# Development, identification, and utilization of wheat–tetraploid Thinopyrum elongatum4EL translocation lines resistant to stripe rust

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**Research Article**

**Keywords:** chromosomal translocation, molecular markers, stripe rust, tetraploid Th. elongatum

**Posted Date:** October 23rd, 2023

**DOI:** https://doi.org/10.21203/rs.3.rs-3453901/v1



**Version of Record:** A version of this preprint was published at Theoretical and Applied Genetics on January 10th, 2024. See the published version at https://doi.org/10.1007/s00122-023-04525-5.

# Abstract

Tetraploid Thinopyrum elongatum is a valuable source of genes useful for improving the resistance of wheat to diseases such as stripe rust, powdery mildew, and Fusarium head blight. There is an urgent need for new tetraploid Th. elongatum disease resistance genes that can be transferred to wheat via alien translocation lines. We previously reported that chromosome 4E of the 4E (4D) substitution line carries all-stage stripe rust resistance genes. To optimize the utility of these genes in wheat breeding programs, we developed translocation lines by inducing chromosomal structural changes through 60Co-γ irradiation and developing monosomic substitution lines. In total, 53 plants with different 4E chromosomal structural changes were identified. Three homozygous translocation lines (T4DS·4EL, T5AL·4EL, and T3BL·4EL) and an addition translocation line (T5DS·4EL) were identified on the basis of GISH, FISH, FISH-painting, and wheat 55K SNP array analyses. These four translocation lines, which contained the chromosome 4EL, exhibited enhanced stripe rust resistance. Thus, the resistance gene (tentatively named Yr4EL) was localized to chromosome 4EL of tetraploid Th. elongatum. By examining the diploid Th. elongatum genome, 48 SSR markers specific to chromosome 4E were obtained, of which 32 co-segregated with chromosome 4EL. Furthermore, the 4DS·4EL translocation line had better agronomic traits than the wheat parents. After transferring Yr4EL into wheat cultivars SM482, CM42, and SM51, the derived progenies had diverse wheat genetic backgrounds, but they were all resistant to stripe rust and there were no yield penalties. These lines may be useful for breeding wheat varieties resistant to stripe rust.


# Key Message

**The new stripe rust resistance gene *Yr4EL* in tetraploid *Th. elongatum* was identified and transferred into common wheat via 4EL translocation lines.**

# Introduction

Wheat stripe rust, which is caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is a devastating fungal disease worldwide. Stripe rust mainly affects photosynthesis in wheat leaves, leading to severe yield losses (Chen et al. 2005; Hou et al. 2016), which typically reach 5%–20%, but may exceed 30% or even lead to crop failure in severe cases (Wellings 2011). Enhancing host immunity is the most cost-effective, environmentally friendly, and efficient strategy for controlling wheat stripe rust (Chen et al. 2013; Jiang et al. 2023). To date, 86 stripe rust resistance genes (*Yr1*– *Yr86*) have been formally cataloged, while more than 100 provisionally named genes and more than 350 quantitative trait loci (QTLs) have been identified, 42 of which mediate all-stage resistance (Jan et al. 2021; Feng et al. 2023; jin et al. 2023; Zhu et al. 2023). Eighteen of these genes are from the progenitors and relatives of wheat, including *Triticum spelta*, *Triticum durum*, *Triticum dicoccoides*, *Aegilops tauschii*, *Secale cereale*, and *Thinopyrum ponticum*, suggesting that wheat relatives carry many stripe rust resistance genes (McIntosh et al. 2000; Singh et al. 2000; Shi et al. 2001; Sun et al. 2002; Hou et al. 2016; Klymiuk et al. 2022). However, only a few genes conferring stable resistance have been deployed and used in wheat breeding programs

because of the evolution of virulent pathogen mutants or other genetic changes (Bai et al. 2014; Sharma-Poudyal et al. 2013; Yuan et al. 2012). To overcome the rapid loss of disease resistance and enhance wheat production, new sources of wheat stripe rust resistance must be identified and the associated genes will need to be transferred to commercial wheat varieties.

*Thinopyrum elongatum* (Host) D. R. Dewey is a wild relative of wheat with multiple desirable agronomic traits, such as the resistance to a variety of diseases (stripe rust, Fusarium head blight, leaf rust, powdery mildew, and stem rust) and abiotic stresses (salinity and drought), making it very useful for wheat breeders (Dvořák and Chen 1984; Dvořák et al. 1988; Fedak et al. 1999; Liu 2022). In addition, the E genome of *Th. elongatum* is closely related to the A, B, and D genomes of wheat. Hence, it is relatively easy to obtain fertile progenies from crosses between *Th. elongatum* and common wheat. Many lines derived from the wheat × *Th. elongatum* hybridization carry valuable genes and have been used as bridge materials for improving the resistance of common wheat to stripe rust, stem rust, powdery mildew, Fusarium head blight, and salt stress (Wang et al. 2020; Borrajo et al. 2022; Gong et al. 2023). Examples include 5E, 6E, and 7E disomic addition lines and 1E, 3E, 4E, 6E, and 7E substitution lines (Li et al. 2016; Zhu et al. 2017; Yin et al. 2019; Yang et al. 2021, 2022; Gong et al. 2023; Guo et al. 2023; Zeng et al. 2023). However, relatively few *Th. elongatum* disease resistance genes have been successfully transferred into wheat lines and used for breeding, likely because of the rapid loss of disease resistance or the linkage drag associated with *Th. elongatum* chromosomes. Therefore, identifying new resistance genes in tetraploid *Th. elongatum* genotypes and developing wheat–tetraploid *Th. elongatum* translocation lines with stable disease resistance and excellent agronomic traits are critical for improving wheat germplasm resources.

In a previous study, we developed a wheat–tetraploid *Th. elongatum* 4E (4D) substitution line (K16-730-3) that was highly resistant to stripe rust and powdery mildew at all stages and determined that the resistance was conferred by the alien chromosome 4E (Gong et al. 2022). In the current study, K16-730-3 plants were irradiated with $^{60}$Co-γ rays and 4E monosomic substitution lines were generated. The objectives of this study were to (1) reveal the genome constitution of the translocation lines, (2) evaluate the stripe rust resistance and agronomic traits of the translocation lines, (3) develop chromosome-specific simple sequence repeat (SSR) markers for translocated alien segments, and (4) exploit *Yr4EL* for wheat breeding. The new germplasm resources obtained from these translocations will be useful for breeding stripe rust-resistant wheat.

# Materials and methods

## Plant materials

*Triticum durum*–tetraploid *Th. elongatum* partial amphidiploid line 8801 (2n = 6x = 42, AABBEE), which is highly resistant to stripe rust, powdery mildew, and Fusarium head blight, was kindly provided by Dr. George Fedak (Eastern Cereal and Oilseed Research Centre, Ottawa, Canada) (Li et al. 2018a). The wheat–tetraploid *Th. elongatum* 4E (4D) substitution line (K16-730-3) highly resistant to wheat stripe rust

and powdery mildew used in this study was generated in an earlier study (Gong et al. 2022). Genomic DNA extracted from a tetraploid *Th. elongatum* line (2n = 4x = 28, EEEE; PI 531750) was used as the genomic in situ hybridization (GISH) probe. The stripe rust-susceptible wheat lines Shumai482 (SM482), Chuanmai42 (CM42), and Shumai51 (SM51) were used as rotating parents. Wheat line Avocet S served as the stripe rust-susceptible control. Genomic DNA from wheat cultivar Chinese Spring (CS) was used as the blocking DNA for GISH. The above-mentioned plant materials were stored at the Triticeae Research Institute, Sichuan Agricultural University, China.

## Development of translocation lines

The K16-730-3 plants at the heading stage were treated with $^{60}$Co-γ rays (18 Gy) at a dose rate of 1 Gy/min. The irradiation was performed at the Institute of Biotechnology and Nuclear Technology Research, Sichuan Academy of Agricultural Sciences, China. The irradiated K16-730-3 plants were used as the female parent in crosses with CM42, which resulted in 479 $M_1$ seeds. Additionally, a cross between K16-730-3 and SM482 produced an $F_2$ population comprising 1,000 individuals. The markers *TTE4E-77* and *TTE4E-112* (Gong et al. 2022) on diploid *Th. elongatum* chromosomes 4ES and 4EL, respectively, were used to detect stripe rust-resistant plants in the $F_2$ generation.

