Molecular cytogenetic characterization of a novel wheat–Psathyrostachys huashanica Keng T3DS-5NsL•5NsS and T5DL-3DS•3DL dual translocation line with powdery mildew resistance

Jiachuang Li¹, Li Zhao¹, Xueni Cheng², Guihua Bai³, Mao Li¹, Jun Wu¹, Qunhui Yang¹, Xinhong Chen¹, Zujun Yang⁴ and Jixin Zhao*¹

Abstract

Background: Psathyrostachys huashanica Keng (2n = 2x = 14, NsNs) carries many outstanding agronomic traits, therefore is a valuable resource for wheat genetic improvement. Wheat–P. huashanica translocation lines are important intermediate materials for wheat breeding and studying the functions of alien chromosomes. However, powdery mildew resistance in these translocation lines has not been reported previously.

Results: This study developed a novel wheat–P. huashanica translocation line TR77 by selecting a F7 progeny from the cross between heptaploid hybrid H8911 (2n = 7x = 49, AABBDDNs) and durum wheat line Trs-372. Chromosome karyotype of 2n = 42 = 21II was observed in both mitotic and meiotic stages of TR77. Genomic in situ hybridization analysis identified two translocated chromosomes that paired normally at meiosis stage in TR77. Molecular marker analysis showed that part of chromosome 5D was replaced by part of alien chromosome fragment 5Ns. It meant replacement made part 5DL and part 5NsL•5NsS existed in wheat background, and then translocation happened between these chromosomes and wheat 3D chromosome. Fluorescence in situ hybridization demonstrated that TR77 carries dual translocations: T3DS-5NsL•5NsS and T5DL-3DS•3DL. Analysis using a 15 K-wheat-SNP chip confirmed that SNP genotypes on the 5D chromosome of TR77 matched well with these of P. huashanica, but poorly with common wheat line 7182. The translocation was physically located between 202.3 and 213.1 Mb in 5D. TR77 showed longer spikes, more kernels per spike, and much better powdery mildew resistance than its wheat parents: common wheat line 7182 and durum wheat line Trs-372.

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Conclusions: TR77 is a novel stable wheat–P. huashanica T3DS-5NsL5NsS and T5DL-3DS-3DL dual translocation line and showed significant improved spike traits and resistance to powdery mildew compared to its parents, thus, it can be an useful germplasm for breeding disease resistance and studying the genetic mechanism of dual translocations.

Keywords: In situ hybridization, Dual translocation line, Psathyrostachys huashanica, Single nucleotide polymorphism array, Wheat powdery mildew

Background
Wheat (Triticum aestivum L, 2n = 42, AABBDD) is one of the most widely cultivated crops throughout the world. Also, wheat yield can be influenced by heat, drought, insects and diseases, while relatively narrow genetic variation in wheat makes it even more difficulty to identify sources of resistance to combat these biotic and abiotic stresses [1, 2]. Wheat wild relatives, however, carry many beneficial genes that can be used to improve wheat resistance to these stresses as well as grain yield and quality [3]. For example, a wheat–Agropyron 6P chromosome disomic addition line has more kernels per spike than its wheat parent [4]; a wheat–Aegilops substitution line called PRH5 showed high iron and zinc content [5]; a wheat–Haynaldia villosa 4DL–4VS translocation line called NAU413 exhibited high resistance to wheat spindle streak mosaic virus [6]; and wheat–rye 1BL/1RS translocation lines have been widely used in wheat breeding to improve resistance to multiple diseases and abiotic stress [3, 7].

Psathyrostachys huashanica Keng (2n = 2x = 14, NsNs) is an endangered species of Poaceae, Triticeae found only in high mountains of China. As a wild relative of wheat, it owns many outstanding traits, such as high tolerance to abiotic stress, i.e. salinity, alkalinity, cold and drought and high resistance to normal wheat diseases, i.e. rust, take-all and powdery mildew [8–11]. Our laboratory made a wide cross between P. huashanica and common wheat line 7182 in the 1980s and obtained the F1 hybrid, H811 (2n = 28, ABDNs), using in vitro culture of young embryos. The colchicine treated H811 was crossed and backcrossed to 7182 to get heptaploid hybrid H8911 (2n = 49, AABBDDNs). Subsequently, H8911 was then backcrossed to 7182 or crossed to several other wheat cultivars, and generated a series of wheat–P. huashanica derived lines including wheat–P. huashanica 1Ns–7Ns disomic addition lines [12–17], 2Ns(2D) disomic substitution line [18], 5Ns(5D) disomic substitution line [19], and translocation lines [20, 21]. These wheat–P. huashanica derived lines have more desired agronomic traits than their wheat parents, demonstrating that P. huashanica is a useful wild relative for wheat improvement. However, all the known wheat–P. huashanica translocation lines are poorly characterized [20–23].

Although previous studies identified the transposition of wheat and P. huashanica chromosomes in some wheat–P. huashanica derived lines, stability of these alien chromosomes in wheat, their homeologous relationship to wheat chromosomes, and actual translocation sites in wheat chromosomes remain to be investigated. Therefore, determining chromosomal composition of stable wheat–P. huashanica translocation lines may facilitate applications of this type of resources in wheat breeding.

Disease resistance genes from wild relatives of wheat play important roles in control of powdery mildew. Among the 25 wheat closely related genera, 14 demonstrated immunity to powdery mildew, including Secale [24], Aegilops [25], Thinopyrum [26], and Haynaldia villosa [27]. P. huashanica is also immune to powdery mildew but wheat–P. huashanica derived lines with powdery mildew resistance have not been reported to date.

In this study, we developed a novel wheat–P. huashanica translocation line with resistance to powdery mildew from a F7 progeny of wheat–P. huashanica heptaploid line H8911 × durum wheat (Triticum durum L, 2n = 28, AABB) line Trs-372. The objectives of this study were to: (a) determine the pairing and inherent stability of the transposed alien chromosome segment using cytogenetic methods; (b) examine the chromosomal composition of the derived line using molecular markers and fluorescence in situ hybridization (FISH); (c) determine the physical locations of the translocation sites on the chromosomes using a wheat 15K single nucleotide polymorphism (SNP) array; and (d) evaluate the agronomic traits of the new line to predict its potential use in wheat breeding.

