

Characterization of Chromosome Constitution in Three Wheat-*Thinopyrum intermedium* Amphiploids Revealed Frequent Rearrangement of Alien and Wheat Chromosomes

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Abstract

Background: *Thinopyrum intermedium* (2n=6x=42) is an important wild perennial *Triticeae* species exhibiting many potentially favorable traits for wheat improvement. Wheat-*Th. intermedium* partial amphiploids serve as a bridge to transfer desirable genes from *Th. intermedium* into common wheat.

Results: Three octoploid *Trititrigia* accessions (TE261-1, TE266-1, and TE346-1) with good resistances to stripe rust, powdery mildew and aphids were selected from hybrid progenies between *Th. intermedium* and the common wheat variety 'Yannong 15' (YN15). Genomic *in situ* hybridization (GISH), fluorescence *in situ* hybridization (FISH) and multicolor GISH (McGISH) analyses demonstrated that the three octoploid *Trititrigia* possess 42 wheat chromosomes and 14 *Th. intermedium* chromosomes. The 14 alien (*Th. intermedium*) chromosomes belong to a mixed genome consisting of J-, J^S- and St-genome chromosomes rather than a single J, J^S or St genome. Different types of chromosomal structural variation were also detected in the 1A, 6A, 6B, 2D and 7D chromosomes via FISH, McGISH and molecular marker analysis. The identity of the alien chromosomes and the variations in the wheat chromosomes in the three *Trititrigia* octoploids were also different.

Conclusions: The wheat-*Th. intermedium* partial amphiploids possess 14 alien chromosomes which belong to a mixed genome consisting of J-, J^S- and St- chromosomes, and 42 wheat chromosomes with different structural variations. These accessions could be used as genetic resources in wheat breeding for the transfer of pest resistance genes from *Th. intermedium* to common wheat.

Background

Thinopyrum intermedium (Host) Barkworth & D.R. Dewey [syn. *Agropyron intermedium* (Host) Beauvior and *Elytrigia intermedia* (Host) Nevski] (2n = 6x = 42; genome formula **E^eE^eE^bE^bStSt, JJJ^SJ^SStSt, EEVStSt** or **J^fJ^rJ^{vs}J^{vs}StSt**) is considered as a segmental autoallohexaploid, and its genome constitution hanging in doubt is a research hot subject in *Triticeae* crop research. The presence of **S** genome (later changed to **St** by Wang *et al.* 1994) in *Th. intermedium* was first reported by Liu and Wang (1993) and confirmed by Zhang *et al.* (1996) as well as Wang and Zhang (1996), leading to the genome formula **JJJJStSt** or **EEEEStSt**, where **J** genome is from *Th. bessarabicum* (2n = 14, **JJ** or **E^bE^b**), **St** genome is from *Pseudoroegneria strigosa* (2n = 14, **StSt**) and **E** genome is from *Th. elongatum* (2n = 14, **EE** or **E^eE^e**). Also by using genomic *in situ* hybridization, Chen *et al.* (1998a) considered that the genome constitution of *Th. intermedium* is **JJJ^SJ^SStSt** where **J^S** might be a modified **J** or **E**. Ji *et al.* (2001) concluded that the genome constitution of *Th. intermedium* is **E^eE^eE^bE^bStSt** by using multicolor GISH (McGISH). Analyzing *trnL-F*, *GBSS* and GISH, Mahelka *et al.* (2011) inferred that genomes of *Th. intermedium* are related to *Ps. strigosa* (**StSt**), *Dasyphyrum villosum* (2n = 14, **VV**), and a complex origin from *Th. elongatum* (**EE**) and *Aegilops tauschii* (2n = 14, **DD**). **J^fJ^rJ^{vs}J^{vs}StSt** was proposed by Wang *et al.* (2015), who made use of EST-SSR makers to analyze the genome evolution of *Th. intermedium*, and **J^f** and **J^{vs}** refer to ancestral genomes of **J^e(E)** and **J^b(J)**, respectively.

Th. intermedium is described as one of the most important perennial *Triticeae* species, possessing many potentially favorable traits for wheat improvement (Gupta 2016, Han *et al.* 2003, Jiang *et al.* 1993, Li and Wang 2009, Wang 2011). Due to its crossability with common wheat, the transfer and utilization of *Th. intermedium* disease resistance genes, including the leaf rust resistance gene Lr38 (Friebe *et al.* 1992); the stem rust resistance gene Sr44 (Friebe *et al.* 1996); the stripe rust resistance gene Yr50 (Liu *et al.* 2013); and the barley yellow dwarf resistance genes Bdv2 (Banks *et al.* 1995), Bdv3 (Sharma *et al.* 1995) and Bdv4 (Lin *et al.* 2010), have been accomplished.