## GISH, FISH, and FISH chromosome painting (FISH-painting) analyses

Seeds were incubated at 4 °C to break dormancy and then germinated at 22 °C in an incubator. When the roots of the resulting seedlings reached 1–2 cm in length, they were treated with $N_2O$ for 2 h and 20 min and then immediately fixed in acetic acid for 5 min and digested with pectinase and cellulase (Komuro et al. 2013). The slides for the GISH and fluorescence in situ hybridization (FISH) assays were prepared as previously described (Han et al. 2006). Tetraploid *Th. elongatum* genomic DNA was labeled with dUTP-ATO-550 (Jena Bioscience, Jena, Germany) and used as the probe, whereas CS DNA was used as the blocking DNA (1:150). The GISH analysis was performed according to a published procedure (Han et al. 2009). Briefly, 1 μL tetraploid *Th. elongatum* genomic DNA probe was mixed with 9 μL hybridization mixture (1 g dextran sulfate, 5 mL formamide, 1 mL 20× SSC, 1 mL salmon sperm, and 2 mL dd$H_2O$) and denatured at 90 °C for 10 min. The sample was cooled on ice for 5 min, after which 3 μL CS DNA was added. The solution was mixed and added to the slides, which were covered with coverslips, heated at 85 °C for 5 min, and incubated overnight at 50 °C (Gong et al. 2023). The slides were immersed in 2× SSC and incubated at 50 °C water bath for 10 min. They were then sequentially washed with dd$H_2O$, 75% ethanol, and 100% ethanol for 5 min each. The chromosomes were counterstained with 4',6-diamidino-2-phenylindole (DAPI; Vector Laboratories, Burlingame, CA). The slides were examined using the BX63 fluorescence microscope (Olympus, Tokyo, Japan) and images were captured using the DP-80 camera.

After the GISH analysis, the slides were recycled as previously described (Gong et al. 2023) before adding the Oligo-pSc119.2 and Oligo-pTa535 FISH probes to determine the chromosomal composition of the translocation lines. The FISH analysis was conducted as described by Li et al. (2019). The FISH signals were captured using the same method as that used to capture GISH signals.

The bulk oligonucleotide libraries Chr1–Chr7 (1E–7E) for the whole genome sequence of the diploid *Th. elongatum* were used for the FISH painting analysis, which was completed to determine the homologous group relationships among the alien chromosomes carried by all translocation lines (Chen et al. 2023) as described by Bi et al. (2020) and Han et al. (2015). Micrographs were obtained as described for the GISH analysis.

### 55K SNP array analysis

A wheat 55K SNP array with 53,063 single nucleotide polymorphisms (SNPs) (China Golden Marker Biotechnology Co., Ltd., Beijing, China) was used to genotype the translocation lines. The SNP sites in the array were distributed on 42 common wheat chromosomes, with AA, TT, CC, GG, AT, and AG indicating different genotypes at a particular position and NA indicating the absence of the position on the chromosome. The NA ratio was calculated by dividing the number of SNP markers with the NA genotype by the total number of SNP markers. The NA ratio for each chromosome was calculated in 10 Mb sliding windows with 10 Mb steps. The chromosomal regions with NA ratios that were significantly higher than the average of the other chromosomal regions were considered to be affected by deletion and translocation events.

### Assessment of stripe rust resistance

The responses of the translocation lines and their parents to *Pst* race CYR-34 at the seedling stage were examined in an artificial climate chamber. The plants were inoculated and their response to the stripe rust pathogen were evaluated as previously described (Li et al. 2019). The infection types (ITs) were determined using the following 0–9 scale as described by Line and Qayoum (1992): 0 and 1: immune; 2–6: resistant; 7–8: susceptible; 9: highly susceptible.

The responses of the translocation lines and their parents to a stripe rust infection at the adult plant stage in the field were evaluated using a mixture of *Pst* races (CYR 32, CYR 33, CYR 34, Sull-4, Sull-5, Sull-7, and G22-14) at Sichuan Agricultural University, Chengdu, Sichuan, China. Plants were inoculated as described by Carter and Chen (2009) and Gong et al. (2023). The IT scale used to assess the adult plants was the same as the IT scale used to evaluate the seedlings.

### Evaluation of agronomic performance

The following agronomic traits of the translocation lines and their parents were evaluated at the Wenjiang Experimental Station of Sichuan Agricultural University, China: tiller number, spikelets per spike, plant height, spike length, grains per spike, and 1,000-grain weight. Three replicates were prepared during the 2022–2023 growing season. For each replicate, 15 seeds per line were sown uniformly in 1.5 m rows with 0.3 m between rows. The agronomic traits of 10 plants in the middle of the middle row were recorded and analyzed using the SPSS Statistics 24.0 software.

### Development of chromosome 4E-specific molecular markers

Simple sequence repeats on diploid *Th. elongatum* chromosome 4E (NCBI BioProject ID PRJNA540081) were detected using the Perl-based MISA tool (http://pgrc.ipkgatersleben.de/misa/download/misa.pl); the detection criteria were as described by Gong et al. (2023). The obtained SSR sequences were used to develop SSR markers using Primer 3 Plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). The specificity of the SSR markers for the CS genome was determined via e-PCR. The markers that did not amplify the target fragments in the CS genome were retained. The SSR sequences corresponding to these markers were used as queries for a BLAST search of the diploid *Th. elongatum* genome sequence. The sequences uniquely mapped to chromosome 4E were selected. All markers were synthesized by Sangon Biotech (Chengdu, China). The PCR program and product detection method were as described by Gong et al. (2022). The 4E-specific SSR markers were mapped to the diploid *Th. elongatum* 4E chromosome using the TBtools (version 1.120) software developed by Chen et al. (2020).

### Breeding-based transfer of *Yr4EL*

On the basis of the double top-cross and two-phase selection strategy (Hao et al. 2019), the obtained disease-resistant translocation lines were crossed with stripe rust-susceptible varieties SM482, SM969, and CM42 to transfer the resistance gene *Yr4EL*. The progeny were screened by molecular markers and GISH identification to obtain wheat varieties or lines with excellent agronomic traits.

## Results

### Development and identification of translocation lines

We irradiated the 4E (4D) substitution line K16-730-3 with $^{60}$Co-γ rays to obtain the $M_1$ population, while also generating the K16-730-3 × SM482 $F_2$ population. The GISH analysis of the $M_1$ population was conducted using tetraploid *Th. elongatum* genomic DNA as the probe and CS DNA as the blocking DNA. Among the 134 $M_1$ plants, 46 carried different 4E chromosome segments, with chromosome numbers ranging from 40 to 43. The 43 translocation lines had a translocation frequency of 32.1% (Supplementary Fig. S1). The *TTE4E-77* (4ES) and *TTE4E-112* (4EL) markers were used to detect 600 stripe rust-resistant plants in the K16-730-3 × SM482 $F_2$ population (Supplementary Fig. S2), of which 10 plants carried chromosome 4EL, but not chromosome 4ES. According to the GISH analysis, K23-163-5 and K23-43-79-7 from the $M_1$ population and K23-161-16 from the K16-730-3 × SM482 $F_2$ population carried 42 chromosomes, including two E chromosome arms that translocated with the wheat chromosome arm (Fig. 1a, c, e). In addition, K23-55-62-25 from the $M_1$ population had 44 chromosomes, two of which were E chromosome arms connected to the common wheat chromosome (Fig. 1g).

