

A chromosome arm from *Thinopyrum intermedium* × *Thinopyrum ponticum* hybrid confers increased tillering and yield potential in wheat

Edina Türkösi

Agricultural Institute, Centre for Agricultural Research, ELKH

Éva Szakács

Agricultural Institute, Centre for Agricultural Research, ELKH

László Ivanizs

Agricultural Institute, Centre for Agricultural Research, ELKH

András Farkas

Agricultural Institute, Centre for Agricultural Research, ELKH

Eszter Gaál

Agricultural Institute, Centre for Agricultural Research, ELKH

Mahmoud Said

Institute of Experimental Botany of the Czech Academy of Sciences, Centre of Plant Structural and Functional Genomics
Genomická akademie ve České republice

Éva Darkó

Agricultural Institute, Centre for Agricultural Research, ELKH

Mónika Cséplő

Agricultural Institute, Centre for Agricultural Research, ELKH

Péter Mikó

Agricultural Institute, Centre for Agricultural Research, ELKH

Jaroslav Doležal

Institute of Experimental Botany of the Czech Academy of Sciences, Centre of Plant Structural and Functional Genomics

Márta Molnár-Láng

Agricultural Institute, Centre for Agricultural Research, ELKH

István Molnár (✉ molnar.istvan@atk.hu)

Agricultural Institute, Centre for Agricultural Research, ELKH <https://orcid.org/0000-0002-7167-9319>

Klaudia Kruppa

Agricultural Institute, Centre for Agricultural Research, ELKH

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Abstract

Tiller number is a key component of plant architecture with direct effect on grain yield of wheat. Interspecific hybridization using wild relative species are promising approach for increasing wheat genetic diversity, including yield potential. *Agropyron glael*, a perennial hybrid of *Thinopyrum intermedium* and *Th. ponticum* has five subgenomes (J, Jst, J^{vs}, J^r, and St), making *A. glael* an important gene source for transferring useful agronomical traits into wheat.

This work reports on development of a wheat-*A. glael* translocation line WT153397. Sequential *in situ* hybridizations with J-, St-, and D-genomic DNA probes and pSc119.2, Afa family, pTa71, and (GAA)₇ DNA repeats, as well as an analysis using molecular markers specific for the wheat 6D chromosome, revealed the presence of a 6DS.6J^{vs} centric fusion in the translocation line.

Field trials in low-input and high-input breeding nurseries over three growing seasons demonstrated high compensating ability of the *Agropyron* chromosome arm for the missing 6DL as spike morphology and fertility of WT153397 did not differ significantly from those of wheat parents. Moreover, the introgressed 6J^{vs} chromosome arm significantly increased the number of productive tillers, which manifested in the significantly higher grain yield potential relative to the parental wheat cultivars.

The translocated chromosome could be flow-sorted in high purity providing an opportunity to employ chromosome genomics to identify *Agropyron* gene variant(s) responsible for the tillering capacity. The translocation line WT153397 represents an important genetic stock for functional genetic studies of tiller formation and useful breeding material to increase wheat yield potential.

Introduction

Hexaploid wheat (*Triticum aestivum* L., 2n = 6x = 42, BBAADD) is one of the most important grain crops, grown on about 220 million hectares worldwide (FAOSTAT 2020). In order to feed the world's growing population, the current 763.7 million tons yield production of wheat (International Grain Council, 2020/2021) should be increased by 60% until 2050 (<https://iwyw.org/global-challenge>). However, wheat domestication and thousands of years of cultivation have resulted in a decrease in genetic diversity (Tiwari et al. 2014), hampering the development of high-yielding cultivars and maintaining yield stability under biotic and abiotic stresses (Sears 1981; Jiang et al. 1993; Friebe et al. 1996; Oliver et al. 2006).

Increasing crop yield potential (YP), which is the maximum yield per unit land area achieved under optimal environmental conditions, includes improving wheat structural and reproductive traits (Reynolds et al. 2011). Among the morphological traits, tiller formation correlates linearly with the number of spikes and has a strong effect on grain yield (Darwinkel 1980). In wheat, two types of tillers form: productive tillers, which lead to the formation of spikes and thus significantly contribute to the total number of grains per plant and grain yield, and non-productive tillers, which reduce grain yield (Kirby and Jones 1977; Kulshrestha and Chowdhury 1987).

Wild relatives of wheat represent a valuable reservoir of genetic variation for expanding the genetic basis of bread wheat (Tester and Langridge 2010; Mujeeb-Kazi et al. 2013) and improving its morphological and reproductive traits (Reynolds et al. 2011). Genus *Thinopyrum*, which belongs to the tertiary genepool of wheat, has a variable genome structure ranging from diploid to auto- and allodecaploid species (Ceoloni et al. 2014). For example, the exact genomic constitution of the perennial *Th. intermedium* (Host) Barkworth & D.R. Dewey, a segmental autoallohexaploid (2n = 6x = 42) species, has shown ambiguous features. Liu and Wang (1993) proposed the genome constitution J^eJ^eJ^eJ^eSS, with the subgenome J^e originating from *Th. elongatum* (Host) D. Dewey (2n = 2x = 14, EE), and the subgenome S originating from *Pseudoroegneria spicata* (Pursh) A. Löve (2n = 2x = 14, SS or StSt). Later, it was suggested to rename the S genome with St, since the genomes of the *Aegilops* species in the section Sitopsis were also assigned S letter. Using genomic *in situ* hybridization (GISH), Chen et al. (1998) found that signals of the St-genomic probe were located at the centromeric region on 11 out of the 28 J-genome chromosomes, prompting them to rename the genome symbol to JJJ^sJ^sStSt. Based on the findings of Deng et al. (2013), Kruppa and Molnár-Láng (2016), and Cseh et al. (2019), who found only 8 to 10 centromeric St GISH signals, the number of *Th. intermedium* chromosomes with St centromeres varies by genotype. A GISH analysis performed by Kishii et al. (2005) suggested that *Dasypyrum villosum* L. (2n = 2x = 14, VV) has also been involved in the evolution of *Th. intermedium* as the V-genomic probe showed strong signals along the length but lacking signals around centromeric regions in several chromosomes and telomeric regions of all chromosomes. Wang

et al. (2015) proposed the most recent change to the genomic designation as $J^{Vs}J^rSt$, where J^{Vs} and J^r represent the ancestral genomes of present-day J^b of *Th. bessarabicum* (Savul. & Rayss) Á.Löve ($2n = 2x = 14$) and J^e of *Th. elongatum*, respectively, with J^{Vs} being more ancient.

Th. intermedium and *Th. ponticum* (Podp.) Z.-W. Liu & R.-C. Wang ($2n = 10x = 70$; JJJJJJJ J^s) are valuable gene sources for wheat as they have useful agronomic and quality traits such as high grain protein content, bread-making quality, resistance to rust diseases, and tolerance to low temperature, drought, moisture, and salinity (Dubcovsky et al. 1994; Tang et al. 2000; Colmer et al. 2006; Garg et al. 2014; Tong et al. 2021). However, only a few studies have been published on beneficial effect of wild alien chromatin on wheat yield potential (Monneveux et al. 2003; Kuzmanović et al. 2014; Gao et al. 2021). The 7DL.7Ag translocation from *Lophopyrum elongatum* (syn: *Thinopyrum ponticum*), which carries the leaf rust resistance gene *Lr19*, was found to increase grain yield significantly (Monneveux et al. 2003).

In order to pyramid genes and QTLs encoding useful agronomic traits of *Th. intermedium* and *Th. ponticum* in a bridge material that can be used as a gene source for introgression breeding of wheat, N. V. Tsitsin developed an artificial hybrid, *Agropyron glael* ($2n = 8x = 56$) in the 1930s by crossing *Th. intermedium* [syn: *A. glaucum* (Desf. ex DC.) Roem. & Schult] and *Th. ponticum* [syn: *A. elongatum* (Host) P. Beauv.]. A detailed molecular cytogenetic analysis of *A. glael* revealed that this hybrid carried 18 J, 28 J St , and 8 St chromosomes, including J-St and J St St translocations and chromosomes with decreased fluorescence intensity, resulting in unique hybridization patterns (Kruppa and Molnár-Láng 2016).

The *A. glael* clone No. 8 obtained from the Moscow Main Botanical Garden was used in the wheat introgression breeding program at the Centre for Agricultural Research (Kruppa et al. 2016). In 2001, the winter wheat line 'Martonvásári 9 kr1' (Mv9kr1) containing recessive crossability alleles (*kr1kr1kr2kr2*) (Molnár-Láng et al. 1996) was crossed with *A. glael*. Backcrossing and selfing the hybrid plants resulted in the selection of several partial amphiploid lines (Kruppa et al. 2016), which were backcrossed with wheat to select addition, substitution, or translocation lines.

The present study reports on the development of a wheat/*A. glael* disomic translocation (WT153397) with positive effects on reproductive tiller formation and grain yield when compared to the wheat parents. Further aims were to (1) identify the chromosome composition of the WT153397 introgression line using a three-step molecular cytogenetic and flow cytometric analysis and molecular markers, and (2) evaluate the effect of the added alien chromosome segment on wheat agronomic performance in field trials carried out in three growing seasons.

