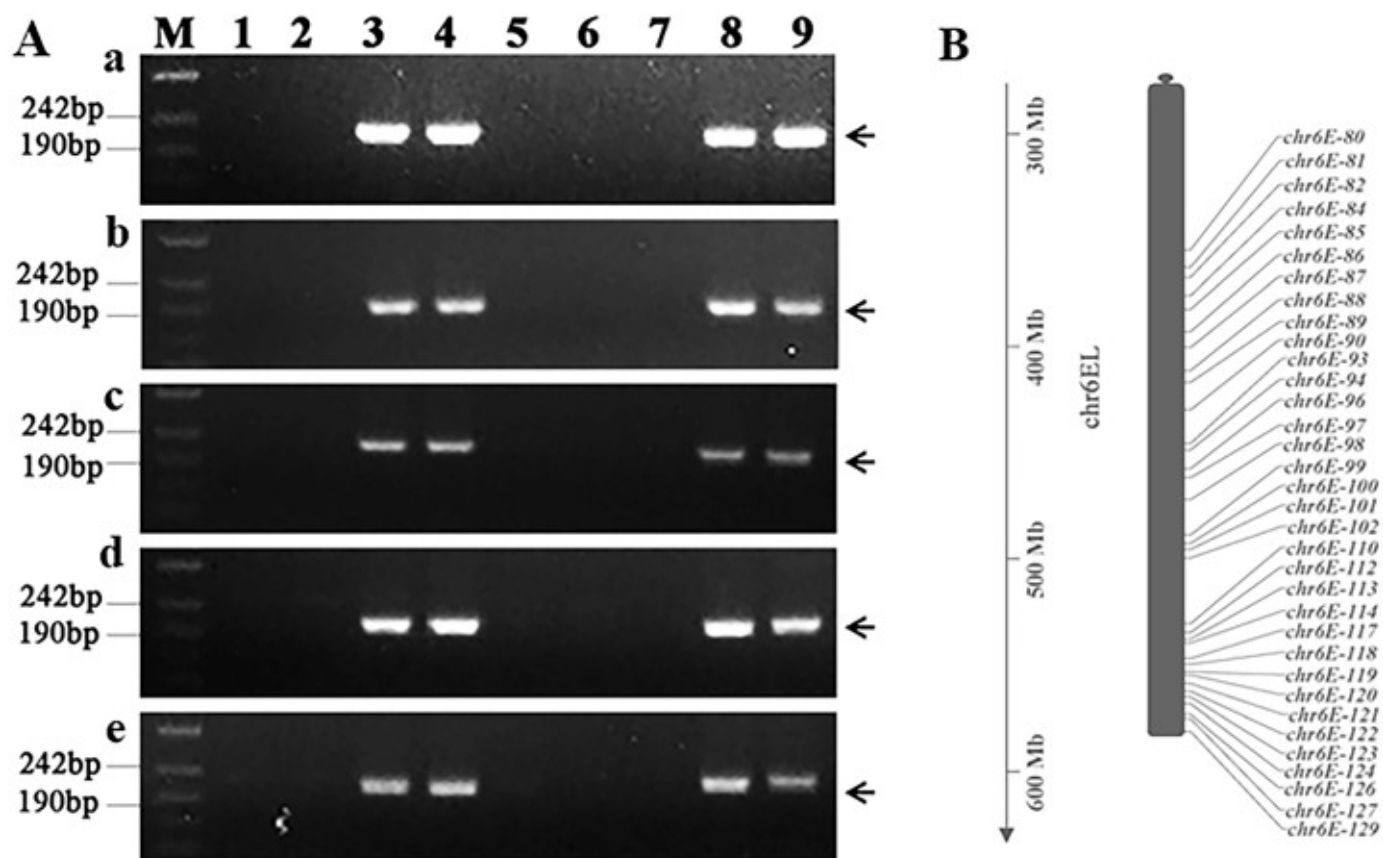


Figure 4

(A) Validation of SSR markers in parents and translocation lines; (B) physical bin map of the 6EL chromosome. (a) *Chr6E-86*; (b) *Chr6E-88*; (c) *Chr6E-94*; (d) *Chr6E-110*; (e) *Chr6E-112*. M, marker (500 bp); 1, CS; 2, CS*ph1b*; 3, 8801; 4, K17-1065-4; 5, SM482; 6, SM482; 7, K227-48; 8, K242-82; 9, K246-6. Arrows indicate the amplification bands of tetraploid *Th.elongatum* chromosome 6E



Figure 5

Plant morphology of lines K227-48, K242-82, K246-6 and their parents. (a) Adult plants; (b) spikes; (c) spikelets; (d) grains. 1, SM482; 2, SM921; 3, *CSph1b*; 4, K17-1065-4; 5, K227-48; 6, K242-82; 7, K246-6

Supplementary Files

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