The FISH analysis of these translocation lines using Oligo-pSc119.2 (green) and Oligo-pTa535 (red) as well as the FISH karyotypes of CS and tetraploid *Th. elongatum* as references (Tang et al. 2014; Li et al. 2018b) showed that chromosome 4EL replaced chromosome arms 4DL and 3BS in K23-163-5 and K23-161-16, respectively (Table 1; Fig. 1a, b, e, f). The analysis of K23-43-79-7 revealed the chromosome

5AL·4EL and 5AS·5DL translocations and the deletion of chromosome 5DS (Table 1; Fig. 1c, d). Furthermore, K23-55-62-25 carried 42 complete wheat chromosomes and a pair of 5DS·4EL translocated chromosomes (Table 1; Fig. 1g, h). The alien chromosomes of these translocation lines were analyzed by FISH-painting using the probes Chr1–Chr7. A strong signal was detected for probe Chr4 (green) (Fig. 2a–h). Thus, K23-163-5, K23-43-79-7, and K23-161-16 were wheat–tetraploid *Th. elongatum* T4DS·4EL, T5AL·4EL, and T3BL·4EL translocation lines, respectively, while K23-55-62-25 was a T5DS·4EL addition translocation line. A wheat–tetraploid *Th. elongatum* T4ES·2AL translocation line (KW-2-3) was also detected (Supplementary Fig. S3a, c).

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

The wheat 55K SNP array was used to analyze the T4DS·4EL, T5AL·4EL, and T3BL·4EL chromosomal structures. The analysis of the distribution and frequency of the effective SNPs showed that chromosomes 4DL and 3BS were missing from T4DS·4EL and T3BL·4EL, respectively (Fig. 3). The number of detected SNPs on the translocated chromosomes of T4DS·4EL and T3BL·4EL (10 Mb sliding windows, with 10 Mb steps) indicated that the breakpoints of the translocated chromosomes in T4DS·4EL and T3BL·4EL were located in the 4D (185.4–206.1 Mb) (Fig. 3a, b, c) and 3B (344.2–348.2 Mb) (Fig. 3g, h, i) centromeric regions, respectively (IWGSC RefSeq v1.0). In addition, many SNPs on chromosome 5D were missing because of the deletion of chromosome 5DS in T5AL·4EL. Because both chromosomes 5AS and 5AL were present in T5AL·4EL, there was no significant deletion of SNPs on chromosome 5A (Fig. 3d, e, f). This is consistent with the GISH and FISH results, which provided additional evidence that T4DS·4EL, T5AL·4EL, and T3BL·4EL were the 4DS·4EL, 5AL·4EL, and 3BL·4EL translocation lines, respectively.

## Evaluation of stripe rust resistance

The seedlings of all (addition) translocation lines and their parents were inoculated with *Pst* race CYR-34 to evaluate their susceptibility to stripe rust. Similar to the susceptible wheat line Avocet S, the common wheat parents (SM51, SM482, and CM42) and translocation line T4ES·2AL were susceptible to CYR-34, with an IT of 8. In contrast, the 4E (4D) substitution line K16-730-3 and the newly generated 4EL (addition) translocation lines (T4DS·4EL, T5AL·4EL, T3BL·4EL, and T5DS·4EL) were highly resistant, with an IT of 1 (Fig. 4a).

All lines/cultivars were inoculated with a mixture of *Pst* strains/isolates (CYR 32, CYR 33, CYR 34, Sull-4, Sull-5, Sull-7, and G22-14) at the adult plant stage. As expected, Avocet S was highly susceptible to stripe rust (IT of 9). According to their ITs, SM51, SM482, CM42, and T4ES·2AL were susceptible to stripe rust (IT of 8), whereas K16-730-3, T4DS·4EL, T5AL·4EL, T3BL·4EL, and T5DS·4EL were resistant to stripe rust (IT of 1) (Fig. 4b). The all-stage stripe rust resistance of these lines carrying 4EL was due to a gene that was tentatively named *Yr4EL*.

## Agronomic traits of the translocation lines

The agronomic traits varied substantially among T4DS·4EL, T5AL·4EL, T3BL·4EL, and T5DS·4EL (Table 2; Figs 5, 6). The agronomic traits of T4DS·4EL were better than those of T5AL·4EL, T3BL·4EL, and T5DS·4EL, especially the tiller number and spike morphology (Table 2; Fig. 6c). The tiller number and grains per spike were significantly higher for T4DS·4EL than for T5AL·4EL, T3BL·4EL, T5DS·4EL, and the parents (Table 2; Fig. 6a, e). The 1,000-grain weight of T4DS·4EL was significantly higher than that of SM482, T5AL·4EL, T3BL·4EL, and T5DS·4EL, but not significantly different from that of SM51, CM42, and K16-730-3 (Table 2; Fig. 6f). In terms of plant height, T4DS·4EL was similar to CM42, but it was significantly shorter than K16-730-3 (Table 2; Fig. 6b). Considered together, these results reflected the excellent agronomic traits of translocation line T4DS·4EL, which may be a useful genetic resource for improving wheat stripe rust resistance.

## Development of specific molecular markers

A total of 77,106 SSRs were retrieved by analyzing the diploid *Th. elongatum* 4E reference genome. Molecular markers were developed for these SSRs using Primer 3 Plus. The 15,301 SSR markers that were generated were tested for their specificity to the *Th. elongatum* genome. According to the e-PCR results, the markers that did not amplify the target fragments in the CS genome were retained. Finally, 3,949 specific molecular markers with a unique physical location on chromosome 4E were obtained.

To efficiently transfer and detect the 4E translocated chromosome, 146 SSR markers with different physical locations on chromosome 4E were randomly selected, among which 48 SSR markers were able to amplify specific fragments on the tetraploid *Th. elongatum* 4E chromosome (Fig. 7; Supplementary Table S1). Specifically, 16 SSR markers, including *TTE4E-731*, *TTE4E-2040*, and *TTE4E-3288*, were able to amplify the target fragment in 8801, K16-730-3, and T4ES·2AL, but not in CS, SM482, SM51, CM42, T4DS·4EL, T5AL·4EL, T3BL·4EL, and T5DS·4EL; these markers are specific to the tetraploid *Th. elongatum* 4ES chromosome (Fig. 7a–d). Moreover, 32 SSR markers, such as *TTE4E-6405*, *TTE4E-7000*, and *TTE4E-7342*, amplified specific fragments in 8801, K16-730-3, T4DS·4EL, T5AL·4EL, T3BL·4EL, and T5DS·4EL, but not in CS, SM482, SM51, CM42, and T4ES·2AL; these markers are specific to the tetraploid *Th. elongatum* 4EL chromosome (Fig. 7a, e–g).

## Utility of *Yr4EL* for breeding

Using the double top-cross and two-phase selection strategy, the wheat–tetraploid *Th. elongatum* 4DS·4EL chromosome homologous line K23-163-5 and the heterozygous line KW-7-2-5 (Supplementary Fig. S3a, b) were crossed with the stripe rust-susceptible varieties SM482, SM969, and CM42 (Fig. 8). The 4EL chromosome-specific SSR marker *TTE4E-3552* was used to identify the progenies carrying alien chromosomes. All plants carrying the 4DS·4EL chromosome were highly resistant to stripe rust, while the plants lacking this chromosome were highly susceptible to stripe rust (Fig. 8a–d). These progenies (e.g., K23-181) represent new breeding materials with *Yr4EL*, excellent agronomic traits, and stable resistance to stripe rust (Fig. 8e, f).