Materials And Methods

Plant materials

The Mv9kr1 $\times$ *A. glael*/BC $_1$ F $_1$ partial amphiploid genotype (Kruppa et al. 2016; Kruppa and Molnár-Láng 2016) was backcrossed with the facultative elite wheat cultivar Mv Karizma (Supplementary Figure S1). The wheat/*A. glael* translocation line WT153397 was selected in the BC $_3$ F $_2$ generation using molecular cytogenetic techniques. The WT153397 line, along with the hexaploid wheat crossing partners Mv9kr1, 'Chinese Spring' (CS), and 'Mv Karizma' was used for field phenotyping experiments over three years (2019, 2021 and 2022).

For molecular marker analyses, genomic DNA isolated from line WT153397, wheat genotypes Mv9kr1, 'Mv Karizma', parental *A. glael* clone No. 8, the *Aegilops tauschii* ($2n = 2x = 14$, DD) accession MvGB605, and the durum wheat (*T. turgidum* ssp. *durum*) cultivar Langdon ($2n = 4x = 28$, BBAA) was used.

Genomic DNA isolated from *Th. bessarabicum*, *Ps. spicata*, *Ae. tauschii*, *Secale cereale* L., *Oryza sativa* L., *T. aestivum*, and *T. turgidum* ssp. *durum* was used as probes or blocking DNA for GISH, or as templates to amplify specific DNA sequences for fluorescence *in situ* hybridization (FISH).

Probe preparation and fluorescence in situ hybridization

Mitotic chromosome preparations were made from germinating root tips, as described by Lukaszewski et al. (2004). In order to visualize *Thinopyrum* (*A. glael*) chromatin in the wheat genetic background, the following genomic probes were applied: total genomic DNA from *Th. bessarabicum* was labeled with biotin-11-dUTP (Roche, Mannheim, Germany) and used as J-genomic probe, while DNA from *Ps. spicata* was labeled with digoxigenin-11-dUTP (Roche) or biotin-11-dUTP using random priming labeling and used as St-genomic probe, as described by Kruppa and Molnár-Láng (2016). Digoxigenin-11-dUTP-labeled DNA isolated from *Ae. tauschii* was used as a D-genomic probe.

For the FISH experiments, Afa family and pSc119.2 DNA repeats were amplified from genomic DNA of *Ae. tauschii* and *S. cereale*, respectively, and labeled using PCR (Nagaki et al. 1995; Contento et al. 2005) with digoxigenin-11-dUTP (Roche) and biotin-16-dUTP (Roche, Mannheim, Germany), respectively. The 18S unit of 45S rDNA was amplified using PCR from rice genomic DNA (Chang et al. 2010) and labeled with 50% biotin-16-dUTP and 50% digoxigenin-11-dUTP. Microsatellite (GAA)₇ was amplified from *T. aestivum* genomic DNA and labeled with digoxigenin-11-dUTP (Roche) using PCR. Anti-digoxigenin-rhodamine Fab fragments (Roche) and streptavidine-Alexa Fluor 488 (Molecular Probes, Waltham, USA) were used to detect digoxigenin and biotin, respectively.

Chromosomal composition of the line WT153397 was determined using sequential multicolor genomic *in situ* hybridization (mcGISH) and fluorescence *in situ* hybridizations (FISH). GISH reaction mix (15 µL per slide) containing 50% formamide, 2xSSC, 10% dextran sulphate, 100 ng each of the J- and St-genomic probes and 3 µg *T. aestivum* blocking DNA was allowed to hybridize overnight at 42°C. The hybridization mixes in the GISH experiments used to visualize St- and D-genomic parts of the translocated chromosome contained St- and D-genomic probes together with durum wheat blocking DNA in the same amount as described above. GISH signals were microscopically documented after signal detection and counterstaining with 2 µg mL⁻¹ DAPI (4',6-diamidino-2-phenylindole, Amersham, Germany). Following washing (3x30 min in 4xSSC Tween, 2x5 min 2xSSC at 25°C), the slides were re-hybridized with DNA repeat probes.

The protocol for the FISH experiments was modified as follows. The hybridization temperature was 37°C for probes pSc119.2, Afa family, and pTa71, and 42°C for probe (GAA)₇ (in a third step hybridization). The hybridization mixture (30 µL per slide) contained 20 ng of pTa71, 70 ng of each of the pSc119.2 and Afa family probes, and salmon sperm as blocking DNA.

The GISH and FISH hybridization sites were documented using an AxioImager M2 fluorescence microscope (Zeiss, Oberkochen, Germany) equipped with an AxioCam MRm CCD camera (Zeiss, Oberkochen, Germany) and filter sets appropriate for DAPI (Zeiss Filterset 49), Alexa Fluor 488 (Zeiss Filterset 38), and Rhodamine (Zeiss Filterset 20). The images were processed with the AxioVision 4.8.2. software (Zeiss, Oberkochen, Germany).

Flow cytometric chromosome analysis and sorting

Suspensions of intact mitotic metaphase chromosomes from the line WT153397 were prepared from synchronized root tips cells of young seedlings following Vrána et al. (2016a; b). Prior to flow cytometric analysis, chromosomes in suspension were fluorescently labeled by FISHIS (Giorgi et al. 2013) with oligonucleotide probe 5'-FITC-(GAA)₇-FITC-3' (Integrated DNA Technologies, USA) and stained with DAPI. Bivariate flow karyotyping and chromosome sorting were carried out using a FACSAria II SORP flow cytometer and sorter (Becton Dickinson Immunocytometry Systems, San José, USA) as described by Molnár et al. (2016) and Said et al. (2019, 2022). The chromosome samples were analyzed at a rate of 900-1,400 particles per second, and bivariate flow karyotypes of FITC vs. DAPI fluorescence were acquired. The sorting window was set to the chromosome population of interest on the dot plots, and chromosomes were flow-sorted at a rate of 15–24 per second. Approximately 3,000 chromosomes from each chromosome fraction were flow-sorted onto a microscope slide into a 3 µL drop of PRINS buffer supplemented with 2.5% sucrose (Kubaláková et al. 1997). The chromosome content of the flow-sorted fractions was determined using FISH, as described by Kruppa et al. (2016).

Molecular marker analysis

A total of 26 SSR and two COS markers previously mapped on chromosome 6D were selected from the GrainGenes database (<https://wheat.pw.usda.gov/GG3/>) and used to characterize the wheat/*A. glael* translocation in the line WT153397. Genomic DNA was extracted from fresh young leaves of wheat genotypes Mv9kr1 and 'Mv Karizma', from the parental *A. glael* clone No. 8, from the *Ae. tauschii* accession MvGB605, the durum wheat cultivar Langdon, and from the WT153397 translocation line using a Quick Gene-Mini80 device (FujiFilm, City, Japan) and a QuickGene DNA tissue kit (FujiFilm) according to the manufacturer's instructions. The PCR reactions were carried out in an Eppendorf Mastercycler (Eppendorf, City, Germany). Reaction mixes (15 µL) contained 20 ng of template DNA, 1.5 µL of 10× key reaction buffer (MgCl₂ final concentration of 1.5 mmol/L), 200 µmol/L of each dNTP, 0.2 µmol/L of forward and reverse primers, and 0.375 U of TEMPase Hot Start DNA Polymerase (VWR International, City, Belgium). The PCR profiles and annealing temperatures of the primer pairs used in the reactions are available in the GrainGenes database (<https://wheat.pw.usda.gov/GG3/>) and are summarized in Supplementary Table S1. The PCR amplicons were separated using a Fragment Analyzer Automated CE System (Advanced Analytical Technologies, City, USA) equipped with a 96-capillary array cartridge (effective length 33 cm) and analyzed with the PROsize v2.0 software (Advanced Analytical Technologies).

The physical positions of the markers on chromosome 6D were determined *in silico* using a sequence similarity approach. The publicly available primer sequences of the markers (Supplementary Table S1) were used as queries in BLASTn searches (Ensembl Plants release 46; <https://plants.ensembl.org/Multi/Tools/Blast>) against the reference pseudomolecules of A, B and D genomes of hexaploid wheat (International Wheat Genome Sequencing Consortium (IWGSC) et al. 2018). The start position of each primer sequence was determined by the best hits obtained on the wheat reference sequence using the BLASTn package of the Blast Command Line Application 2.9.0 (<ftp://ftp.ncbi.nlm.nih.gov/>) with the following parameters: -task 'BLASTn'; -evalue 1e-5; -max target seqs 2; -max hsp 1.

Field phenotyping experiments

Morphological traits of the line WT153397, together with the wheat genotypes Mv9kr1, 'Chinese Spring', and 'Mv Karizma' were evaluated in a low-input, pesticide-free location (Tükrös nursery, Martonvásár, Hungary; geographic coordinates: 47°18'40"N 18°46'56"E) in the 2018–2019, 2020–2021, and 2021–2022 growing seasons and also in a high-input nursery (Bolgárföld, Martonvásár, Hungary, geographic coordinates: 47°19'39"N, 18°47'01"E) in the 2021–2022 season. In the low-input 'Tükrös' nursery, 40 grains of each genotype were sown (4 rows; 10 grains per row) with a 0.15 m row spacing, whereas in the high-input experiments 250 grains of each genotype were sown in 2 m² plots (5 rows; 50 grains per row) with a row distance of 0.15 m, as previously described by Mikó et al. (2014).