Results
Mitotic cells of TR77
About 98% of metaphase mitotic root tip cells (RTCs) of TR77 had a chromosome number of 2n = 42 (Fig. 1a, Table 1) among 136 microscopically screened mitotic RTCs that were randomly selected from different plants. All the chromosomes were clearly separated from each other in metaphase. Genomic in situ hybridization (GISH) analysis using the whole genomic DNA from P. huashanica as the probe and wheat 7182 as the block on the same slide
identified a pair of chromosomal segments with strong yellow-green hybridization signals (Fig. 1b). Each of the signals was emitted from nearly half of the wheat chromosome, and clearly covered the entire short arm and partial long arm of the chromosome that were connected through the centromere (Fig. 1c), indicating the wheat chromosomes were evidently transposed with *P. huashanica* chromosomal segments. Therefore, TR77 was a wheat--*P. huashanica* line with a large segment translocation.

**Chromosomes in meiosis cells of TR77**

Pollen mother cells (PMCs) in metaphase I of TR77 had a chromosome configuration of $2n = 21I$ (Table 1, Fig. 2a).

In meiosis anaphase I, trivalents or quadrivalents and lagging chromosomes were not observed (Fig. 2b). GISH analysis on PMCs in the important fission periods detected one tetrad with a semicircular fluorescent signal in meiosis prophase I (Fig. 2c), and then a rod chromosome with a partial yellow-green signal appeared when the chromosomes were arranged on the equatorial plate (Fig. 2d), separated into two chromosomes, and finally moved to the two poles of the cell in meiotic anaphase I (Fig. 2e). In meiosis telophase II, each of the four sperms carried a fluorescent segment (Fig. 2f). The pair of transposed *P. huashanica* chromosome fragments showed normal synapsis, pairing, segregation, and inheritance in the wheat

**Table 1** Chromosome numbers and pairing status in the meiotic and meiotic phases of TR77

| Material | No. of counted cells | $2n$ | Chromosome configuration | Univalent | Bivalent | Trivalent | Quadrivalent |
|----------|----------------------|------|--------------------------|-----------|----------|-----------|--------------|
|          |                      |      |                          | Rod       | Ring     | Total     |              |
| TR77     | 128                  | 42   |                          | 0.12 (0–2)| 1.31 (1–3)| 19.54 (19–21)| 20.85 (20–21) |

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**Fig. 1** Cytological and genomic in situ hybridization (GISH) analyses of root tip cells from line TR77. a Mitotic metaphase, $2n = 42$. b GISH analysis of TR77. GISH was conducted using the total DNA from *P. huashanica* as the probe and 7182 as the block. Two chromosomal segments with fluorescent hybridization signals (yellow-green) were detected as alien chromosomes in the mitotic metaphase. c Enlarged a pair of the translocated chromosomes. The alien chromosome segment consists of the short arm and a small portion of the long arm attached to the centromere. Chromosomes were counterstained with Propidium Iodide (red).
background, therefore, they came from the same homoeologous groups, which confirmed that TR77 was a cytogenetically stable wheat–*P. huashanica* translocation line.

**Analysis of molecular markers in TR77**

We screened 384 pairs of simple sequence repeat (SSR) primers distributed among the long and short arms of the wheat chromosomes to determine the homoeologous groups for the transposed wheat chromosomes in TR77 (Table S1, Figure S1). Except for 5D that have only a few markers amplified on 5DL of TR77, the markers on other wheat genomes amplified the same wheat chromosome specific bands after the chromosomes arranged on the equatorial plate. Two chromosomes with partial hybridization signals moved to the cell poles during meiotic anaphase I. Each of the four progeny cells had a fluorescent signal in the meiosis telophase II period. *P. huashanica* chromosomes were labeled by the yellow-greenish fluorescent signals.

Among the 78 pairs of expressed sequence tag-sequence tagged site (EST-STS) markers from seven wheat homoeologous groups, three pairs of primers, BF146187, BE444644 and CD452608, from the wheat homoeologous group 5 (5AS, 5BS, and 5DS) (Table 2, Figure S1 and Fig. 4) amplified in *P. huashanica* and TR77, but not in wheat line 7182, suggesting that the three markers are Ns genome-specific and alien chromosome segment in line TR77 was from *P. huashanica* 5Ns chromosome.

**FISH analysis of TR77**

FISH analysis was performed to determine the transposed wheat chromosomes using repetitive oligonucleotide sequences pSc119.2 and pTa535 as probes. FISH results of line TR77, common wheat 7182 and *P. huashanica* could be seen in Fig. 5a, b and c, respectively. All the chromosomes of TR77 showed the same fluorescent karyotype as wheat line
7182 [28, 29], except for the chromosomes 3D and 5D (Fig. 5d) that showed translocations. One breakage site was observed between the long arm of 5D chromosome and short arm of 3D chromosomes of wheat (Fig. 5e), which was caused by loss of most portion wheat 5D chromosome and that remaining 5DL segment was combined with most portion of chromosome 3D (3DS·3DL) to become 5DL-3DS·3DL. The other breakage site was observed between the wheat 3DS chromosome and most portion of newly acquired *P. huashanica* chromosome 5Ns (NsL·NsS) segment (Fig. 5e) due to reciprocal translocations and recombination between remaining 3DS segment and the 5Ns segment (NsL·NsS) of *P. huashanica* to form a new translocation chromosome 3DS-NsL·NsS. Thus, TR77 was a translocation line with dual translocations.

Comparison of fingerprints of TR77 and its parents using a SNP Array
To determine the chromosomal composition of TR77, wheat 15 K SNP arrays were employed to genotype TR77 and its parents (Table S2). In general, significantly higher percentages of common SNP sequences on all 21 chromosomes were observed between TR77 and 7182 than between TR77 and *P. huashanica* except for the 5th group chromosomes that had the lowest percentages of the same SNP loci between TR77 and 7182 (Table 3, Fig. 6a) and the chromosome 5D that had the highest percentage of the same SNP loci between TR77 and the alien parent *P. huashanica*, confirming the translocation occurred between wheat and *P. huashanica* on the chromosome homologous 5. After comparison of the corresponding SNP positions in chromosome 5D between TR77 and its parent 7182 (Fig. 6b), we found an obvious boundary between Affymetrix SNP ID Affx-111,836,242 and Affx-111,527,395 located between 202.3 Mb and 213.1 Mb of 5D chromosome and assumed that translocation occurred in this interval.