# Discussion

## Importance of compensatory translocation lines for wheat breeding

The development of wheat–relative translocation lines can decrease the effect of unfavorable genes on alien chromosomes and introduce genes associated with desirable traits into wheat (Duan 2018). For example, wheat–rye 1RS·1BL chromosome translocation lines with multiple genes conferring stripe rust and powdery mildew resistance were developed in the 20th century and then widely used in wheat breeding programs worldwide, with the 1RS·1BL translocation detected in approximately 30% of wheat varieties (Ko et al. 2002; Howell et al. 2014; Wang et al. 2017). In the 1990s, breeders produced a wheat–*Haynaldia villosa* 6VS·6AL translocation line carrying a new powdery mildew resistance gene (*Pm21*) and various genes underlying ideal traits; this line has since been used by wheat breeders to produce more than 40 new wheat varieties in China (Chen et al. 1995a; Xing et al. 2021). The wheat–*Aegilops ventricosa* 2NS·2AS translocation line resistant to various biotic stresses, including wheat blast, has been used to develop multiple wheat varieties, including Jagger, Milan, and Hyak (Guo et al. 2023; Helguera et al. 2003). The Pubing series of wheat lines were derived from the recently generated wheat–*Agropyron cristatum* translocation lines with excellent traits (e.g., high yield and disease and stress resistance) (Zhang et al. 2019; Zhou et al. 2019). Tetraploid *Th. elongatum* is a wild germplasm resistant to a variety of wheat diseases, but there are no reports of the successful application of its stress resistance genes for improving wheat lines with desirable agronomic traits. In the current study, three wheat–tetraploid *Th. elongatum* 4EL chromosome translocation lines (T4DS·4EL, T5AL·4EL, and T3BL·4EL) and a wheat–tetraploid *Th. elongatum* addition translocation line (T5DS·4EL) exhibiting all-stage resistance to stripe rust were developed. The agronomic traits of T4DS·4EL were superior to those of the parents and other translocation lines, especially the tiller number, grains per spike, 1,000-grain weight, and spike shape (i.e., compact). Thus, the 4DS·4EL translocation may have decreased the genetic drag caused by chromosome 4E and resulted in improved genetic compensation. The relatively poor agronomic traits associated with the T5AL·4EL and T3BL·4EL translocations may be due to the additional introgressed genes/QTLs related to agronomic traits on chromosomes 5D and 3B (Liu et al. 2023; Liao et al.2023). Overall, the translocation lines developed in this study will provide the foundation for future studies conducted to exploit the resistance genes on chromosome 4E of the tetraploid *Th. elongatum*. Moreover, the T4DS·4EL translocation line with desirable agronomic traits may be applicable for breeding wheat varieties resistant to stripe rust.

## Utility of *Yr4EL* for wheat breeding to enhance stripe rust resistance

To date, many wheat stripe rust resistance genes have been identified, some of which were derived from the progenitors or wild relatives of wheat, including *Yr5*, *Yr8*, *Yr9*, *Yr15*, *Yr17*, *Yr19*, *Yr28*, *Yr35*, *Yr36*, *Yr37*, *Yr38*, *Yr40*, *Yr42*, *Yr50*, *Yr69*, *Yr70*, *Yr83*, and *Yr84* (Gong et al. 2022; Klymiuk et al. 2022; Li et al. 2020). Some of these resistance genes have been widely used by wheat breeders (e.g., *Yr5*, *Yr9*, *Yr15*, *Yr17*, *Yr24*, *Yr28*, and *Yr36*) (Bariana and Mcintosh 1994; He et al. 2020; Zhou et al. 2017). Among the 348 wheat germplasm resources analyzed by Dong et al. (2019) for the presence of stripe rust resistance genes, 122

were revealed to carry *Yr5*. In other studies, *Yr15* was detected in eight of the 72 materials collected from wheat-growing regions in Sichuan province, China (Wu et al. 2007), while *Yr17* was detected in Zhongmai 175, Mengmai 58, Huaiyang 1, and Shanong 33 (Huang et al. 2021; Lu et al. 2016; Wang et al. 2018). Because *Pst* races mutate rapidly, the extensive use of a single resistance gene for breeding stripe rust-resistant wheat varieties may be ineffective, leading to stripe rust pandemics (Han et al. 2010). For example, *Yr19* from rye was widely used in breeding programs in China, but it became ineffective after the appearance and spread of CYR29 in 1985 (Luo et al. 2005). Strain TSA-6, which is unaffected by *Yr5*, was recently detected for the first time in China (Zhang et al. 2020). Therefore, new stripe rust resistance genes must continue to be identified and applied. In the current study, *Yr4EL* was identified as an all-stage stripe rust resistance gene on the tetraploid *Th. elongatum* 4EL chromosome. Although some stripe rust resistance genes from *Thinopyrum* species have been reported, including *Yr69* derived from *Th. ponticum* in the wheat deletion bin 2AS-5 (FL of 0.78–1.00) (Hou et al. 2016) and *YrE* and *Yr2E* on chromosomes 3E and 2E, respectively (Liu 2007; Ma et al. 1999). In addition, *Yrx9366* on chromosome 2AL provides resistance to CYR25, CYR27, CYR30, and CYR31 (Wang et al. 2013). *Yrxy1* on chromosome 7A was localized to a 10.3 cM region between SSR markers *Xbarc49* and *Xwmc422* (Zhou et al. 2011). However, none of these loci are from the group 4 chromosome. Furthermore, the all-stage stripe rust resistance genes *Yr22* and *Yr28* were localized in homologous group 4, whereas *Yr22* and *Yr28* were derived from *Triticum aestivum* and *Ae. tauschii*, respectively. The effects of *Yr22* and *Yr28* on *Pst* races CYR 32, CYR 33, CYR 34, Sull-4, Sull-5, Sull-7, and G22-14 reportedly differ from those of *Yr4EL* (Chen et al. 1995; Singh et al. 2000; Wang et al. 2021). Thus, we propose that *Yr4EL* is not an ortholog of *Yr22* or *Yr28*. Instead, it is a new gene mediating stripe rust resistance.

### The development of stable, reliable, and specific molecular markers can accelerate wheat breeding

High-density and stable specific molecular markers are effective tools for identifying and localizing superior genes in the wheat genetic background. There are currently various molecular markers specific to the E genome of *Th. elongatum*. For example, Liu et al. (2018) developed 573 specific-locus amplified fragment sequencing (SLAF-seq) markers and constructed a physical map of chromosome 4Ag by analyzing eight wheat– *Th. ponticum* 4Ag chromosome translocation lines, which localized the blue-grain gene to bin 4AgL-6 (FL of 0.75–0.89) and revealed 89 markers related to the blue-grain trait. Luo et al. (2017) detected 35,193 E genome-specific SNPs on the basis of a comparative analysis of the transcripts from CS and CS– *Th. elongatum* octoploids, validated 420 randomly selected SNPs, and developed 33 specific SNP markers for chromosome 3E. We previously conducted genotyping-by-sequencing analyses and developed 132 and 74 chromosome-specific molecular markers for tetraploid *Th. elongatum* 1E and 4E, respectively (Gong et al. 2022; Li et al. 2019). More recently, 33 tetraploid *Th. elongatum* 6E chromosome-specific molecular markers that were developed by analyzing the diploid *Th. elongatum* genome sequence were linked to disease resistance genes on the corresponding chromosomes (Gong et al. 2023). However, there is a lack of highly specific, reliable, and stable molecular markers useful for exploiting the many valuable genes in *Th. elongatum*. Thus, on the basis of the diploid *Th. elongatum* genome sequence, we developed 48 SSR markers specific for chromosome 4E of tetraploid *Th. elongatum*, of which 16 and 32 markers on chromosomes 4ES and 4EL are effective markers for

detecting chromosomes 4ES and 4EL in different wheat genetic backgrounds. These specific molecular markers may be applicable for molecular marker-assisted selection and the localization of agronomically important genes on chromosome 4E. As part of our future research, we will generate stable high-generation lines from T4DS·4EL, T5AL·4EL, T3BL·4EL, and T5DS·4EL. Furthermore, we will construct 4EL small-fragment translocation lines resistant to stripe rust and develop additional 4EL chromosome-specific markers useful for locating and cloning the stripe rust resistance genes on chromosome 4E of tetraploid *Th. elongatum* for the subsequent breeding of disease-resistant wheat lines.

# Declarations

### Author Contributions

Biran Gong, Linfeng Chen, Hao Zhang, Dandan Wu, and Houyang Kang conducted the experiment, analyzed the data, and drafted the manuscript. 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 Zhouprovided technique guidance. Dandan Wu and Houyang Kang designed the experiment and formulated the questions. All the authors read and approved the manuscript.

### Acknowledgments

This work was supported by the Major Program of National Agricultural Science and Technology of China (NK20220607), and the National Natural Science Foundation of China (No. 31971883, No. 32200180), and the Science and Technology Bureau of Sichuan Province (2023NSFSC1995, 2022YFH0069, 2022NSFSC1671), and the Science and Technology Bureau of Chengdu City (2022-YF05-00449-SN). We thank Dr. George Fedak, Eastern Cereal and Oilseed Research Center, Ottawa, Canada, for kindly supplying the *Trititrigia* 8801 material used in this study. We also thank Prof. Q. Z. Jia, Plant Protection Institute of Gansu Academy of Agricultural Sciences, Gansu, China, for kindly supplying the urediniospores of the *P. striiformis* f. sp. *tritici* races.