For phenotypic characterization, ten typical plants were selected from each genotype in each location and season. Plant height and tillering (number of tillers per plant) were measured in the field immediately before harvest. After harvest, the length of the main spike, the number of spikelets per main spike, the number of seeds per main spike, the number of seeds per spikelets, and the number of seeds per plant were determined. The total number of spikes and the yield of the plots were also measured in the high-input experiment.

Statistical analysis

The significant differences between the line WT153397 and the wheat genotypes Mv9kr1, 'Chinese Spring', and 'Mv Karizma' were calculated from 10 biological replicates for each year using the Tukey's post hoc test of the IBM SPSS Statistics 20.0 software (SPSS Inc., Chicago, IL, USA).

Results

Development and molecular cytogenetic analysis of the line WT153397

Schematic representation of the crossing program used to develop wheat/*A. glael* introgression lines is shown in Supplementary Figure S1. Partial amphiploids of the BC₁F₇ generation were backcrossed with the elite wheat cultivar Mv Karizma and selfed in

two cycles to produce BC₂F₁ and BC₃F₁ populations. Molecular cytogenetic screening for the presence of *Agropyron* chromosomes and wheat/*Agropyron* translocations was carried out on the BC₃F₂ generation. Because *A. glael* contains genomes J, Js, and St, a mixture of St-genomic probe from digoxigenin-labeled *Ps. spicata* genomic DNA (red) and J-genomic probe from biotin-labeled *Th. bessarabicum* DNA (green) was used. GISH analysis using this probe combination (combination 1) identified a wheat line (WT153397) with 42 chromosomes, including a disomic wheat/*A. glael* translocation chromosome. Red fluorescent signal indicated that a St-genomic segment had been terminally translocated to a wheat chromosome, whereas the lack of green hybridization signals suggested that no J-genomic fragment was involved in the translocation (Fig. 1a).

In order to identify the wheat chromosome involved in the translocation, the slides used for GISH were then subjected to sequential FISH using repetitive DNA probes pSc119.2, Afa family and pTa71 (Fig. 1b). The resulting FISH hybridization patterns confirmed the presence of 14 A-genome and 14 B-genome chromosomes. Strong Afa signals also confirmed the presence of one pair for each 1D-5D and 7D chromosomes with normal FISH karyotype and the absence of 6D chromosomes. However, a strong Afa signal was detected on the telomeric part of the wheat chromosome arm involved in the translocation, which corresponded to the short arm of 6D, suggesting that the *A. glael* chromosome segment was translocated to the 6D wheat chromosome.

In a third step, using the (GAA)₇ microsatellite probe, intense GAA clusters were detected along the recombinant chromosome arm from the near centromeric region to the telomeric part (Fig. 1c), which is not typical for the D genome (Pedersen and Langridge 1997). This result suggests that the rearranged chromosome arm does not contain D-genome fragments.

To further investigate the structure of the translocation and to visualize its D-genomic part, we used a second combination of biotin-labeled St-genomic probe and digoxigenin-labeled D-genomic probe, along with A- and B-genomic blocking DNA from *T. turgidum* ssp. *durum*. The GISH analysis demonstrated that only the short arm of the translocation originated from the D genome, while the long arm came from *A. glael*, where the terminal part belongs to the St genome (Fig. 1d).

Flow cytometric analysis and chromosome sorting

The presence of large GAA clusters on the wheat/*A. glael* translocated chromosome was further confirmed by flow cytometric analysis and chromosome sorting, in which the chromosome suspension was labeled by FISHIS using a FITC conjugated (GAA)₇ oligonucleotide probe. Bivariate flow karyotype of wheat/*A. glael* translocation line WT153397 (Fig. 2a) showed a chromosome population that is not typical for bivariate flow karyotype of hexaploid wheat with standard karyotype (Said et al. 2022). The position of the translocated chromosome on the dot plot indicated that its relative DNA content was lower as compared to the B-genome chromosomes, but similar to that of the A- and D-genome chromosomes of wheat. However, because the *Agropyron* chromosome segment has prominent (GAA)₇ clusters, its FITC fluorescence was much higher than that of the wheat A- and D-genome chromosomes. These differences allowed the 6D/*A. glael* translocated chromosome to be distinguished from the wheat A-, B-, and D-genome chromosomes (Fig. 2a). The analysis of 126 chromosomes flow-sorted from the candidate population onto microscopy slides after FISH with DNA repeat probes pSc119.2, Afa family and pTa71 (Fig. 2b) confirmed that the translocation could be sorted at high purity (94.4%) with negligible contamination by wheat chromosomes 2D (2.3%), 4A (1.65%) and 4B (1.65%).

The structure of the wheat/ *Agropyron* translocation

The homoeologous relationships between the introgressed *Agropyron* chromatin and wheat were also investigated using molecular markers specific for wheat group 6 chromosomes. Twenty-six SSR and two COS markers previously mapped on chromosome 6D were used, and their physical positions on 6D were determined using BLASTn (Supplementary Table S2).

All 11 markers specific for the short arm of chromosome 6D were detected in the introgression line WT153397, but none of the 17 markers specific for 6DL amplified PCR products, indicating that the long arm of the 6D chromosome was missing from the translocation (Supplementary Figure S2, Fig. 3). The fact that five of the 17 markers mapped on the 6DL chromosome arm produced polymorphic fragments on *A. glael* and that all of these markers amplified *Agropyron*-specific products in the line WT153397 suggests that the missing chromosome arm 6DL was replaced by an *Agropyron* chromosome segment homeologous

for wheat group 6 chromosomes (Fig. 3). As a result, the translocated chromosome can be identified as a T6DS.6Ag centric fusion.

Morphological traits and yield components of the WT153397 line

In order to clarify whether the introgressed *Agropyron* chromosome segment influences wheat morphological traits, the line WT153397 was compared with the parental wheat genotypes Mv9kr1, 'Chinese Spring', and 'Mv Karizma' in three field trials (2019, 2021, 2022) under low-input, and one trial (2022) under high-input conditions (Table 1).

Table 1

Morphological traits of the wheat/*A.glae*/ WT153397 line and the parental wheat genotypes Mv9kr1, 'Mv Karizma', and 'Chinese Spring' in three growing seasons (2019, 2021, 2022) and in two locations (low- and high-input). Values are the means $\pm$ standard deviations of 10 measurements. Values with the same letters in a column do not differ significantly by Tukey's post hoc test ($\alpha = 0.05$). Values of WT153397 significantly different from the wheat genotypes have been highlighted by bold letters.

Field trial	Genotype	Plant height (cm)	Tillering (spikes/plant)	Length of main spike (cm)	Spikelet/main spike	Seeds/main spike	Seeds/plant	Fertility (seeds/spikelet)
Low-input 2019	Mv9kr1	88.1 $\pm$ 4.72 b	8.9 $\pm$ 2.46 bc	10.5 $\pm$ 1.08 b	21.1 $\pm$ 1.13 a	43.5 $\pm$ 5.42 ab	321.0 $\pm$ 36.09 c	2.09 $\pm$ 0.30 ab
	Mv Karizma	89.0 $\pm$ 5.56 b	9.8 $\pm$ 2.08 b	11.2 $\pm$ 0.79 ab	21.0 $\pm$ 1.41 ab	47.4 $\pm$ 7.52 a	439.1 $\pm$ 60.7 b	2.28 $\pm$ 0.20 a
	CS	107.4 $\pm$ 8.63 a	6.8 $\pm$ 1.83 c	8.6 $\pm$ 0.64 c	19.2 $\pm$ 1.46 b	36.0 $\pm$ 10.41 b	237.7 $\pm$ 72.5 c	1.84 $\pm$ 0.42 b
	WT153397	90.6 $\pm$ 6.15 b	13.0 $\pm$ 1.73 a	11.5 $\pm$ 0.70 a	22.0 $\pm$ 1.84 a	44.6 $\pm$ 5.24 ab	599.3 $\pm$ 94.0 a	2.47 $\pm$ 0.25 a
Low-input 2021	Mv9kr1	91.0 $\pm$ 5.46 b	7.0 $\pm$ 2.00 c	10.6 $\pm$ 0.74 b	21.1 $\pm$ 1.13 b	43.5 $\pm$ 5.42 a	303.7 $\pm$ 87.35 c	2.06 $\pm$ 0.22 a
	Mv Karizma	91.6 $\pm$ 4.40 b	9.6 $\pm$ 2.37 b	11.8 $\pm$ 1.18 a	21.0 $\pm$ 1.41 b	47.4 $\pm$ 7.37 a	445.3 $\pm$ 80.27 b	2.25 $\pm$ 0.31 a
	CS	118.7 $\pm$ 11.61 a	8.3 $\pm$ 0.67 bc	8.6 $\pm$ 0.80 c	23.4 $\pm$ 1.42 a	32.1 $\pm$ 10.97 b	243.1 $\pm$ 59.82 c	1.37 $\pm$ 0.44 b
	WT153397	81.4 $\pm$ 2.72 c	14.0 $\pm$ 1.49 a	11.2 $\pm$ 1.09 ab	22.2 $\pm$ 1.72 ab	44.8 $\pm$ 4.97 a	616.4 $\pm$ 81.94 a	2.01 $\pm$ 0.15 a
Low-input 2022	Mv9kr1	87.8 $\pm$ 5.47 b	8.2 $\pm$ 1.07 b	9.0 $\pm$ 1.07 c	17.9 $\pm$ 1.64 b	47.3 $\pm$ 6.82 bc	366.7 $\pm$ 78.0 b	2.64 $\pm$ 0.26 ab
	Mv Karizma	81.3 $\pm$ 3.37 b	8.7 $\pm$ 0.74 b	10.1 $\pm$ 0.45 b	19.2 $\pm$ 0.74 b	39.0 $\pm$ 2.75 c	291.5 $\pm$ 68.2 b	2.03 $\pm$ 0.20 c
	CS	133.5 $\pm$ 9.82 a	8.1 $\pm$ 1.42 b	8.4 $\pm$ 0.53 c	23.2 $\pm$ 0.87 a	52.6 $\pm$ 3.74 b	373.0 $\pm$ 59.6 b	2.32 $\pm$ 0.27 bc
	WT153397	83.0 $\pm$ 6.40 b	12.2 $\pm$ 1.86 a	12.2 $\pm$ 0.95 a	22.8 $\pm$ 1.66 a	61.9 $\pm$ 11.5 a	524.0 $\pm$ 86.3 a	2.70 $\pm$ 0.39 a
High-input 2022	Mv9kr1	90.6 $\pm$ 5.23 b	7.9 $\pm$ 1.60 b	11.1 $\pm$ 0.84 b	24.1 $\pm$ 1.75 ab	58.1 $\pm$ 9.40 b	375.3 $\pm$ 89.7 b	2.39 $\pm$ 0.35 b
	Mv Karizma	92.7 $\pm$ 1.73 b	8.2 $\pm$ 1.66 b	13.3 $\pm$ 0.97 a	23.9 $\pm$ 1.22 ab	67.5 $\pm$ 10.80 ab	422.9 $\pm$ 64.2 b	2.61 $\pm$ 0.90 ab
	CS	116.3 $\pm$ 2.83 a	8.7 $\pm$ 0.97 b	8.9 $\pm$ 0.84 c	22.0 $\pm$ 2.44 b	69.7 $\pm$ 9.12 ab	419.0 $\pm$ 66.3 b	3.19 $\pm$ 0.47 a