Trititrigia octoploids were developed from hybrid progenies between *Th. intermedium* and common wheat and inherited numerous excellent traits from *Th. intermedium*, such as resistance to powdery mildew, leaf rust, stem rust and stripe rust (Kruppa *et al.* 2016, Wang and Wang 2016). Furthermore, it is easier to successfully cross common wheat with *Trititrigia* octoploids than with *Th. intermedium*. Thus, *Trititrigia* octoploids are valuable germplasm to hybridize with common wheat, continually producing wheat-alien addition, substitution and translocation lines that can transfer beneficial traits from alien species into wheat (Bao *et al.* 2014, Han *et al.* 2003, Han *et al.* 2004, Tang *et al.* 2000).

Many *Trititrigia* octoploids have been produced in the past five decades and used as intermediary parents to facilitate the transfer of excellent genes from *Th. intermedium* to wheat. These *Trititrigia* octoploids include TAF46 (Cauderon *et al.* 1966), 78829 (Zhang *et al.* 1996), the 'Zhong' series of *Trititrigia* octoploids (Sun 1980) and the TE series of *Trititrigia* octoploids (Bao *et al.* 2009, Bao *et al.* 2014, Liu *et al.* 2005, Qi *et al.* 2017, Wang *et al.* 2006). The TE series of *Trititrigia* octoploids was developed from hybrid progenies between *Th.*

intermedium and the common wheat variety 'Yannong 15' (YN15) and inherited many characteristics from *Th. intermedium*, such as large spikes, multi-florets, multi-tillers, and good resistance to stripe rust, powdery mildew and leaf rust. Thus, this TE series of *Trititrigia* octoploids represent an excellent genetic resource for wheat improvement (Bao et al. 2009, Bao et al. 2014, Liu et al. 2005, Qi et al. 2017, Wang et al. 2006).

Genomic *in situ* hybridization (GISH) is generally used to distinguish alien chromosomes in the common wheat background (Cai et al. 2001, Le et al. 1989). When *Pseudoroegneria strigosa* ($2n = 2x = 14$, **StSt**) genomic DNA was used as a probe in GISH analysis of *Th. intermedium*, the karyotype of *Th. intermedium* chromosomes could be classified into three groups. The first group is the **St** genome, completely labeled by probe signals, the second group is the **J^S** genome labeled only in the centromere areas, and the third group is the **J** genome labeled only in the subtelomeric position (Chen et al. 1998a, Chen et al. 1998b, Zhang et al. 1996). As GISH with the **St**-genome DNA probe could effectively distinguish the three sub-genome of *Th. intermedium*, it was widely used in the identification of *Th. intermedium*-derived wheat germplasms (Bao et al. 2014, Chen et al. 2003, Kruppa et al. 2016, Qi et al. 2017).

Fluorescence *in situ* hybridization (FISH) and multicolor GISH (McGISH) are generally used to distinguish different chromosomes, analyze the chromosomal constitution and detect chromosomal variations in cytological studies (Guo et al. 2015, Kruppa et al. 2016, Wang and Wang 2016, Zheng et al. 2015). The available FISH probes that reveal fine banding patterns in wheat chromosomes include the *Aegilops tauschii* clone pAs1 (Rayburn and Gill 1986a, Rayburn and Gill 1986b), the rye clone pSc119.2 (Mukai et al. 1993), the GAA-satellite sequence (Pedersen et al. 1996), *T. aestivum* clone pTa535 (Komuro et al. 2013) and many other oligonucleotides (Du et al. 2017, Tang et al. 2014). Wheat chromosomes can be clearly distinguished using a combination of FISH probes including the pAs1 clone and the GAA-satellite sequence. Chromosome painting using this combination of FISH probes has been achieved in the common wheat cultivar 'Chinese Spring' (CS) (Cabrera et al. 2002, Pedersen and Langridge 1997) in which all chromosomes of A, B and D subgenomes were labeled with specific signals, with the signals on the B- and D-genome chromosomes being particularly abundant. Through combined McGISH and FISH analysis, the positions and characteristics of chromosomal structural variations in such materials can be revealed.

In this study, the chromosomal constitution and chromosomal variations in three *Trititrigia* octoploids (TE261-1, TE266-1 and TE346-1) exhibiting good resistance to stripe rust, powdery mildew and aphids were investigated. The genetic characteristics, types of chromosome variation of the three *Trititrigia* octoploids were revealed using GISH, FISH, McGISH and molecular markers.