**Conflict of interest** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Data availability** The whole genome sequence of diploid *Th. elongatum* reported in this study can be found in the National Center for Biotechnology Information, NCBI BioProject ID PRJNA540081. Data supporting the results of this study are in the manuscript or supplemental file.

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# Tables

**Table 1** Genetic genealogy and chromosome composition of four 4EL translocation lines

| No. | Line | Pedigree | Generation | chromosome composition |
|---|---|---|---|---|
| K23-163-5 | T4DS·4EL | K16-730-3 (irradiate) × CM42 $M_2$ × CM42 | $F_3$ | 2n=42=40W+II 4DS·4EL |
| K23-43-79-7 | T5AL·4EL | K16-730-3 (irradiate) × CM42 × CM42 $BC_1F_2$ × CM42 | $F_2$ | 2n=42=40W+II 5AL·4EL |
| K23-161-16 | T3BL·4EL | K16-730-3 × SM482 | $F_4$ | 2n=42=40W+II 3BL·4EL |
| K23-55-62-25 | T5DS·4EL | K16-730-3 (irradiate) × CM42 × CM42 | $BC_1F_3$ | 2n=44=42W+II 5DS·4EL |
| KW-2-3 | T4ES·2AL | K16-730-3 (irradiate)×CM42×CM42 | $BC_1F_1$ | 2n=40=39W+I 4ES·2AL |

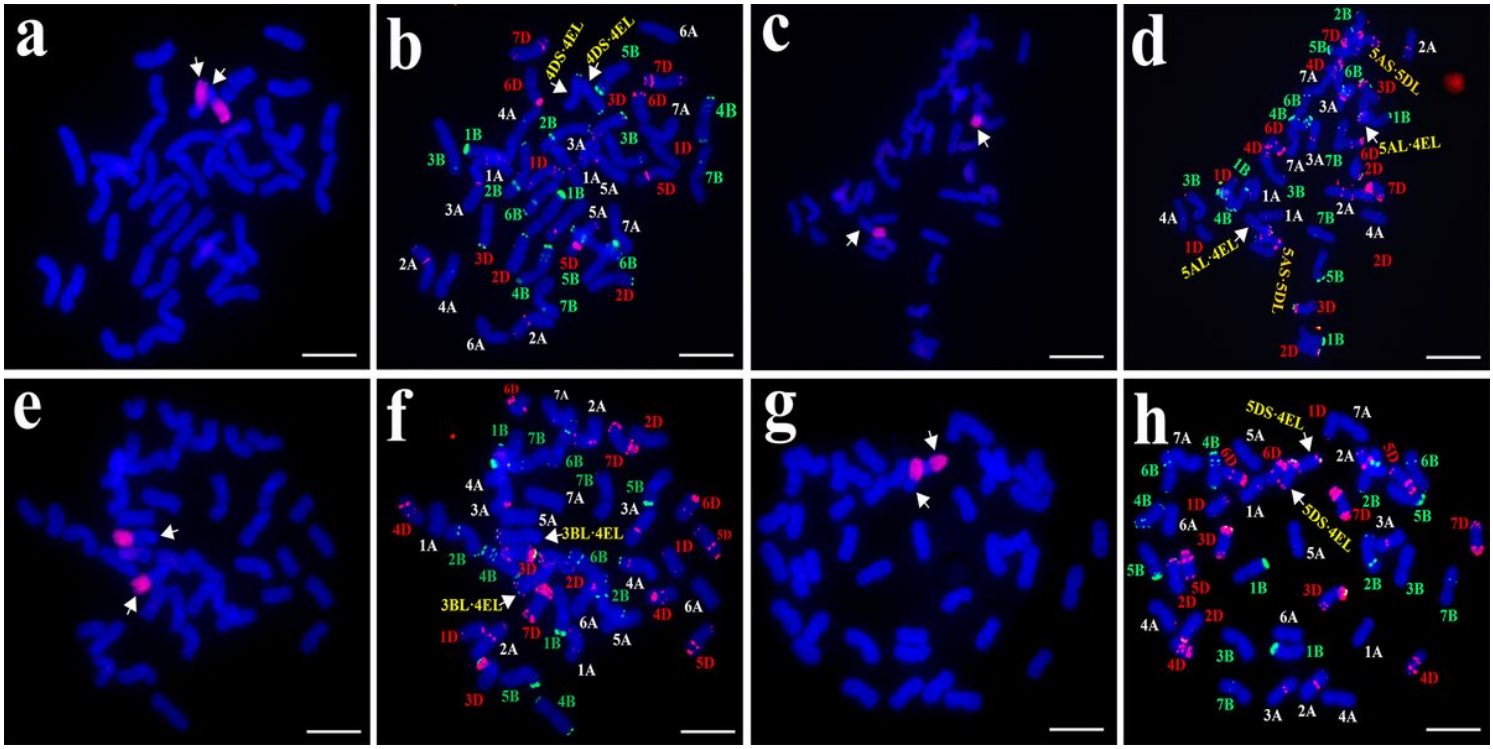
W: Common wheat chromosome

**Table 2** Agronomic traits of four wheat-tetraploid *Th. elongatum* 4EL chromosome translocation lines and their parents [z].

| Line | Tiller number | Plant Height (cm) | Spike Length (cm) | Spikelets per spike | Grains per spike | 1000-grain weight (g) |
|---|---|---|---|---|---|---|
| SM482 | 6.8±1.0c | 76.6±2.6de | 14.5±0.4a | 24.0±2.2a | 37.3±2.3d | 35.7±0.3b |
| SM51 | 8.0±0.9bc | 84.5±2.0c | 12.6±1.2c | 19.7±1.4d | 52.3±1.5c | 48.3±0.6a |
| CM42 | 8.3±1.2b | 90.2±1.5b | 13.8±0.4ab | 20.3±0.8d | 52.8±0.8c | 48.0±0.6a |
| K16-730-3 | 9.5±1.0a | 94.9±0.3a | 13.0±0.7bc | 22.7±1.6ab | 74.2±1.2b | 47.4±0.9a |
| T4DS·4EL | 10.7±1.2a | 90.6±1.3b | 12.8±0.7c | 20.8±1.0cd | 77.5±1.4a | 48.2±0.5a |
| T5AL·4EL | 4.7±0.5d | 75.0±1.2e | 11.0±0.4de | 21.0±0.9cd | 7.0±0.6g | 34.0±1.5c |
| T3BL·4EL | 9.7±1.0a | 50.4±1.4f | 11.4±0.9d | 22.0±0.6bc | 9.2±0.8f | 26.5±1.6d |
| T5DS·4EL | 7.0±0.6bc | 77.9±1.3d | 10.3±0.5e | 19.3±1.2d | 17.8±1.3e | 35.2±0.8b |