Field trial	Genotype	Plant height (cm)	Tillering (spikes/plant)	Length of main spike (cm)	Spikelet/main spike	Seeds/main spike	Seeds/plant	Fertility (seeds/spikelet)
	WT153397	81.7 ± 4.10 c	12.2 ± 2.47 a	13.45 ± 0.75 a	24.8 ± 2.27 a	71.0 ± 10.38 a	621.2 ± 141.6 a	2.88 ± 0.44 ab

In all trials, the plant height measured for the line WT153397 (81.3–92.7 cm) did not differ from that of ‘Mv Karizma’ and Mv9kr1, but it was significantly higher for ‘Chinese Spring’ (107.4–133.5 cm). On the other hand, Tukey's post-hoc analysis revealed high tillering capacity for WT153397, with values ranging from 12.2 to 14. This significantly exceeded the number of productive tillers per plant of the wheat genotypes Mv9kr1 and ‘Mv Karizma’ (7.0–9.8 for both), as well as ‘Chinese Spring’ (6.8–9.6) under both low- and high-input conditions in all growing seasons. At the same time, the parameters of WT153397 related to spike morphology and fertility did not differ significantly from those of Mv9kr1 and ‘Mv Karizma’. WT153397 and ‘Mv Karizma’ had a weak tendency for longer spikes and a higher number of seeds per main spike compared to the other wheat genotypes, but differences between WT153397 and the parental wheat genotypes were significant only in 2022 (Table 1, Fig. 4). There were no significant differences in the parameters of spikelets number per spike and seeds per spikelet between WT153397 and its wheat parents. The increased number of productive tillers resulted in the highest grain yield (seed/plant) value of the line WT153397, which was 36–79% higher than the values of ‘Mv Karizma’ under low-input conditions, and 46% higher under high-input condition. The higher tillering ability of the line WT153397 relative to Mv9kr1 and ‘Mv Karizma’ was also manifested in the total number of fertile spikes (WT153397: 1191, Mv9kr1: 780, ‘Mv Karizma’: 962) and the yield values per plot (WT153397: 1720 g, Mv9kr1: 1523 g, ‘Mv Karizma’: 1460 g) measured at maturity for each plot under high-input conditions, where the plants (250 seeds per genotypes) were sown at a normal density. This implies that the normal sowing density has no effect on the tillering ability and yield potential of the newly developed wheat/*A.glael* translocation line.

All of these morphological traits of WT153397 indicate a high compensating ability of the *Agropyron* segment for the missing 6DL chromosome arm, supporting the close homoeologous relationship between the introgressed *Agropyron* chromosome segment and wheat group 6 chromosomes.

Discussion

A. glael is a promising reservoir of gene variants for wheat breeding. The present study is the first report on a genetically stable introgression line with 42 chromosomes, including a homozygous translocated chromosome carrying a chromosome arm from *A. glael*.

Molecular cytogenetic analysis revealed that wheat chromosome arm 6DS was involved in the translocation. The introgressed *Agropyron* chromosome arm was identified by a terminal St-genomic signal, missing GISH signals with *Th. bessarabicum* DNA, and massive GAA clusters from the near centromeric region to the telomere. These results are consistent with those of Cseh et al. (2019), who found that all of the J^{vs}-genome chromosomes of *Th. intermedium*, the parental species of *A. glael*, have telomeric hybridization signals with St-genomic probe, eight of them also have centromeric signals, and all of them were labeled by V-genomic probe from *D. villosum*.

Giorgi et al. (2013) also reported that V-genome chromosomes of *D. villosum* contains a significant amount of GAA microsatellite repeats. The strong GAA clusters allowed fluorescent labeling of the V chromosomes in FISHIS using FITC-conjugated GAA oligonucleotide probes and discrimination of the V chromosomes on the bivariate (FITC vs. DAPI) flow karyotype (Giorgi et al. 2013).

Previous studies also indicated that J^{vs} genome preserved repetitive sequences from the *D. villosum* V genome (Kishii et al. 2005; Mahelka et al. 2011; Cseh et al. 2019). It seems reasonable to conclude that GAA microsatellites are common repetitive DNA fractions in *D. villosum* and the *Th. intermedium* J^{vs} genome. Molecular cytogenetic analysis, which aimed to describe the karyotype with repetitive DNA probes of diploid ancestors of St- and J^b-genomes, *Ps. spicata* and *Th. bessarabicum*, respectively,

revealed that only one St-genome chromosome contains GAA signal, while the J^b chromosomes do not contain microscopically detectable GAA clusters (Linc et al. 2017).

Based on all of the previous findings and our present results showing the presence of GAA clusters in the introgressed chromosome arm of WT153397, we conclude that the chromosome arm transferred from *A. glael* belongs to the J^{vs} genome of the parental *Th. intermedium*. Our molecular marker analysis confirmed the presence of the 6DS chromosome arm in the line WT153397, as well as the fact that the introgressed chromosome arm is homoeologous with the missing 6DL. The homoeologous relationship between diploid *Thinopyrum* genomes and the wheat genome has been established in several publications (Wang et al. 2010; Gaál et al. 2018; Grewal et al. 2018; Baker et al. 2020; Yang et al. 2021). A comparison of the chromosomes of *Th. bessarabicum* and wheat revealed that the macrosynteny between the two species is preserved, and most of the chromosome arms of *Th. bessarabicum* are collinear with their corresponding A-, B-, and D-genome chromosomes of hexaploid wheat (Grewal et al. 2018). Baker et al. (2020) found that the D genome had the most significant BLAST hits for the markers on the diploid *Th. elongatum* genetic map and that good overall synteny is maintained between wheat and *Th. elongatum*, showing the close relationship of the two genomes. Yang et al. (2021) also confirmed that the 2A and 2E chromosomes, as well as the 5D and 5E chromosomes, appear to have a good collinearity relationship, based on synteny analyses between the wheat genome and the *Th. elongatum* genome. Their results agreed with those of Gaál et al. (2018), who used EST sequences to study the macrosyntenic relationship between the wheat and *Th. elongatum* genomes. Even in polyploid wheatgrasses, the homoeology of their genomes with wheat genomes is well preserved.

Kantarski et al. (2017) applied genotyping-by-sequencing (GBS) technology to develop a consensus genetic map for *Th. intermedium*, and significant syntenic relationships between the linkage groups of *Thinopyrum* and barley were discovered by aligning the mapped 10,029 markers to the reference sequence of barley. Later, using an Axiom 35K Wheat-Relative Genotyping Array to characterize 187 wheat/*Th. intermedium* introgression lines, 634 chromosome-specific SNPs were validated (Cseh et al. 2019). The authors found a significant macrosyntenic relationship between the 21 chromosomes of *Th. intermedium* and their homoeologues from the wheat D genome, including the 6J^{vs}-6D synteny.