Agronomic traits and resistance to powdery mildew in TR77
The agronomic traits were evaluated for TR77 and their parents (common wheat 7182, durum wheat Trs-372, *P. huashanica*).

### Table 2

| markers | locus     | 7182 | Trs-372 | TR77 | Primer (5'→3')                       | Tm (°C) |
|---------|-----------|------|---------|------|--------------------------------------|--------|
| Barc130 | 5DS       | +    | −       | −    | F: CGGCTAGTAGTGGAGTTGTTGG R: ACCGCTCTTAGTTATTGCTCTC | 50     |
| Xcfd189 | 5DS       | +    | −       | −    | F: GCTAAAGCCACATAGGACGG R: GCACAAGTATTGGAAGGCTGCT | 60     |
| Xwmc318 | 5DL       | +    | −       | −    | F: CGTAAGCTATCTGAGTTATGAT R: GTGACGGGTTTGTTGTTTGG | 60     |
| Xgdm153 | 5DL       | +    | −       | +    | F: TAGTTGATGAGCAGCGGAGAGG R: TTGACGAGAGGGGGGGGCA | 60     |
| Xcfd8   | 5DL       | +    | −       | +    | F: ACACCGGATCTGACTTCACGG R: GTGACGAGAGGGGGGGGCA | 60     |
| Xgwm182 | 5DL       | +    | −       | −    | F: TAGTTGATGAGCAGCGGAGAGG R: TTGACGAGAGGGGGGGGCA | 60     |
| Xwmc357 | 5DL       | −    | −       | +    | F: TAGTTGATGAGCAGCGGAGAGG R: TTGACGAGAGGGGGGGGCA | 60     |
| BF146187| 5AS 5BS 5DS | −    | −       | +    | F: CAGGTTGCAACAGTCTGATG R: GTGACGAGAGGGGGGGGCA | 60     |
| BE444644| 5AS 5BS 5DS | −    | −       | +    | F: AAGCTTGCTGACCTTCTTCTG R: TTGACGAGAGGGGGGGGCA | 60     |
| CD452608| 5AS 5BS 5DS | −    | −       | +    | F: TGTGATGAGCAGCGGAGAGG R: TTGACGAGAGGGGGGGGCA | 60     |

*P* indicates *P. huashanica*; + means marker is present; − means marker is absent.
huashanica) (Table 4 and Fig. 7). TR77 exhibited completely different spike traits from its wheat parent. TR77 had conical and awnless spikes, taller plant, longer spikes, and more kernels per spike than its parents ($P < 0.01$) between TR77 and its common wheat parent 7182 and Trs-372, indicating that addition of 5Ns chromosome of *P. huashanica* in TR77 increased spike length and spikelets number per spike, but also plant height.

The response of TR77 to powdery mildew infection was evaluated in both a growth chamber and a field. Control varieties, TR77, and its parents were grown under the same conditions to ensure accurate results. In the seedling age, susceptible mildew infection types (IT) were observed in Huixianhong (IT = 4), Mianyang 11 (IT = 4), line 7182 (IT = 3) and Trs-372 (IT = 3), whereas *P. huashanica* (IT = 0) and TR77 (IT = 0) were almost immune to powdery mildew (Fig. 7d). In the adult stage, the leaves of TR77 clearly had less spores than those of the control materials Mianyang 11 and Chinese Spring at the same positions on each plant (Fig. 7e).

**Discussion**

Wheat is a self-pollinating plant, and thus it may not evolve rapidly enough to meet the needs of modern humans. Modern wheat breeders usually create desired genetic variations by making crosses between breeding lines within bread wheat or using mutagenesis, transgenic approach, etc. [30]. However, for mutagenesis, controlling the direction of mutations is very difficult due to the random nature of this process, the large and complex genome of allopolyploid wheat and quantitative nature of the most target traits, and transformation genotype specificity limit transgenic wheat breeding to a few traits in a few genotypes [31], making crossing breeding the most widely used strategy for creation of new variations in wheat breeding. However, long time interbreeding has narrowed the wheat genetic diversity, and caused loss of many useful genes for disease resistance, and plant adaptation and productivity. Wide crosses between wheat and its relatives can introduce alien genetic materials into common wheat to generate desired genetic variations and create new germplasm, which is important for the sustainable development of new wheat cultivars [32]. *P. huashanica* is a wheat relative species with many desirable traits that are valuable for wheat improvement. Many wheat lines carrying genetic materials from *P. huashanica* show outstanding agronomic traits. For example, a wheat–*P. huashanica* 1Ns disomic addition line exhibits increased storage of microelements in the seeds [10]. In addition, 2Ns, 3Ns, 4Ns, and 5Ns disomic addition lines and a 2Ns(2D) substitution line are resistant to stripe rust [13–16, 18]. A 6Ns disomic addition line and a small segment translocation line possess twin spikelets and more kernels per spike than its wheat parent [13, 21], and a 7Ns disomic addition line showed high resistance to leaf rust [12]. However, only a few wheat–*P. huashanica* translocation lines are available for breeding despite its importance. In the current study, we identified and characterized a novel wheat–*P. huashanica* translocation line that carries part of *P. huashanica* 5Ns chromosome and underwent dual reciprocal translocations in the
wheat 3D and 5D chromosomes. This translocation line exhibited resistance to powdery mildew, an important wheat leave disease.

Whether alien chromosome segments can stably transmitted to their wheat offspring in a wheat background determines usefulness of the derived line in breeding [33]. In this study, the chromosomal compositions and behavior in the RTCs and PMCs of TR77 indicated 42 chromosomes with 21 bivalents in meiosis prophase I of TR77, with no lagging chromosomes during anaphase I. GISH analysis suggested that TR77 carries a pair of unique chromosomes that consist of chromosomal segments from both P. huashanica and wheat with the segment of P. huashanica from fifth homoeologous group (5Ns). The translocated 5Ns chromosome segment in TR77 paired normally in metaphase I and synchronized with the spliced wheat chromosome during the duplication and division phases, therefore, is stably inherited.