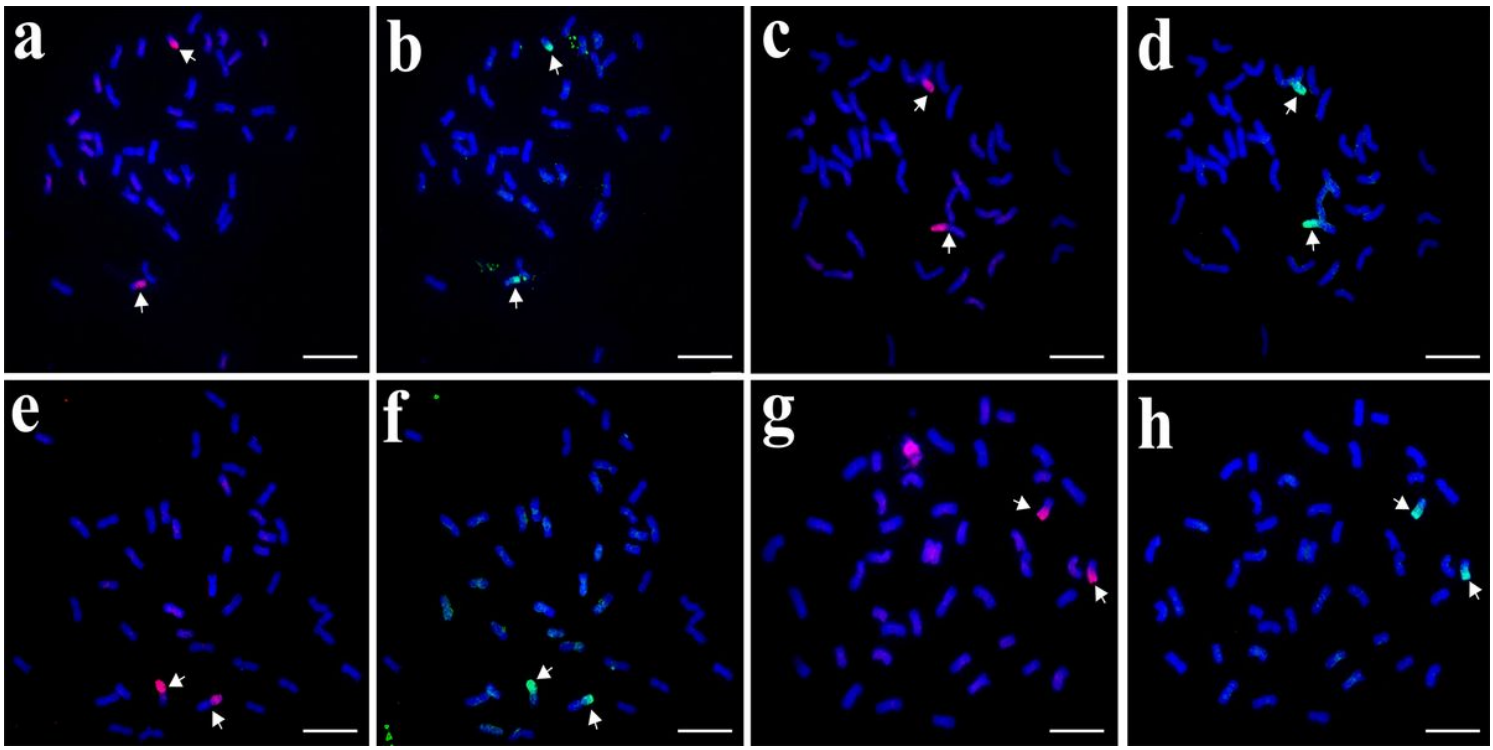
[z] Data in the columns indicate means ± standard errors. Lowercase letters after the means indicate significant differences at $P < 0.05$ as determined by the least significant differences.

# Figures



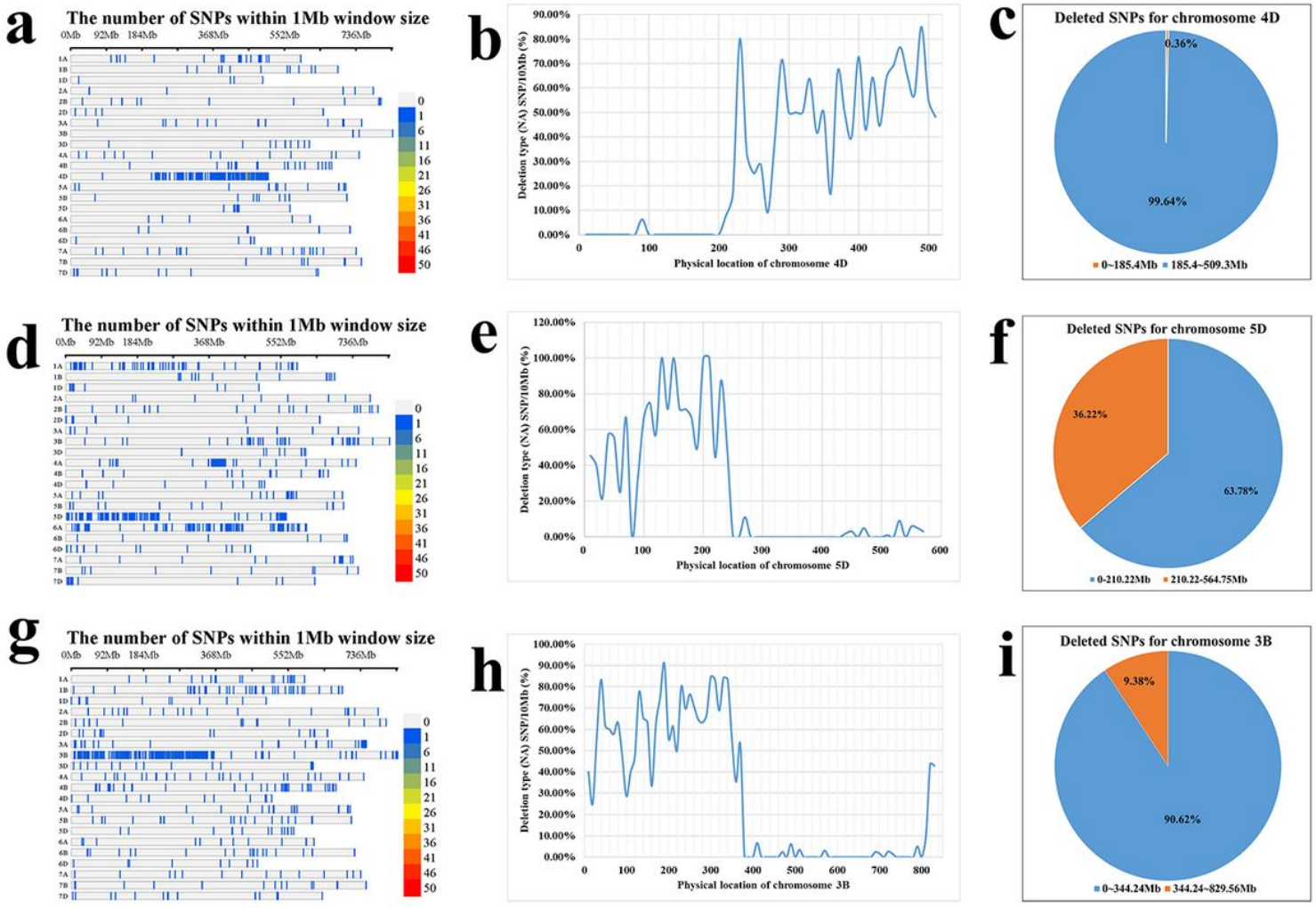

## Figure 1

Identification of wheat-tetraploid *Th. elongatum* 4EL translocation lines by GISH and FISH. Tetraploid *Th. elongatum*genome labeled in red as a GISH probe (a, c, e, g). Oligo-pSc119.2 (green) and Oligo-pTa535 (red) as FISH probes (b, d, f, h). Arrows indicate translocation chromosome of 4E. Scale bar: 10 μm.




## Figure 2

FISH-painting identification of four wheat-tetraploid *Th. elongatum* 4EL translocation lines. Tetraploid *Th. elongatum* genome labeled in red as a GISH probe (a, c, e, g). Chr4 (green) as a FISH-painting probe (b, d, f, h). Arrows indicate translocation chromosome of 4E. Scale bar: 10 μm.




## Figure 3

Deleted SNP analysis of T4DS·4EL, T5AL·4EL, and T3BL·4EL using wheat 55K SNP array. a, d, g, distribution of SNPs on chromosomes. b, e, h, scanning for SNPs detected on translocated chromosomes in 10-Mb sliding windows, with 10-Mb steps. g, h, I, different regions of deleted SNPs counted on translocated chromosomes.