The close 6J^{vs}-6D homoeologous relationship is also supported by the present work, as we found good compensation ability of the introgressed *A. glael* chromosome arm for the missing 6DL for morphological traits and fertility. After three seasons of field trials, there were no significant differences in the spike morphology (spike length, spikelets per spike) and fertility between the introgression line WT153397 and the wheat genotypes Mv9kr1 and 'Mv Karizma'. It is important to note that 93.75% of the genes in Mv9kr1 come from the wheat cultivar Mv9, which adapted well to the agro-ecological conditions of Central Europe (Molnár-Láng et al. 1996), while 'Mv Karizma' is a modern elite wheat cultivar registered in 2009. However, field experiments confirmed the translocation line's outstanding tillering ability, which significantly exceeded the capacity of productive tiller formation in all wheat control genotypes under both low- and high-input conditions.

Tillering is a complex trait that is determined by the combined effects of multiple genes. Although four single genes (*tin1*, *tin2*, *tin3*, *ftin*) responsible for tiller inhibition have been identified on wheat chromosomes 1A, 2A, and 3A (Spielmeyer and Richards 2004; Kuraparthi et al. 2007), it was found that a majority of underlying variation for tillering was controlled by quantitative trait loci (QTL) primarily distributed on chromosomes 1A, 2A, 2D, 6A, 6D, 7A, and 7D (Li et al. 2010; Ren et al. 2018; Wang et al. 2018). Our results that the substitution of 6J^{vs} chromosome arm of 6DL resulted in an increased capacity of tiller formation is consistent with the finding of Bilgrami et al. (2020), who identified two metaQTLs, MQTL6D-3 and MQTL6D-4, on the terminal position of wheat chromosome 6DL. Our results indicate that the 6J^{vs} chromosome arm contains QTLs affecting more positively on the tiller formation than those of 6DL.

The fact that the 6DS-6J^{vs} translocated chromosome can be purified at high purity by flow cytometric sorting will facilitate identification of key genes on 6J^{vs} responsible for tiller formation using Mutant Chromosome Sequencing (MutChromSeq). As demonstrated by Dong et al. (2023) for the cloning of a key tiller number regulatory gene *TN1*, EMS mutagenesis is a suitable method to produce low-tillering mutants of wheat. Combining EMS mutagenesis with flow cytometric chromosome sorting and sequencing MutChromSeq is a powerful approach for rapid gene isolation and identification (Sánchez-Martín et al. 2016) as demonstrated by cloning of disease resistance genes (Yu et al. 2022, Holden et al. 2022), or genes related with plant morphology

(Borrill et al. 2022). The production of low-tillering knock-out EMS mutants followed by the flow sorting and sequencing of 6DS·6J^{vs} translocated chromosomes from the mutant and wild type WT153397 can be an effective strategy in the future to obtain gene content of 6J^{vs} and identify the candidate gene(s) affecting the tillering.

Conclusion

Wheat/*Thinopyrum* introgressions can be used to increase not only stress resistance but also yield components of bread wheat. The translocation line WT153397 is genetically stable, with no evidence of linkage drag. The presence of the *A.glael* 6J^{vs} chromosome arm in subsequent generations can be tracked using selected molecular markers as well as molecular cytogenetic methods. Even in environments with low and high nutrient supply, it may still generate tillers with a 35–45% higher efficiency. These advantages enable the use of this line in both wheat breeding programs and functional genomic studies intended to clarify the genetic controls over tillering.

Declarations

Main conclusion Wheat/*Agropyron glael* translocation line WT153397 exhibits increased productive tillers and is an excellent gene source for wheat improvement and attractive model to study genetic control of tillering.

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Conflict of interest The authors declare that they have no competing interests.

Data availability statement: Seeds of the described translocation line are freely available to any researcher wishing to use them for non-commercial purposes. Email: molnar.istvan@atk.hu

References

1. Baker L, Grewal S, Yang C-Y, Hubbart-Edwards S, Scholefield D, Ashling S, Burridge AJ, Przewieslik-Allen AM, Wilkinson PA, King IP, King J (2020) Exploiting the genome of *Thinopyrum elongatum* to expand the gene pool of hexaploid wheat. *Theor Appl Genet* 133:2213–2226. <https://doi.org/10.1007/s00122-020-03591-3>
2. Bilgrami SS, Ramandi HD, Shariati V, Razavi K, Tavakol E, Fakheri BA, Mahdi Nezhad N, Ghaderian M (2020) Detection of genomic regions associated with tiller number in Iranian bread wheat under different water regimes using genome-wide association study. *Sci Rep* 10:14034. <https://doi.org/10.1038/s41598-020-69442-9>
3. Borrill P, Mago R, Xu T, Ford B, Williams SJ, Derkx A, Bovill WD, Hyles J, Bhatt D, Xia X, MacMillan C, White R, Buss W, Molnár I, Walkowiak S, Olsen O-A, Doležel J, Pozniak CJ, Spielmeyer W (2022) An autoactive NB-LRR gene causes *Rht13* dwarfism in wheat. *Proc. Nat. Acad. Sci. USA (PNAS)*, 119: e2209875119. <https://doi.org/10.1073/pnas.2209875119>
4. Ceoloni C, Kuzmanović L, Gennaro A, Forte P, Giorgi D, Rosaria Grossi M, Bitti A (2014) Genomes, chromosomes and genes of the wheatgrass genus *Thinopyrum*: the value of their transfer into wheat for gains in cytogenomic knowledge and

- sustainable breeding. Genomics of plant genetic resources: Crop productivity, food security and nutritional quality, pp 333–358. https://doi.org/10.1007/978-94-007-7575-6_14
5. Chang KD, Fang S, an, Chang FC, Chung MC (2010) Chromosomal conservation and sequence diversity of ribosomal RNA genes of two distant *Oryza* species. *Genomics* 96:181–190. <https://doi.org/10.1016/j.ygeno.2010.05.005>
 6. Chen Q, Conner RL, Laroche A, Thomas JB (1998) Genome analysis of *Thinopyrum intermedium* and *Thinopyrum ponticum* using genomic in situ hybridization. *Genome* 41:580–586. <https://doi.org/10.1139/g98-055>
 7. Colmer TD, Flowers TJ, Munns R (2006) Use of wild relatives to improve salt tolerance in wheat. *J Exp Bot* 57:1059–1078. <https://doi.org/10.1093/jxb/erj124>
 8. Contento A, Heslop-Harrison JS, Schwarzach T (2005) Diversity of a major repetitive DNA sequence in diploid and polyploid Triticeae. *Cytogenet Genome Res* 109:34–42. <https://doi.org/10.1159/000082379>
 9. Cseh A, Yang C, Hubbart-Edwards S, Scholefield D, Ashling SS, BurrIDGE AJ, Wilkinson PA, King IP, King J, Grewal S (2019) Development and validation of an exome-based SNP marker set for identification of the S^t, J^r and J^{vs} genomes of *Thinopyrum intermedium* in a wheat background. *Theor Appl Genet* 132:1555–1570. <https://doi.org/10.1007/s00122-019-03300-9>
 10. Darwinkel A (1980) Ear development and formation of grain yield in winter wheat. *NJAS* 28:156–163. <https://doi.org/10.18174/njas.v28i3.17029>
 11. Deng C, Bai L, Fu S, Yin W, Zhang Y, Chen Y, Wang RR-C, Zhang X, Han F, Hu Z (2013) Microdissection and chromosome painting of the alien chromosome in an addition line of wheat–*Thinopyrum intermedium*. *PLoS ONE* 8:e72564. <https://doi.org/10.1371/journal.pone.0072564>
 12. Dong C, Zhang L, Zhang Q et al (2023) *Tiller Number1* encodes an ankyrin repeat protein that controls tillering in bread wheat. *Nat Commun* 14:836. <https://doi.org/10.1038/s41467-023-36271-z>
 13. Dubcovsky J, Galvez AF, Dvořák J (1994) Comparison of the genetic organization of the early salt-stress-response gene system in salt-tolerant *Lophopyrum elongatum* and salt-sensitive wheat. *Theor Appl Genet* 87:957–964. <https://doi.org/10.1007/BF00225790>
 14. FAOSTAT (2020) Food and Agriculture Organization of the United Nations Food and agriculture data. <https://www.fao.org/faostat/en/#data/QCL>. Accessed 19 December 2022
 15. Friebe B, Jiang J, Raupp WJ, McIntosh RA, Gill BS (1996) Characterization of wheat-alien translocations conferring resistance to diseases and pests: current status. *Euphytica* 91:59–87. <https://doi.org/10.1007/BF00035277>
 16. Gaál E, Valárik M, Molnár I, Farkas A, Linc G (2018) Identification of COS markers specific for *Thinopyrum elongatum* chromosomes preliminary revealed high level of macrosyntenic relationship between the wheat and *Th. elongatum* genomes. *PLoS ONE* 13:e0208840. <https://doi.org/10.1371/journal.pone.0208840>
 17. Gao L, Koo D-H, Juliana P et al (2021) The *Aegilops ventricosa* 2NvS segment in bread wheat: cytology, genomics and breeding. *Theor Appl Genet* 134:529–542. <https://doi.org/10.1007/s00122-020-03712-y>
 18. Garg M, Yanaka M, Tanaka H, Tsujimoto H (2014) Introgression of useful genes from *Thinopyrum intermedium* to wheat for improvement of bread-making quality. *Plant Breed* 133:327–334. <https://doi.org/10.1111/pbr.12167>
 19. Giorgi D, Farina A, Grosso V, Gennaro A, Ceoloni C, Lucretti S (2013) FISHIS: fluorescence *in situ* hybridization in suspension and chromosome flow sorting made easy. *PLoS ONE* 8:e57994. <https://doi.org/10.1371/journal.pone.0057994>
 20. Grewal S, Yang C, Edwards SH, Scholefield D, Ashling S, BurrIDGE AJ, King IP, King J (2018) Characterisation of *Thinopyrum bessarabicum* chromosomes through genome-wide introgressions into wheat. *Theor Appl Genet* 131:389–406. <https://doi.org/10.1007/s00122-017-3009-y>
 21. Holden S, Bergum M, Green P, Bettgenhaeuser J, Hernández-Pinzón I, Thind A, Clare S, Russell JM, Hubbard A, Taylor J, Smoker M, Gardiner M, Civolani L, Cosenza F, Rosignoli S, Strugala R, Molnár I, Šimková H, Doležel J, Schaffrath U, Barrett M, Salvi S, Moscou MJ (2022) A lineage-specific *Exo70* is required for receptor kinase-mediated immunity in barley. *Sci Adv* 8:eabn7258. [10.1126/sciadv.abn7258](https://doi.org/10.1126/sciadv.abn7258)
 22. International Wheat Genome Sequencing Consortium (IWGSC), Appels R, Eversole K et al (2018) Shifting the limits in wheat research and breeding using a fully annotated reference genome. *Science* 361. <https://doi.org/10.1126/science.aar7191>