Genome specific DNA markers can be used to identify specific wheat chromosomes and determine homoeology of alien chromosome(s) with wheat. Du et al. [13] identified a wheat–P. huashanica 5Ns disomic addition line using EST-STS markers; Li et al. [19] identified a wheat–P. huashanica 5Ns(5D) disomic substitution line using SSR, EST-STS, sequence characterized amplified region (SCAR) markers. In this study, we used wheat genome-specific SSRs and P. huashanica genome specific EST-STSs to determine the chromosomal composition of TR77 and found that SSRs on 5DS and 5DL near the centromere amplified wheat genome-specific bands in parent 7182, but not in the translocation line TR77, whereas three EST-STSs on the fifth homoeologous group [13] amplified Ns genome-specific bands in both TR77 and P. huashanica. These results indicate that the short arm and partial long arm of wheat chromosome 5D close to the centromere were replaced by P. huashanica.

Fig. 5 Fluorescence in situ hybridization (FISH) analysis of TR77. Oligo-primers pSc119.2 (green) and pTa535 (red) were used as probes for wheat chromosomes during the mitosis metaphase in the root tip cells. a line TR77, b common wheat 7182, c P. huashanica. Wheat 3D and 5D chromosomes and chromosomes from two groups underwent double translocations to yield the 3DS-NsL.NsS and 5DL-3DS.3DL chromosomes as pointed by arrows. d Comparison of special chromosomes in common wheat 7182 and TR77. e Diagrammatic sketch of the two translocations, 3DS-NsL.NsS and 5DL-3DS.3DL, in TR77 to show breakage sites in each chromosome as pointed by arrows and chromosome rearrangement. Chromosomes were stained with 4′,6-diamidino-2-phenylindole (blue)
chromosome 5Ns. Many studies found that homoeologous chromosomes are remarkably conserved and any missing genetic content is usually complemented by corresponding homoeologous chromosomes [34–37]. In the H8911 × Trs-372 cross, because Trs-372 only carries A and B genomes, chromosome recombination mainly occurred between the D and Ns genomes, in particular, the reciprocal translocation between wheat 5D and P. huashanica 5Ns chromosomes that generated a new 5DL-5NsS chromosome.

High-density SNP arrays can track sources of chromosomes from different species by comparing marker sequence identity between progeny and their donor parents. In the present study, comparison of a wheat 15 K SNP array data between TR77 and its parent 7182 confirmed that the D genome of TR77 was from 7182, whereas the A and B genomes were from chromosome recombination between wheat line 7182 and durum Trs-372. TR77 genome had extremely low similarity with the genome of P. huashanica except for the chromosome 5D of TR77 that had high percentage of SNP loci in common with P. huashanica. Also, 5D of TR77 had lower percentage of SNP loci in common with its wheat parent 7182 compared with the other chromosomes. These results indicated the translocation most likely occurred in wheat chromosome 5D, which was supported by the results from previous studies of DNA markers and GISH. To determine the translocation site, we arranged all SNP markers on the chromosome 5D based on their physical locations in Chinese Spring reference genome and identified an obvious physical boundary between 202.3 Mb and 213.1 Mb. The P. huashanica chromosomal segment was located before 202.3 Mb including the centromere region from 185.6 Mb to 188.7 Mb [38] and the common wheat (5DL) segment was located after 213.1 Mb of the chromosome. Therefore, translocation break position in TR77 is near the centromere of wheat 5D chromosome.