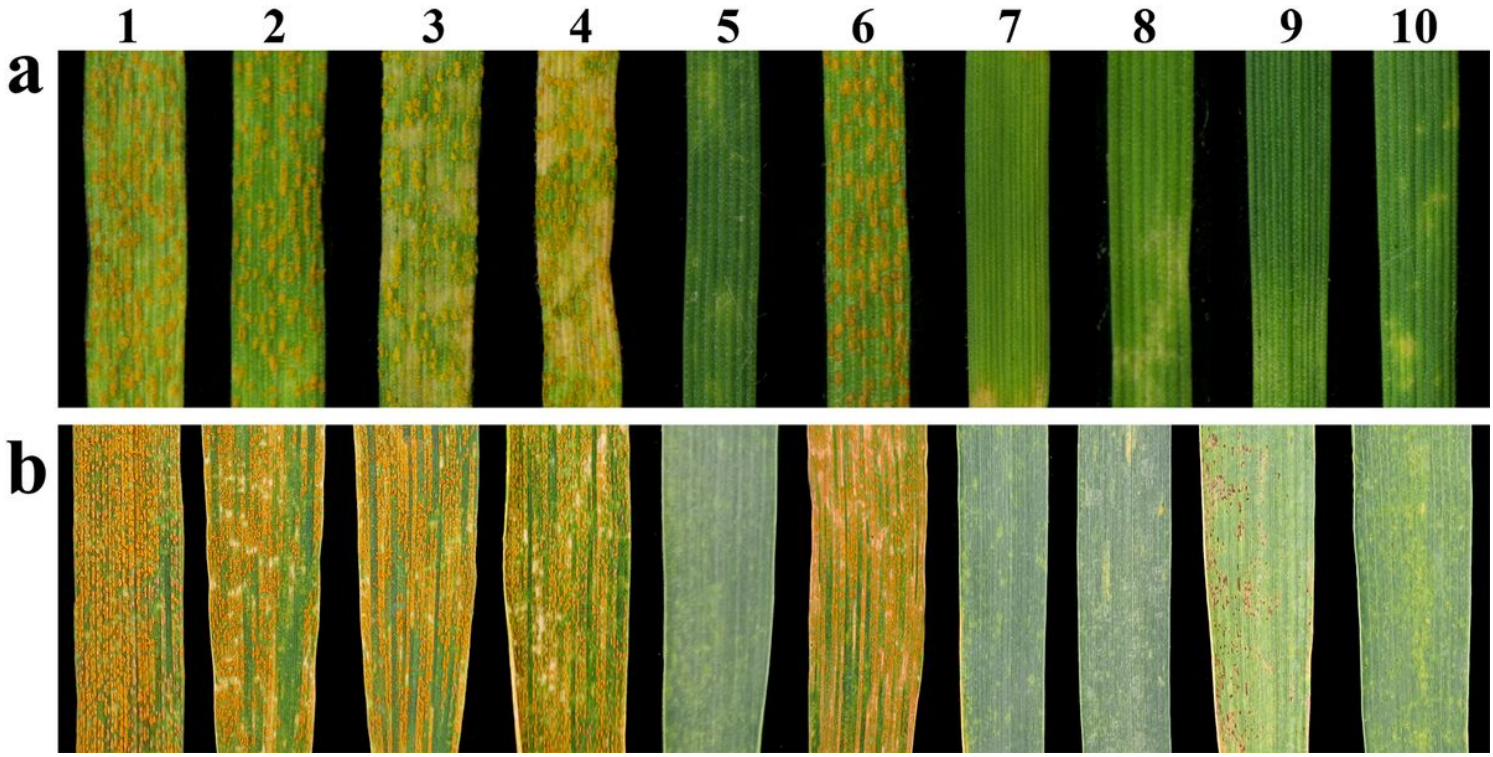
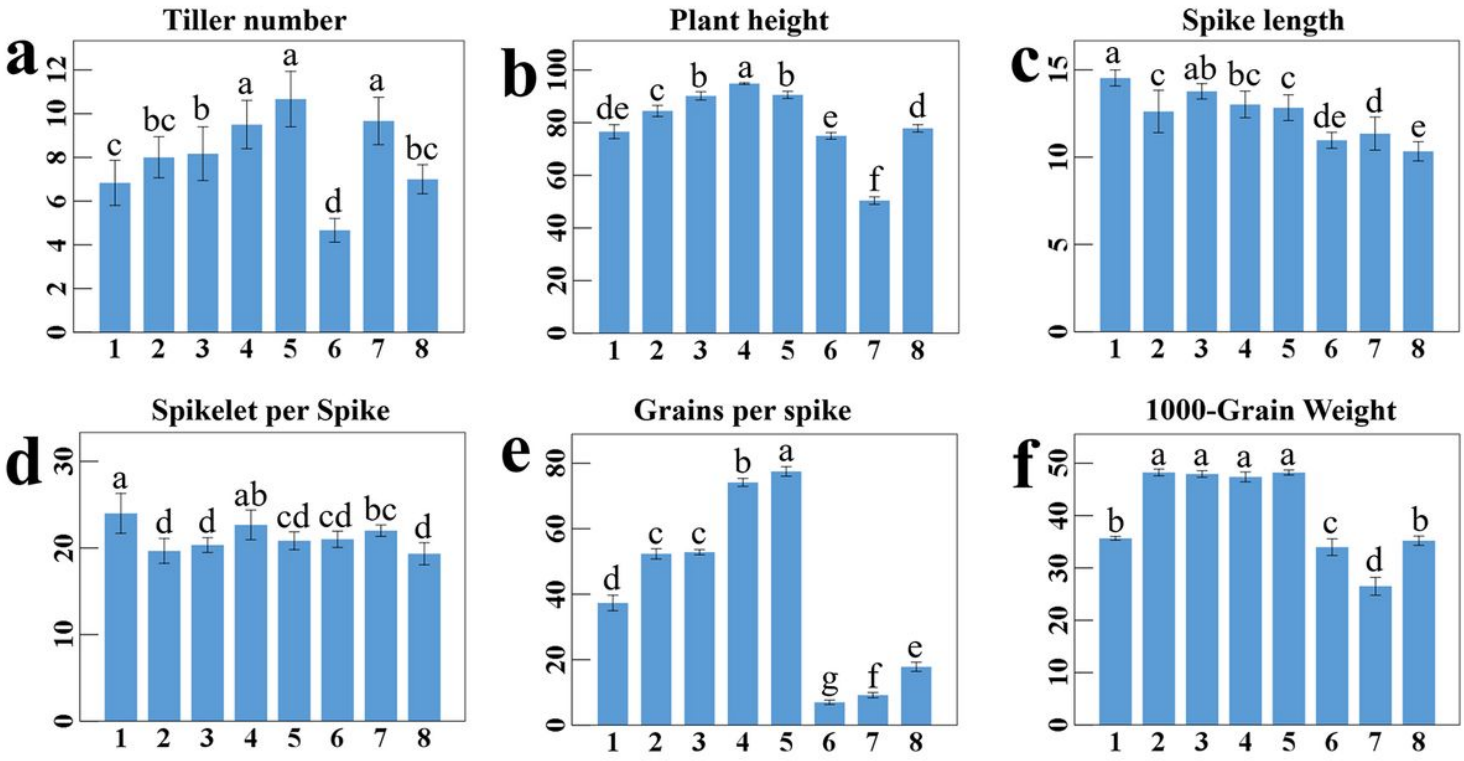



**Figure 4**

Evaluation of four different types of wheat-tetraploid *Th. elongatum* 4EL chromosome translocation lines and their parents for stripe rust resistance. a, seedling stages; b, adult plant. 1, Avocet S; 2, SM51; 3, SM482; 4, CM42; 5, K16-730-3; 6, T4ES·2AL; 7, T4DS·4EL; 8, T5AL·4EL; 9, T3BL·4EL; 10, T5DS·4EL.