23. Jiang J, Friebe B, Gill BS (1993) Recent advances in alien gene transfer in wheat. *Euphytica* 73:199–212. <https://doi.org/10.1007/BF00036700>
24. Kantarski T, Larson S, Zhang X, DeHaan L, Borevitz J, Anderson J, Poland J (2017) Development of the first consensus genetic map of intermediate wheatgrass (*Thinopyrum intermedium*) using genotyping-by-sequencing. *Theor Appl Genet* 130:137–150. <https://doi.org/10.1007/s00122-016-2799-7>
25. Kirby EJM, Jones HG (1977) The relations between the main shoot and tillers in barley plants. *J Agric Sci* 88:381–389. <https://doi.org/10.1017/S0021859600034870>
26. Kishii M, Wang RR, Tsujimoto H (2005) GISH analysis revealed new aspect of genomic constitution of *Thinopyrum intermedium*. *Czech J Genet Plant Breed* 41:91–95
27. Kruppa K, Molnár-Láng M (2016) Simultaneous visualization of different genomes (J, JSt and St) in a *Thinopyrum intermedium* × *Thinopyrum ponticum* synthetic hybrid (Poaceae) and in its parental species by multicolour genomic in situ hybridization (mcGISH). *Comp Cytogenet* 10:283–293. <https://doi.org/10.3897/COMP CYTOGEN.V10I2.7305>
28. Kruppa K, Türkösi E, Mayer M, Tóth V, Vida G, Szakács É, Molnár-Láng M (2016) McGISH identification and phenotypic description of leaf rust and yellow rust resistant partial amphiploids originating from a wheat × *Thinopyrum* synthetic hybrid cross. *J Appl Genet* 57:427–437. <https://doi.org/10.1007/s13353-016-0343-8>
29. Kubaláková M, Macas J, Doležel J (1997) Mapping of repeated DNA sequences in plant chromosomes by PRINS and C-PRINS. *Theor Appl Genet* 94:758–763. <https://doi.org/10.1007/s001220050475>
30. Kulshrestha VP, Chowdhury S (1987) A new selection criterion for yield in wheat. *Theor Appl Genet* 74:275–279. <https://doi.org/10.1007/BF00289980>
31. Kuraparthi V, Sood S, Dhaliwal HS, Chhuneja P, Gill BS (2007) Identification and mapping of a tiller inhibition gene (*tin3*) in wheat. *Theor Appl Genet* 114:285–294. <https://doi.org/10.1007/s00122-006-0431-y>
32. Kuzmanović L, Gennaro A, Benedettelli S, Dodd IC, Quarrie SA, Ceoloni C (2014) Structural-functional dissection and characterization of yield-contributing traits originating from a group 7 chromosome of the wheatgrass species *Thinopyrum ponticum* after transfer into durum wheat. *J Exp Bot* 65:509–525. <https://doi.org/10.1093/jxb/ert393>
33. Li Z, Peng T, Xie Q, Han S, Tian J (2010) Mapping of QTL for tiller number at different stages of growth in wheat using double haploid and immortalized F₂ populations. *J Genet* 89:409–415. <https://doi.org/10.1007/s12041-010-0059-1>
34. Linc G, Gaal E, Molnar I, Icoşo D, Badaeva E, Molnaar-Lang M (2017) Molecular cytogenetic (FISH) and genome analysis of diploid wheatgrasses and their phylogenetic relationship. *PLoS ONE* 12:1–18. <https://doi.org/10.1371/journal.pone.0173623>
35. Liu ZW, Wang RR (1993) Genome analysis of *Elytrigia caespitosa*, *Lophopyrum nodosum*, *Pseudoroegneria geniculata* ssp. *scythica*, and *Thinopyrum intermedium* (Triticeae: Gramineae). *Genome* 36:102–111. <https://doi.org/10.1139/g93-014>
36. Lukaszewski AJ, Rybka K, Korzun V, Malyshev SV, Lapinski B, Whitkus R (2004) Genetic and physical mapping of homoeologous recombination points involving wheat chromosome 2B and rye chromosome 2R. *Genome* 47:36–45. <https://doi.org/10.1139/g03-089>
37. Mahelka V, Kopeck D, Patová L (2011) On the genome constitution and evolution of intermediate wheatgrass (*Thinopyrum intermedium*: Poaceae, Triticeae). *BMC Evol Biol* 11:1–17. <https://doi.org/10.1186/1471-2148-11-127>
38. Mikó P, Löschenberger F, Hiltbrunner J, Aebi R, Megyeri M, Kovács G, Molnár-Láng M, Vida G, Rakszegi M (2014) Comparison of bread wheat varieties with different breeding origin under organic and low input management. *Euphytica* 199:69–80. <https://doi.org/10.1007/s10681-014-1171-8>
39. Molnár I, Vrána J, Burešová V, Cápál P, Farkas A, Darkó É, Cseh A, Kubaláková M, Molnár-Láng M, Doležel J (2016) Dissecting the U, M, S and C genomes of wild relatives of bread wheat (*Aegilops* spp.) into chromosomes and exploring their synteny with wheat. *Plant J* 88:452–467. <https://doi.org/10.1111/tpj.13266>
40. Molnár-Láng M, Linc G, Sutka J (1996) Transfer of the recessive crossability allele *kr1* from Chinese Spring into the winter wheat variety Martonvásári 9. *Euphytica* 90:301–305. <https://doi.org/10.1007/BF00027480>
41. Monneveux P, Reynolds MP, Aguilar JG, Singh RP, Weber WE (2003) Effects of the 7DL.7Ag translocation from *Lophopyrum elongatum* on wheat yield and related morphophysiological traits under different environments. *Plant Breed* 122:379–384.