### Table 3 Comparison of wheat 15 K SNP array data between TR77 and its parents

| Chromosome | No. of markers | No. of valid markers in materials | No. of same markers (TR77 vs 7182) | Percentage of same markers (TR77 vs 7182) | No. of same markers (TR77 vs Trs-372) | Percentage of same markers (TR77 vs Trs-372) | No. of same markers (TR77 vs P. huashanica) | Percentage of same markers (TR77 vs P. huashanica) |
|------------|----------------|-------------------------------|-----------------------------------|------------------------------------------|--------------------------------------|-------------------------------------------|---------------------------------------------|---------------------------------------------|
| 1A         | 607            | 408                           | 211                               | 52%                                      | 304                                  | 75%                                       | 23                                          | 6%                                          |
| 1B         | 670            | 438                           | 228                               | 52%                                      | 229                                  | 52%                                       | 32                                          | 7%                                          |
| 1D         | 337            | 234                           | 171                               | 73%                                      | –                                    | –                                         | 5                                           | 2%                                          |
| 2A         | 907            | 607                           | 389                               | 64%                                      | 166                                  | 27%                                       | 21                                          | 3%                                          |
| 2B         | 736            | 431                           | 289                               | 67%                                      | 275                                  | 64%                                       | 17                                          | 4%                                          |
| 2D         | 597            | 404                           | 353                               | 87%                                      | –                                    | –                                         | 8                                           | 2%                                          |
| 3A         | 594            | 391                           | 294                               | 75%                                      | 149                                  | 38%                                       | 27                                          | 7%                                          |
| 3B         | 997            | 616                           | 398                               | 65%                                      | 432                                  | 70%                                       | 20                                          | 3%                                          |
| 3D         | 505            | 357                           | 319                               | 89%                                      | –                                    | –                                         | 11                                          | 3%                                          |
| 4A         | 766            | 545                           | 406                               | 74%                                      | 395                                  | 72%                                       | 11                                          | 2%                                          |
| 4B         | 589            | 348                           | 201                               | 58%                                      | 196                                  | 56%                                       | 15                                          | 4%                                          |
| 4D         | 248            | 165                           | 148                               | 90%                                      | –                                    | –                                         | 12                                          | 7%                                          |
| 5A         | 690            | 414                           | 166                               | 40%                                      | 203                                  | 49%                                       | 32                                          | 8%                                          |
| 5B         | 763            | 462                           | 222                               | 48%                                      | 286                                  | 62%                                       | 22                                          | 5%                                          |
| 5D         | 518            | 360                           | 180                               | 50%                                      | –                                    | –                                         | 104                                         | 29%                                         |
| 6A         | 463            | 265                           | 209                               | 79%                                      | 80                                   | 30%                                       | 11                                          | 4%                                          |
| 6B         | 768            | 446                           | 306                               | 69%                                      | 226                                  | 51%                                       | 67                                          | 15%                                         |
| 6D         | 404            | 279                           | 260                               | 93%                                      | –                                    | –                                         | 11                                          | 4%                                          |
| 7A         | 735            | 490                           | 326                               | 67%                                      | 320                                  | 65%                                       | 26                                          | 5%                                          |
| 7B         | 640            | 404                           | 354                               | 88%                                      | 228                                  | 56%                                       | 12                                          | 3%                                          |
| 7D         | 665            | 462                           | 412                               | 89%                                      | –                                    | –                                         | 12                                          | 3%                                          |
| A genome   | 4762           | 3120                          | 2001                              | 64%                                      | 1617                                 | 52%                                       | 151                                         | 5%                                          |
| B genome   | 5163           | 3145                          | 1998                              | 64%                                      | 1872                                 | 60%                                       | 185                                         | 6%                                          |
| D genome   | 3274           | 2261                          | 1843                              | 82%                                      | –                                    | –                                         | 163                                         | 7%                                          |
| Total      | 13,199         | 8526                          | 5842                              | 69%                                      | 3489                                 | 41%                                       | 499                                         | 6%                                          |
The FISH probes, Oligo-pSc119.2 and Oligo-pTa535, can accurately distinguish all 42 common wheat chromosomes based on the standard FISH karyotypes in mitotic cells [39, 40]. Many wheat-derived lines including substitution and translocation lines have been identified using this method [41–43]. In addition, structural changes in wheat chromosomes can be detected by FISH. For example, FISH was used to confirm a reciprocal translocation of the 5BL-7BS chromosome in a wheat variety Humai15 and a reciprocal translocation of the 4BL-6AL chromosome in a wheat variety Mingxian 169 [44]. In this study, however, FISH did not detect 5DL-5NsL.5NsS chromosome, therefore, 5NsL·5NsS might undergo another translocation within wheat chromosome, which is confirmed by FISH identification of two translocations, 3DS-5NsL·5NsS and 5DL-3DS·3DL in TR77. A previous FISH karyotype study on line 7182 did not find translocation between wheat chromosomes 3D and 5D [29]. Thus, the first translocation might have occurred between the wheat 5D and P. huashanica 5Ns chromosomes to yield chromosome 5DL-5NsL·5NsS as transition, and then a reciprocal translocation occurred between the wheat 3D and 5DL-5NsL·5NsS chromosomes that produced 3DS·5NsL·5NsS and 5DL-3DS·3DL in TR77. During the two translocation events, TR77 lost its short arm and partial long arm of wheat 5D chromosome and acquired short arm (5NsS) and partial long arm (5NsL) of P. huashanica chromosome 5. Therefore, TR77 is a wheat–P. huashanica translocation line with dual translocations of 3DS·5NsL·5NsS and 5DL-3DS·3DL. The 5NsL·5NsS segment was combined with the wheat 3D chromosome via inter-chromosomal recombination. The fifth group of chromosomes in wheat carries the Ph1 gene that controls the cross ability among species [45]. A previous study found that the wheat 5BL chromosome contained a subtelomeric insertion from wheat chromosome 3A [46]. Also a wheat line carrying T5BS.7BS, T5BL.7BL and T5AS.3BS chromosomes was identified using FISH technology [47]. Those studies support the possible reciprocal translocations between wheat chromosomes 5D and 3D occurred in this study.

![Fig. 6 Chromosomal composition of TR77 analyzed using a wheat 15K SNP array.](image)

**Table 4** Agronomic traits of TR77, its parents, P. huashanica, common wheat 7182, and durum wheat Trs-372

| Material          | Plant height (cm) | Tiller number | Spike length (cm) | Spikelets per spike | Kernels per spike | Thousand kernel weight (g) | Awn type   |
|-------------------|------------------|---------------|-------------------|--------------------|-------------------|----------------------------|------------|
| P. huashanica     | 63.5 ± 2.2Dd     | clump         | 8.26 ± 0.96Bbc    | 16 ± 2Bcb          | 42 ± 6Cc          | 3.44 ± 0.11Cc              | Short awn  |
| 7182              | 83.8 ± 1.4Bb     | 9 ± 2Ab       | 9.05 ± 0.57Bb     | 18 ± 3Aba          | 54 ± 4Bb          | 37.69 ± 0.74Aa             | Long awn   |
| Trs-372           | 76.3 ± 2.9Cc     | 10 ± 2Aab     | 7.91 ± 0.33Cc     | 14 ± 2Cc           | 43 ± 2Cc          | 35.52 ± 0.68Bb             | Long awn   |
| TR77              | 92.6 ± 2.3Aa     | 12 ± 3Aa      | 13.05 ± 0.77Aa    | 19 ± 3Aa           | 67 ± 5Aa          | 37.56 ± 0.69Aa             | Awnless    |

Different uppercase and lowercase letters indicate significant differences at $p < 0.01$ and $p < 0.05$, respectively, between the translocation line TR77 and its parents.
Powdery mildew resistance genes from wild relatives of wheat are usually durable with broad-spectrum of resistance \([48]\). Pm21 was transferred from *Haynaldia villosa* 6VS to wheat and a highly powdery mildew resistant 6VS/6AL translocation line is still effective and widely used in production \([27]\). The current study showed that TR77 was near immune to powdery mildew in both seedling and adult ages, although its two wheat parents exhibited susceptible symptoms, indicating that powdery mildew resistance trait in TR77 was from *P. huashanica*. So, the identification of TR77 filled a gap where no wheat–*P. huashanica* derived lines had excellent powdery mildew resistance before. Agronomic traits are important criteria for assessing the value of a line carrying alien chromosome segments. TR77 had larger spikes and more kernels per spike than its wheat parents, thereby has potential to increase yield. Crossing TR77 with locally adapted varieties can transfer these desired agronomic traits to new wheat cultivars. Line TR77 exhibited many excellent characteristics, which meant these excellent genes most possible introduced from *P. huashanica* because of chromosomal exchange and recombination between wheat and *P. huashanica*. The replaced segment were entire short arm and partial long arm of the chromosome close to centromere which was generally considered to contain few expressible genes. Therefore, the gene(s) that control awn length may be located in chromosome 5DS and raise powdery mildew resistance and spikes development may be located in chromosome 5NsS.