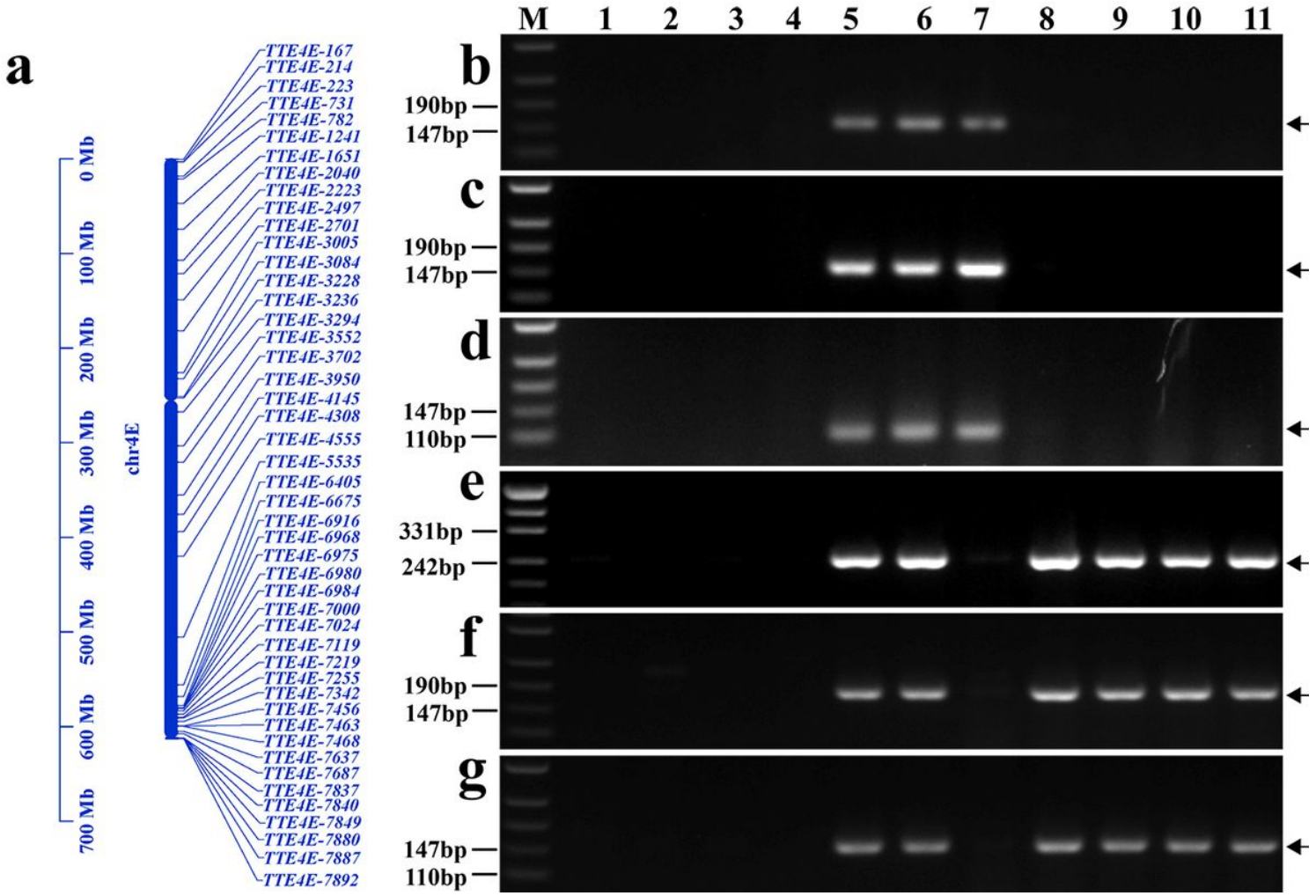

**Figure 5**

Plant morphology of four wheat-tetraploid *Th. elongatum* 4EL chromosome translocation lines and their parents. 1, SM482; 2, SM51; 3, CM42; 4, K16-730-3; 5, T4DS·4EL; 6, T5AL·4EL; 7, T3BL·4EL; 8, T5DS·4EL.



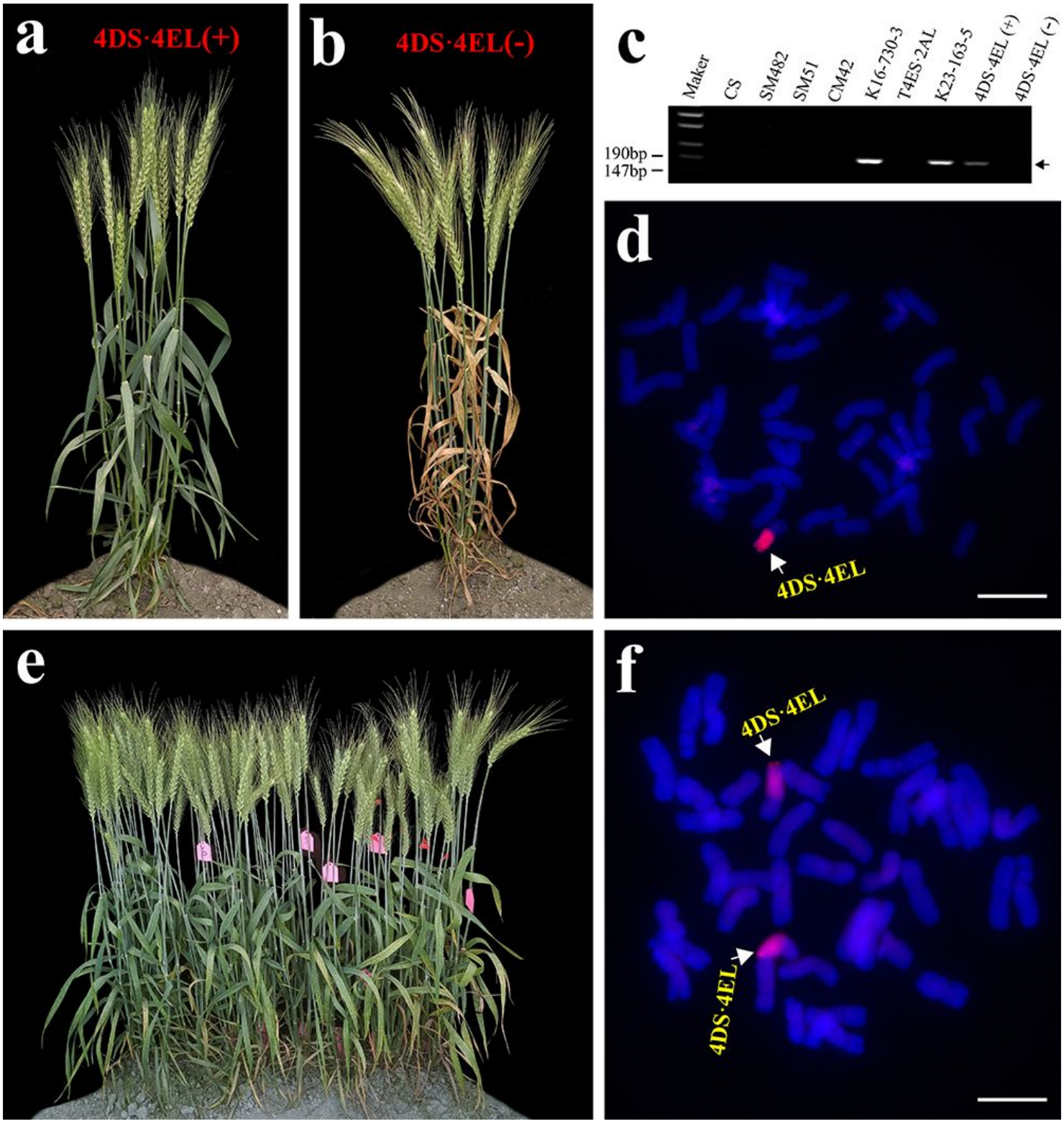

## Figure 6

Statistical analysis of four wheat-tetraploid *Th. elongatum* 4EL chromosome translocation lines for different agronomic traits. 1, SM482; 2, SM51; 3, CM42; 4, K16-730-3; 5, T4DS·4EL; 6, T5AL·4EL; 7, T3BL·4EL; 8, T5DS·4EL. The statistical significance of differences was evaluated by one-way ANOVA (* $p<0.05$).




## Figure 7

*Th. elongatum* chromosome 4E virtual map and amplification patterns of chromosome 4E-specific SSR markers. a, 4E virtual map; b, *TTE4E-731*; c, *TTE4E-2040*; d, *TTE4E-3288*; e, *TTE4E-6405*; f, *TTE4E-7000*; g, *TTE4E-7342*. M, Maker; 1, CS; 2, SM482; 3, SM51; 4, CM42; 5, 8801; 6, K16-730-3; 7, T4ES·2AL; 8, T4DS·4EL; 9, T5AL·4EL; 10, T3BL·4EL; 11, T5DS·4EL.




## Figure 8

Breeding transfer of the gene for stripe rust resistance on chromosome 4E of tetraploid *Th. elongatum*. Plants carrying 4DS·4EL chromosome (resistant to stripe rust) and not carrying 4DS·4EL chromosome (susceptible to stripe rust) (a, b). Molecular marker detection and GISH validation of progeny (c, d, f). K23-181 plant morphology (e).

# Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigure.pdf
- SupplementaryTable.xlsx