<https://doi.org/10.1046/j.1439-0523.2003.00856.x>

42. Mujeeb-Kazi A, Kazi AG, Dundas I, Rasheed A, Ogonnaya F, Kishii M, Bonnett D, Wang RR-C, Xu S, Chen P, Mahmood T, Bux H, Farrakh S (2013) Genetic diversity for wheat improvement as a conduit to food security. Elsevier, pp 179–257.
<https://doi.org/10.1016/B978-0-12-417187-9.00004-8>
43. Nagaki K, Tsujimoto H, Isono K, Sasakuma T (1995) Molecular characterization of a tandem repeat, Afa family, and its distribution among Triticeae. *Genome* 38:479–486. <https://doi.org/10.1139/g95-063>
44. Oliver RE, Xu SS, Stack RW, Friesen TL, Jin Y, Cai X (2006) Molecular cytogenetic characterization of four partial wheat-*Thinopyrum ponticum* amphiploids and their reactions to *Fusarium* head blight, tan spot, and *Stagonospora nodorum* blotch. *Theor Appl Genet* 112:1473–1479. <https://doi.org/10.1007/s00122-006-0250-1>
45. Pedersen C, Langridge P (1997) Identification of the entire chromosome complement of bread wheat by two-colour FISH. *Genome* 40:589–593. <https://doi.org/10.1139/g97-077>
46. Ren T, Hu Y, Tang Y, Li C, Yan B, Ren Z, Tan F, Tang Z, Fu S, Li Z (2018) Utilization of a Wheat55K SNP Array for mapping of major QTL for temporal expression of the tiller number. *Front Plant Sci* 9:333. <https://doi.org/10.3389/fpls.2018.00333>
47. Reynolds M, Bonnett D, Chapman SC, Furbank RT, Manès Y, Mather DE, Parry MAJ (2011) Raising yield potential of wheat. I. Overview of a consortium approach and breeding strategies. *J Exp Bot* 62:439–452. <https://doi.org/10.1093/jxb/erq311>
48. Said M, Cápál P, Farkas A, Gaál E, Ivanizs L, Friebe B, Doležel J, Molnár I (2022) Flow karyotyping of wheat-*Aegilops* additions facilitate dissecting the genomes of *Ae. biuncialis* and *Ae. geniculata* into individual chromosomes. *Front Plant Sci* 13:1017958. <https://doi.org/10.3389/fpls.2022.1017958>
49. Said M, Kubaláková M, Karafiátová M, Molnár I, Doležel J, Vrána J (2019) Dissecting the complex genome of crested wheatgrass by chromosome flow sorting. *Plant Genome* 12:0. <https://doi.org/10.3835/plantgenome2018.12.0096>
50. Sánchez-Martín J, Steuernagel B, Ghosh S, Herren G, Hurni S, Adamski N, Vrána J, Kubaláková M, Krattinger SG, Wicker T, Doležel J, Keller B, Wulff BB (2016) Rapid gene isolation in barley and wheat by mutant chromosome sequencing. *Genome Biol* 17:1–7. <https://doi.org/10.1186/s13059-016-1082-1>
51. Sears ER (1981) Transfer of alien genetic material to wheat. In: Evans LT, Peacock WJ (eds) *Wheat science - today and tomorrow*. Cambridge University Press, New York., pp 75–89
52. Spielmeier W, Richards RA (2004) Comparative mapping of wheat chromosome 1AS which contains the tiller inhibition gene (*tin*) with rice chromosome 5S. *Theor Appl Genet* 109:1303–1310. <https://doi.org/10.1007/s00122-004-1745-2>
53. Tang S, Li Z, Jia X, Larkin PJ (2000) Genomic in situ hybridization (GISH) analyses of *Thinopyrum intermedium*, its partial amphiploid Zhong 5, and disease-resistant derivatives in wheat. *Theor Appl Genet* 100:344–352.
<https://doi.org/10.1007/s001220050045>
54. Tester M, Langridge P (2010) Breeding technologies to increase crop production in a changing world. *Science* 327:818–822.
<https://doi.org/10.1126/science.1183700>
55. Tiwari VK, Wang S, Sehgal S, Vrána J, Friebe B, Kubaláková M, Chhuneja P, Doležel J, Akhunov E, Kalia B, Sabir J, Gill BS (2014) SNP Discovery for mapping alien introgressions in wheat. *BMC Genomics* 15:273. <https://doi.org/10.1186/1471-2164-15-273>
56. Tong K, Wu X, He L, Qiu S, Liu S, Cai L, Rao S, Chen J (2021) Genome-wide identification and expression profile of OSCA gene family members in *Triticum aestivum* L. *Int J Mol Sci* 23:469. <https://doi.org/10.3390/ijms23010469>
57. Vrána J, Cápál P, Šimková H, Karafiátová M, Čížková J, Doležel J (2016a) Flow analysis and sorting of plant chromosomes. *Curr Protoc Cytom* 78:5.3.1–5.3.43. <https://doi.org/10.1002/cpcy.9>
58. Vrána J, Cápál P, Číhalíková J, Kubaláková M, Doležel J (2016b) Flow sorting plant chromosomes. *Methods Mol Biol* 1429:119–134. https://doi.org/10.1007/978-1-4939-3622-9_10
59. Wang RR-C, Larson SR, Jensen KB, Bushman BS, DeHaan LR, Wang S, Yan X (2015) Genome evolution of intermediate wheatgrass as revealed by EST-SSR markers developed from its three progenitor diploid species. *Genome* 58:63–70.
<https://doi.org/10.1139/gen-2014-0186>
60. Wang RR-C, Larson SR, Jensen KB (2010) Analyses of *Thinopyrum bessarabicum*, *T. elongatum*, and *T. junceum* chromosomes using EST-SSR markers. *Genome* 53:1083–1089. <https://doi.org/10.1139/G10-088>

61. Wang R, Liu Y, Isham K, Zhao W, Wheeler J, Klassen N, Hu Y, Bonman JM, Chen J (2018) QTL identification and KASP marker development for productive tiller and fertile spikelet numbers in two high-yielding hard white spring wheat cultivars. *Mol Breed* 38:135. <https://doi.org/10.1007/s11032-018-0894-y>
62. Yang G, Boshoff WHP, Li H, Pretorius ZA, Luo Q, Li B, Li Z, Zheng Q (2021) Chromosomal composition analysis and molecular marker development for the novel Ug99-resistant wheat-*Thinopyrum ponticum* translocation line WTT34. *Theor Appl Genet* 134:1587–1599. <https://doi.org/10.1007/s00122-021-03796-0>
63. Yu G, Matny O, Champouret N et al (2022) *Aegilops sharonensis* genome-assisted identification of stem rust resistance gene *Sr62*. *Nat. Commun.* 13:1607. <https://doi.org/10.1038/s41467-022-29132-8>

Figures

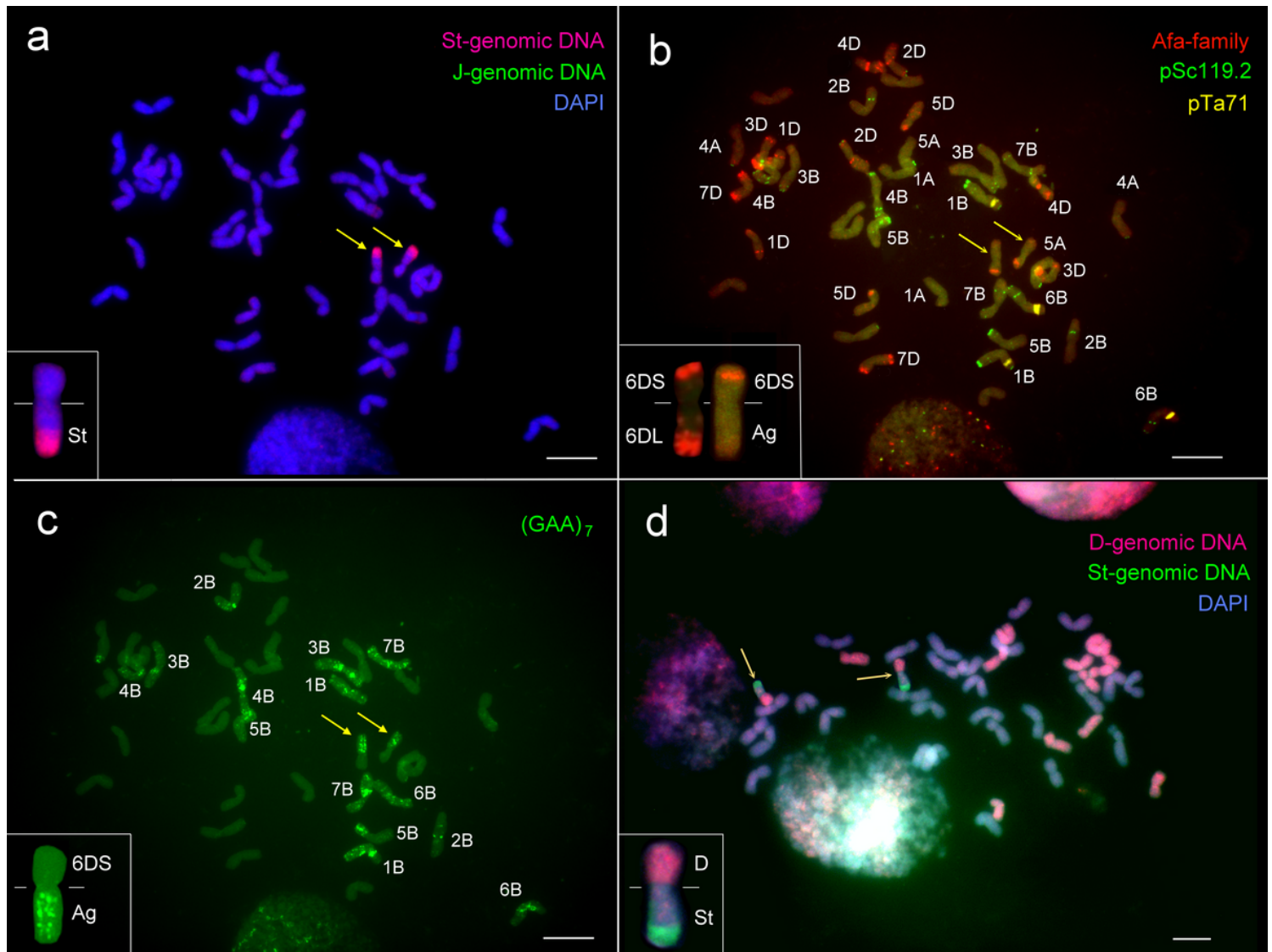


Figure 1

Molecular cytogenetic analysis of the wheat/*A. glael* translocation line WT153397. **a** GISH using St-genomic DNA of *Ps. spicata* (red) discriminated a disomic wheat/*A. glael* translocation, while the labelled J^b-genomic DNA of *Th. bessarabicum* (green) did not produce any hybridization signals. The chromosomes were counterstained with DAPI (blue). Inset: the translocated chromosome. **b** FISH on the same mitotic metaphase cell with the DNA repeat probes Afa family (red), pSc119.2 (green), and pTa71 (yellow). Inset: The FISH pattern of a normal wheat chromosome 6D (left) and the translocated chromosome (right). **c** FISH pattern of the (GAA)₇ microsatellite probe on the same mitotic cell. Inset: the translocated chromosome with intense GAA

clusters on the long arm. **d** McGISH performed with a combination of digoxigenin-labeled D-genomic- (red) and biotin-labeled St-genomic (green) probes together with A- and B-genomic blocking DNA from *T. turgidum* ssp. *durum*. The red fluorescent signal indicates that only the short arm of the translocation derived from the wheat D genome. Inset: the translocated chromosome. Scale bars = 10 mm