**Conclusion**

In this study, TR77 was identified as a new wheat–*P. huashanica* T3DS-5NsL-5NsS and T5DL-3DS-3DL dual translocation line with larger spikes, more kernels per spike, and better powdery mildew resistance than their wheat parents. Molecular, cytogenetic and phenotypic analyses on TR77 determined its chromosomal composition, translocation position and inheritance stability as well as its superior agronomic performance. TR77 can be an important germplasm for improving powdery mildew resistance and yields in wheat breeding, and for dual translocation research.

**Methods**

**Plant materials**

The plant materials include one line of *Psathyrostachys huashanica* Keng \((2n = 14, \text{NsNs})\) from Huashan Mountains, Shaanxi province, China, one common wheat (*Triticum aestivum* L.) line 7182 \((2n = 42, \text{AABBDD})\), one durum (*Triticum durum*) line Trs-372 \((2n = 28, \text{AABB})\), and the wheat–*P. huashanica* translocation line TR77 obtained from the F\(_7\) progeny of the cross between line Trs-372 and a wheat–*P. huashanica* derived line H8911\((2n = 49, \text{AABBDNNs})\). The heptaploid line H8911 was created.
by the wide cross between *P. huashanica* and line 7182 via artificial embryo rescue and backcross [8]. Common wheat varieties Huixianhong, Mianyang 11, and Chinese Spring were used as controls for assessing disease resistance.

The wheat wild related material *P. huashanica* was identified and collected by Shuyang Chen and Langran Xu who were the first researchers to do hybridization between common wheat and *P. huashanica* in China in 1980s [8]. The wheat materials were collected from The National Wheat Improvement Center of China and the wheat–*P. huashanica* derived lines were developed by our lab. All materials were deposited and planted in College of Agronomy, Northwest A&F University, China. The collection and treatment of materials for this experiment complied Wild Plants Protection Regulation of China. Extraction and purification of genomic DNA from leaf tissues used the standard Cetyltrimethylammonium Ammonium Bromide method [49].

### Cytological analysis

The appropriate stages for sampling were length of roots to 1.5 cm and young panicles to 5 cm [50]. The cut roots were immediately soaked in ice-water mixture for 12–20 h before transferring to Carnoy’s fixative fluid I for 24 h, and finally stored at −20 °C. Young panicles at appropriate stage were treated with Carnoy’s fixative fluid II for 24 h and then stored under refrigeration. The root apical meristem was digested in 2% cellulase plus 1% pectinase at 37 °C for more than 50 min before scattering in acetic acid. The chromosomes in root tip cell were observed under a microscope at 400 times magnification (OLYMPUS BH2, Japan). Anthers were stained with 1% acetocarmine and gently broke for cytological observations. The slides with good split phases were dried and marked for the following experiments. Microscopic observations were the first step in order to identify the chromosomal number and behavior in TR77. In this process, each plant was numbered to ensure root and spike from the same plants.

### DNA marker analysis

A total of 384 pairs of SSR primers [51, 52] were selected from 21 wheat chromosomes to determine the translocated wheat chromosomes in TR77. In addition, to identify the homoeologous group of the introduced alien chromosomes in TR77, 87 pairs of EST-STS markers (https://wheat.pw.usda.gov/SNP/new/pcr_primers.shtml) were selected from seven wheat homoeologous groups that had homoeology with corresponding chromosomes of *P. huashanica*. PCR assays were conducted following Doğrur et al. [53] and an ABI PRISM 3730 DNA Analyzer (Applied Biosystems, USA) was adopted for separation of the PCR products. The data of SSR markers were scored by GeneMarker V1.97 (Soft Genetics LLC, USA).

### In-situ hybridization

For the GISH analysis, Mixed the *P. huashanica* genomic DNA and DiG-Nick Translation Mix (Roche, Germany) in a ratio of two to one at 15 °C for 1.5 h as labeling probes following Wetzel et al. [54] and Zhao et al. [55]. The addition of Anti-Digoxigenin–Fluorescein mix (Roche, Germany) made the probes visualize after hybridization. The wheat chromosomes were dyed using Vectashield H1300 (Vector Laboratories, USA). In the FISH ANALYSIS, the match of Oligo-pSc119.2 (6-FAM-5′CCGTTTTGTTG GACTATTACT CACCGC TTG GGTTCCCAATA GCTAT) and Oligo-pTa535–1 (Tamra-5′AAAACCTTGA CGCACGTAC GTACA ATTG GACAAACTCT TTCCGAGTTAT CAGGGTTC) were used to give insight into the chromosomal composition of TR77. After hybridizing with wheat chromosomes, the standard karyotype of the two Oligo-probes could be seen [28, 39]. FISH experiment was conducted as described by Patokar et al. [56] and Lang et al. [57]. The signals were observed and captured using OLYMPUS BX60 fluorescence microscope with a color camera (Penguin, Japan) at 400 times magnification.

### Characterization of wheat-*P. huashanica* translocation using wheat 15 K SNP arrays

High-quality genomic DNA of TR77 and its parents was used to hybrid with Illumina wheat 15 K SNP arrays for loci difference scanning in China Golden Marker Biotechnology Company (Beijing, China). The array contained 13,199 SNP loci, which were dispersed in all 21 wheat chromosomes. The total valid number of SNP loci divided by the loci number that had the same genotype in a chromosome between materials was calculated as the percentage of the same markers in each chromosome. Data analysis used SigmaPlot V12.5 (SYSTAT software, USA) and chromosomal map was drew using MapChart V2.32 (Wageningen University & Research, The Netherlands).

### Characterization of agronomic traits and powdery mildew resistance

*P. huashanica*, TR77 and its wheat parents were evaluated in the field for seven agronomic traits, i.e. spike length, plant height, spikelet number, tiller number, kernel number, thousand-kernel weight and self-fertility. The mean values over five plants were collected in two successive years as repeats to ensure data accuracy.

The powdery mildew resistance was evaluated at the seedling stage in a growth chamber. The powdery mildew Bgt race E09 was used for inoculation with wheat variety Huixianhong as a susceptible control. The pathogen spores were dusted onto wheat leaves at two-leaf stage and incubated at 22 °C and 70% humidity for 15 d. the adult plant powdery mildew resistance was
evaluated in a field in Sichuan province, China using Mianyang 11 as the susceptible control. The field usually had a high incidence of powdery mildew and thus tested plants were infected naturally. The reactions to powdery mildew were assessed following Sheng [58] and An [59]. In brief, wheat responses to infection were scored using infection type (IT) in a 0–4 IT scale at the seedling stage, in which IT = 0, 0.5, 1, 2, 3 and 4 denoting immune, nearly immune, highly resistant, moderately resistant, moderately susceptible and susceptible respectively. In the adult stage, the plant responses to infection were recorded as the percentage of the powdery mildew spores covered the total area of the leaves at the same position on each plant [60].

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12870-020-02366-8.

Additional file 1. Figure S1 TR77-Molecular markers uncropped images.
Additional file 2. Table S1 TR77-SSR Analysis.
Additional file 3. Table S2 TR77-15K SNP array.

Abbreviations

EST-STS: Expressed sequence tag-sequence tagged site; FISH: Fluorescence in situ hybridization; GISH: Genomic in situ hybridization; IT: Immunity type; P. huashanica: Psathyrostachys huashanica Keng; PMC: Pollen mother cell; SCAR: Sequence characterized amplified region; SNP: Single nucleotide polymorphism; SSR: Simple sequence repeat; RTC: Root tip cell

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None.

Authors’ contributions

JL, JZ and ZY conducted experiments. LZ and ML analyzed data. XNC and GB contributed new reagents and analytical tools. JW and QY contributed new methods or models. JL and JZ wrote and GB edited the paper. All authors read and approved the final version of the manuscript.

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Availability of data and materials

All related plant materials are available and comply Wild Plants Protection Regulation of China. The datasets supporting the conclusions of this article are included within the article and its supplementary files.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

1. Shaanxi Key Laboratory of Plant Genetic Engineering Breeding, College of Agronomy, Northwest A&F University, Yangling 712100, Shaanxi, China.
2. College of Life Science, Northwest A&F University, Yangling 712100, Shaanxi, China.
3. USDA, Hard Winter Wheat Genetics Research Unit, 4008 Throckmorton Hall, Manhattan, KS 66506, USA.
4. School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu 610054, Sichuan, China.