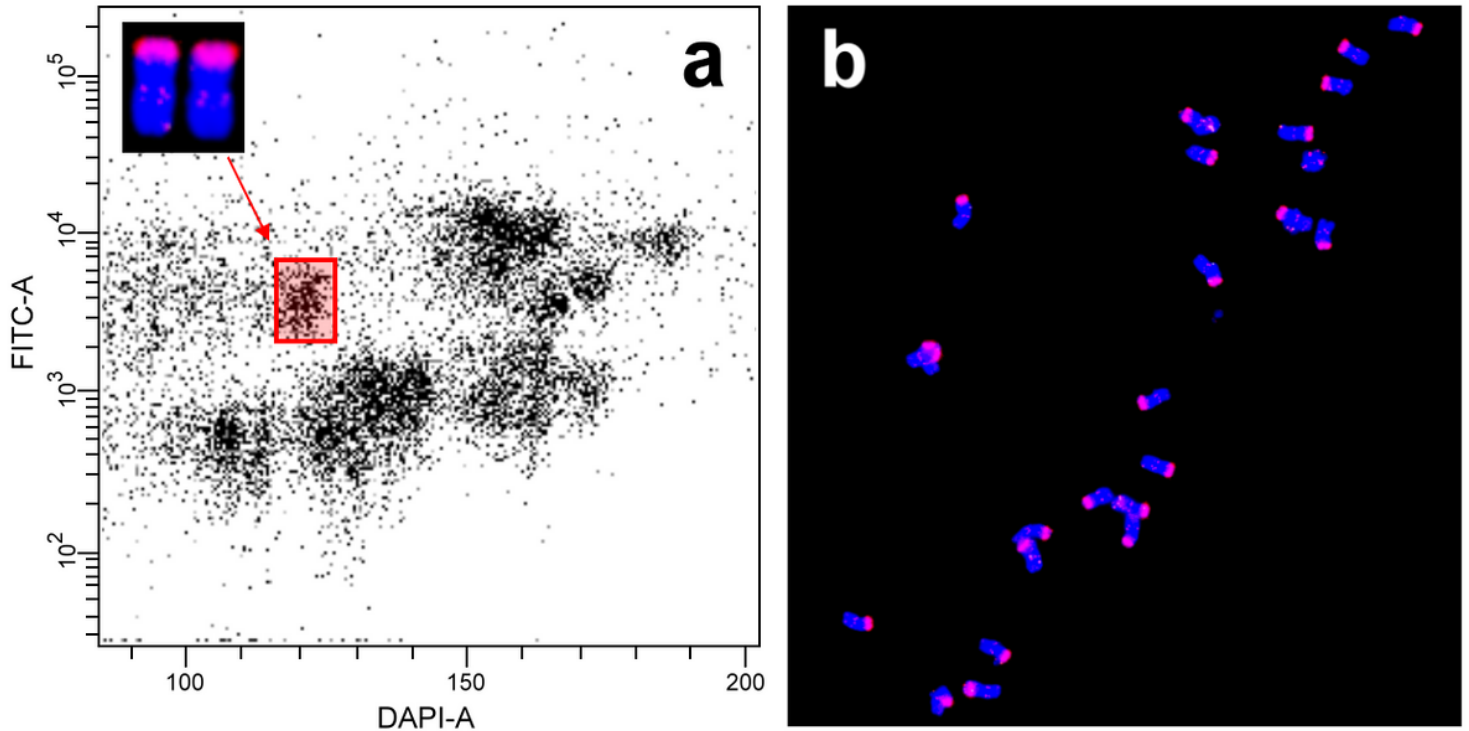


Figure 2

Flow cytometric analysis and sorting of the wheat/*A. glael* chromosome translocation from the WT153397 genotype. **a** Bivariate flow karyotype FITC vs. DAPI fluorescence of the line WT153397. The translocated chromosomes were flow sorted in high purity (94.4%) using the sort window shown as red rectangle. Inset: flow sorted wheat/*A. glael* translocation. **b** Example of translocated chromosomes sorted onto a microscope slide after FISH with probes pSc119.2 (green – not visible), Afa family (red) and 45S rDNA (yellow – not visible).

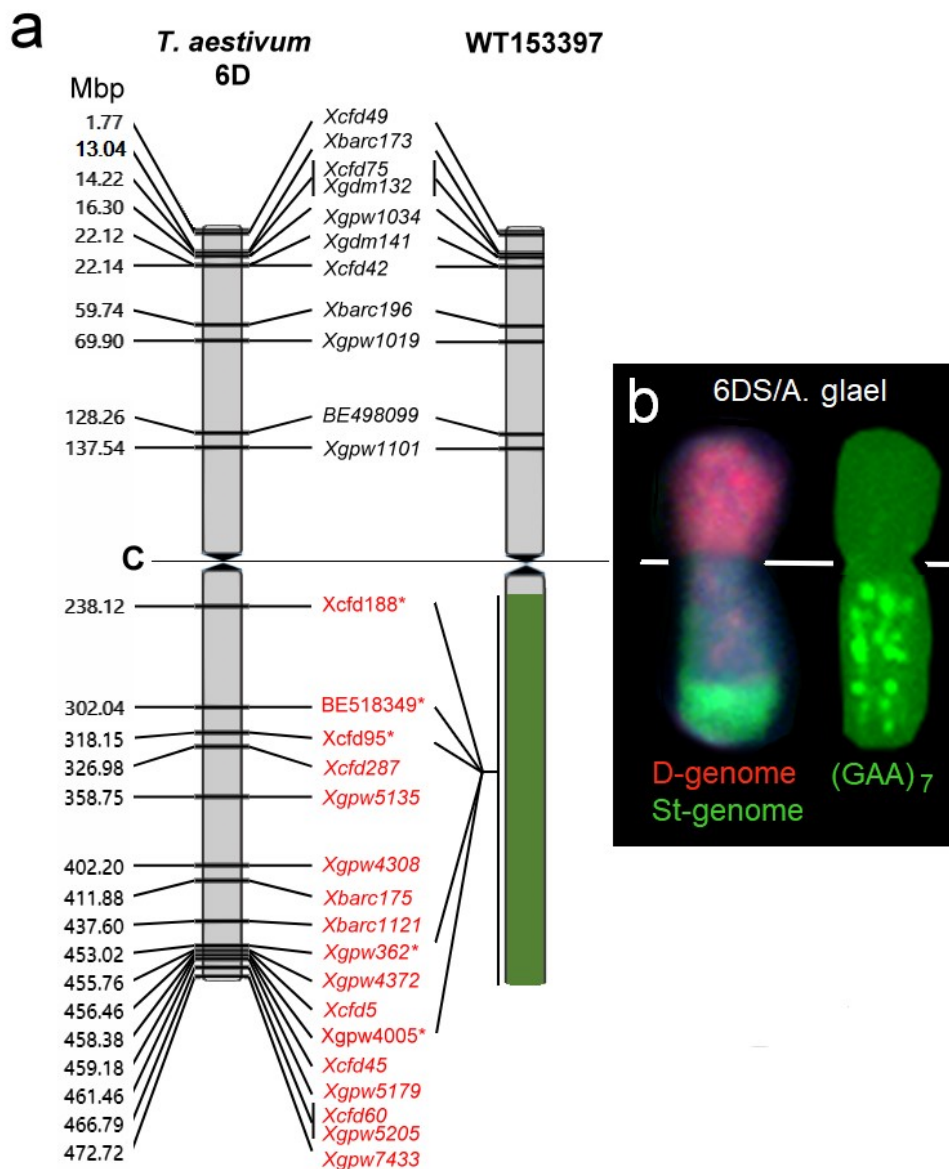


Figure 3

The structure of wheat-*A. glael* T6DS.6Ag centric fusion. **a** Physical locations of twenty-six SSR and two COS markers on the wheat 6D chromosome and the translocated chromosome of the line WT153397. The 6DS-specific markers are present in both wheat and WT153397, but the 6DL-specific markers are absent from the long arm of the translocated chromosome of WT153397, with the exception of five markers (marked with asterisks) that amplified polymorphic PCR fragments. **b** *In situ* hybridization with a D-genomic probe (red) confirms the presence of the 6DS chromosome arm in the line WT153397, but the intense (GAA)₇ clusters on the long arm of the translocated chromosome excludes the presence of the 6D long arm.



Figure 4

Spike morphology of the wheat/*A. glabra* translocation line WT153397 and the parental wheat genotypes Mv9kr1, 'Mv Karizma', and 'Chinese Spring' in the high-input nursery (Martonvásár, 2022)

Supplementary Files

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