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References

1. Fischer RA, Edmeades G. Breeding and cereal yield Progress. Crop Sci. 2010; 50(1):585–98.
2. Mujeeb-Kazi A, Kazi AG, Dundas I, Rasheed A, Ogbonnaya F, Kishii M, Bonnett D, Wang RRC, Xu S, Chen P, et al. Chapter four–genetic diversity for wheat improvement as a conduit to food security. In: Sparks DL, editor. Advances in Agronomy, vol. 122: Academic Press; 2013. p. 179–257.
3. Tester M, Langridge P. Breeding technologies to increase crop production in a changing world. Science. 2010;327(5967):818.
4. Han HM, Li J, Liang J, Liu Y, Song LQ, Gao AN, Yang XM, Li QX, Liu WH, Li LH. Genetic rearrangements of six wheat–Agropyron cristatum 6P addition lines revealed by molecular markers. PLoS One. 2014;9(3):e91066.
5. Sharma P, Sheik K, Kumar S, Verma SK, Kumar R, Vyás P, Dhivalal HS. Precise transfers of genes for high grain iron and zinc from wheat–Aegilops substitution lines into wheat through pollen irradiation. Mol Biol. 2018; 38(6):81.
6. Zhang QP, Li Q, Wang X, Wang HY, Lang SP, Wang SY, Yang Q, Chen XH, Liu DJ. Development and characterization of a Triticum aestivum–Haynaldia villosa translocation line T4VS4DL conferring resistance to wheat spindle streak mosaic virus. Euphytica. 2005;145(3):317–20.
7. Rabinovich SV. Importance of wheat–rye translocations for breeding modern cultivar of Triticum aestivum L. Euphytica. 1998;100(1):323–40.
8. Chen SY. The hybridization between Triticum aestivum and Psathyrostachys huashanica. Acta Genet Sin. 1991;18(6):588–12.
9. Fu J, Wang MN, Zhao JX, Chen SY, Hou WS, Yang QH. Studies on cytogenetics and utilization of resistance-Psathyrostachys huashanica medium material H8911 with resistance to wheat take-all fungus. Acta Botan Boreali-Occiden Sin. 2003;23(12):2157–62.
10. Zhao JK, Ji WQ, Wu J, Cheng XH, Wang X, Peng YH, Liu SH, Yang QH. Development and identification of a wheat–Psathyrostachys huashanica addition line carrying HMW-GS, LMW-GS and gliadin genes. Genet Resour Crop Evol. 2010;57(3):387–94.
11. Li Q, Huang J, Hou L, Liu P, Jing J, Wang B, Kang Z, Genetic and molecular mapping of stripe rust resistance gene in wheat–Psathyrostachys huashanica medium material H9020 with resistance to wheat take-all fungus. Acta Botan Boreali-Occiden Sin. 2003;23(12):2157–62.
12. Zhao JK, Ji WQ, Wu J, Cheng XH, Wang X, Peng YH, Liu SH, Yang QH. Development and characterization of a Psathyrostachys huashanica Keng 7Ns chromosome addition line with leaf rust resistance. PLoS One. 2012; 8(8):70879.
13. Du WL, Wang J, Lu M, Sun SG, Chen XH, Zhao JX, Yang QH, Wu J. Molecular cytogenetic identification of a wheat–Psathyrostachys huashanica Keng 5Ns dicotic addition line with stripe rust resistance. Mol Biol. 2013;31(4):879–88.
14. Du WL, Wang J, Lu M, Sun SG, Chen XH, Zhao JX, Yang QH, Wu J. Molecular characterization of a wheat–Psathyrostachys huashanica Keng 2Ns dicotic addition line with resistance to stripe rust. Mol Gen Genomics. 2014;289(5):735–43.
15. Du WL, Wang J, Lu M, Sun SG, Chen XH, Zhao JX, Yang QH, Wu J. Characterization of a wheat–Psathyrostachys huashanica Keng 4Ns dicotic addition line for enhanced tiller numbers and stripe rust resistance. Planta. 2014;239(1):97–105.
16. Du WL, Wang J, Peng YH, Wang LM, Wu J, Zhao JX, Yang QH, Chen XH. Isolation and characterization of a wheat–Psathyrostachys huashanica Keng 5Ns dicotic addition line with resistance to stripe rust. Genome. 2015;57(1):37–44.
17. Du WL, Wang J, Peng YH, Wu J, Zhao JX, Liu SH, Yang QH, Chen XH. Development and application of PCR markers specific to the 1Ns chromosome of Psathyrostachys huashanica Keng with leaf rust resistance. Euphytica. 2014;200(2):207–20.
18. Du WL, Zhao JX, Wang J, Wang LM, Wu J, Yang QH, Liu SH, Chen XH. Cyto genetic and molecular-based characterization of a wheat- 
Aegilops
huashanica Keng 2N(2D) substitution line. Plant Mol Biol Report. 2015;33(3):414–23.
19. Li JC, Yao XN, Yang ZJ, Cheng XN, Yuan FP, Liu Y, Wu J, Yang QH, Zhao JX, Chen XH. Molecular cytogenetic characterization of a novel wheat-Aegilops
huashanica Keng 5N(5D) disomic substitution line with stripe rust resistance. Mol Breed. 2019;39(7):119.
20. Gao Z, Deng Z, Wang M, Wang X, Jing J, Zhang X, Shang H, Li Z. Inheritance and molecular analysis of an alien stripe-rust resistance gene from a wheat- 
Aegilops
huashanica Keng line. Plant Sci. 2008;174(5):544–9.
21. Kang HY, Zhang ZJ, Xu XL, Qi WL, Tang Y, Wang H, Zhu W, Li DY, Zeng J, Wang Y. Characterization of wheat-Aegilops huashanica small segment substitution line with enhanced kernels per spike and stripe rust resistance. Genome. 2016;59(4): 221–9.
22. Wang Y, Yu K, Xie Q, Kang H, Lin L, Fan X, Shi L, Zhang H, Zhou Y. The 3Ns chromosome of 
Aegilops
huashanica carries the gene(s) underlying different wheat cultivars stripe rust resistance. Cytogenet Genome Res. 2011;134(2):136–43.
23. Ma DF, Zhou XL, Hou L, Bai YB, Li Q, Wang HG, Tang M, Jiang JX. Genetic analysis and molecular mapping of a stripe rust resistance gene derived from 
Aegilops
huashanica Keng in wheat line 9H014-121-S-5-S. Mol Breed. 2013;22(3):365–72.
24. An DG, Zheng Q, Zhou YL, Ma PT, Lv ZL, Li LH, Li B, Luo QL, Xu HX, Xu YF. Molecular cytogenetic characterization of a new wheat-4R chromosome translocation line resistant to powdery mildew. Chromosoma. 2013;121(4):419–32.
25. Wang YJ, Quan W, Peng NN, Wang CY, Yang XF, Liu XL, Zhang H, Chen CH, Ji WQ. Molecular cytogenetic identification of a wheat- 
Aegilops
geniculata Roth 7M disomic addition line with powdery mildew resistance. Mol Breed. 2016;35(4).
26. Zhan HX, Xiaoqian Z, Li GR, Pan ZH, Hu J, Li X, Qiao LY, Jia QJ, Guo HJ, Chang ZJ, et al. Molecular characterization of a new wheat-Thinopyrum intermediate translocation line with resistance to powdery mildew and stripe rust. Int J Mol Sci. 2015;16:162–73.
27. Cao AZ, Xing LP, Wang XY, Yang XM, Wang W, Sun YL, Qian C, Ni L, Chen YP, Liu DJ, et al. Serine/threonine kinase gene Spk-V, a key member of powdery mildew resistance gene Phm21, confers powdery mildew resistance in wheat. Proc Natl Acad Sci. 2011;108:7727–32.
28. Annamaria S, Linc G, Molnar-Lang M, Graner A. Fluorescence in situ hybridization polymorphism using two repetitive DNA clones in different wheat cultivars of 
Triticum aestivum
L.). Theor Appl Genet. 2004;109(6):1105–12.
29. Yang XF, Wang CY, Li X, Chen CH, Tian ZR, Wang YJ, Ji WQ. Development and molecular cytogenetic identification of a novel wheat-Leptus mollis Imil7Ns(7D) disomic substitution line with stripe rust resistance. PLoS One. 2015;10(10):e0140227.
30. Smith EL, Mac Ney J, Qualset C. Conventional methods of wheat breeding. In: Genetic Improvement in Yield of Wheat. America. Crop Science Society of America and American Society of Agronomy; 1986.
31. Bajaj YPS, Gosal SS. Biotechnology of Wheat Improvement. In: YPS B, editor. Crops I. Berlin: Springer Berlin Heidelberg; 1986. p. 3–38.
32. Li Z, Li B, Tong Y. The contribution of distant hybridization with decaploid 
Agropyron secalis elongatum to wheat improvement in China. J Gen Genomics. 2008;35(8):451–6.
33. Cifuentes M, Benavente E. Wheat-alien metaphase I pairing of individual wheat genomes and D genome chromosomes in interspecific hybrids between 
Triticum aestivum
and 
Aegilops geniculata Roth. Theor Appl Genet. 2009;119(5):805–13.
34. Li CZ, Huang HQ, Yin FY, Wang ZY, Peng YK, Xie CJ, Liu ZY, Sun QX, Yang ZM. The effect of 
Hoynickia villosa
v chromosome on the mitochondrial proteome of wheat-H. villosa chromosome substitution line and translocation line. J Mol Cell Biol. 2008;41(2):150–4.
35. Ren TH, Chen F, Yan BJ, Zhang HQ, Ren ZL. Genetic diversity of wheat-4R chromosome translocation lines derived from different wheat and rice sources. Euphytica. 2012;183(2):133–46.
36. Danilova TV, Friebe B, Gill BS, Pollock J, Jackson E. Chromosome rearrangements caused by double monosomy in wheat-barley group-7 chromosome substitution 
Triticum aestivum
Genome. 2018;154(1):45–55.
37. Du YX, Ma X, Min JZ, Zhang XC, Jia ZZ. Development of a wheat-Aegilops searsii substitution line with positively affecting Chinese steamed bread quality. Breed Sci. 2018;68(2):289–93.
38. Appels R, Eversele K, Stein N, Feuillet C, Kerver B, Rogers J, Pozniak CJ, Choulet F, Distelfeld A, Poland J, et al. Shifting the limits in wheat research