Results

Chromosomal constitution of the three *Trititrigia* octoploids

The root tip chromosome analysis showed that chromosome number of TE261-1, TE266-1 and TE346-1 was $2n = 56$. Observation of meiotic chromosomes in PMCs revealed that most chromosomes in the observed cells formed 28 bivalents at meiotic metaphase I, 14 alien chromosomes formed 7 bivalents were observed in GISH analysis, indicating high cytological stability (Supplemental Fig. 1, Supplemental Table 1).

GISH, FISH and McGISH were used to identify the chromosomal constitution of TE261-1, TE266-1 and TE346-1. The GISH (Fig. 1-A1) and FISH (Fig. 1-A2) results indicated that TE261-1 contained 42 wheat chromosomes and 14 *Th. intermedium* chromosomes (Fig. 2), including one pair of **St**-genome chromosomes that were completely labeled with probe signals, one pair of **J**-genome chromosomes labeled only in the telomeres, three pairs of **J^S**-genome chromosomes with obvious labeling in centromere areas, one pair of acrocentric chromosomes from the **J^S** genome, and one pair of **J-St** translocated chromosomes. The **J^S** acrocentric chromosomes (Fig. 2), were first discovered in the TE series of *Trititrigia* octoploids.

The results revealed that TE266-1 also contained 42 wheat chromosomes and 14 *Th. intermedium* chromosomes (Fig. 1-B1, B2, B3 and Fig. 2), including two pairs of **St**-genome chromosomes, one pair of **J**-genome chromosomes, three pairs of **J^S**-genome chromosomes, and one pair of **J-St** translocated chromosomes. As shown in Fig. 1-C1, C2, C3 and Fig. 2, TE346-1 also contained 42 wheat chromosomes and 14 *Th. intermedium* chromosomes, including one pair of **St**-genome chromosomes, one pair of **J**-genome chromosomes, four pairs of **J^S**-genome chromosomes and one pair of **J-St** translocated chromosomes.

Variation in the wheat chromosomes in the three *Trititrigia* octoploids

The wheat chromosome variations in the three *Trititrigia* octoploids were detected based on FISH and McGISH patterns and were compared with their common wheat parent YN15. For the **A**-genome chromosomes, the hybridization signals of pAs1 are not visible on **1A** in TE261-1, TE266-1 and TE346-1 (Fig. 3D). An absence of (GAA)₈ signals on **6AL** and additional pAs1 signals at the **6AS** subtelomeric position were observed in TE261-1 and TE346-1, while only the absence of (GAA)₈ signals on **6AL** was observed in TE266-1 (Fig. 3D).

Compared with YN15, the signals on the **6B** chromosome in TE261-1, TE266-1 and TE346-1 also changed (Fig. 3A-D). Interspersed green signals for the pAs1 probe were detected on **6BS** of TE261-1 and TE266-1, and the length of the **6BS** chromosomes appeared to be extended in TE266-1 and TE346-1. Faint red (GAA)₈ signals were also observed on the **6BL** chromosomes in the three *Trititrigia* octoploids.

The signals of the **D**-genome chromosomes in TE261-1, TE266-1 and TE346-1 appeared to vary significantly. FISH and McGISH results indicated that in TE266-1, the **2D** chromosomes were replaced with one pair of **A-D** translocated chromosomes; the main body of the **A-D** translocated chromosomes was derived from **2A** chromosomes according to the pAs1 signals, while part of the long arm was replaced with a **2D** chromosome segment (Fig. 3-B, 3-D).

The FISH results showed that the original strong pAs1 signals on **7DS** or **7DL** in YN15 were replaced with faint (GAA)₈ and pAs1 signals in TE261-1, TE266-1 and TE346-1. The McGISH results indicated that the partial long arm of the **7D** chromosome in TE261-1 was replaced with the chromosome segment of the **A**-genome chromosome (Fig. 1-A3, Fig. 3-D). However, the translocated **A**-genome chromosome segment was located on the short arm in TE266-1 and TE346-1, not the long arm as in TE261-1. The source of the **A**-genome chromosome segment was uncertain due to the lack of a specific FISH signal in this **A**-genome chromosome segment.

Specific molecular markers of the wheat chromosomes were employed to verify the chromosomal variation and detect the variation type in the three *Trititrigia* octoploids. The specific band (~ 300 bp) of YN15 amplified with the specific marker *Xmag3124*, which was located on **1A**, was not observed in the three *Trititrigia* octoploids (Fig. 4). The specific band (~ 150 bp) of YN15 detected by marker *GPW4344*, which was located on **6A** (Supplemental Fig. 4), were also absent in the three *Trititrigia* octoploids, and a **6A** specific band (~ 300 bp) absent in TE346-1 were detected by *GPW7465*. The **6B** specific band (~ 200 bp) detected by the marker *Xgwm219* showed a 10 bp insertion in three *Trititrigia* octoploids (Supplemental Fig. 4). Two specific bands (240, 200 bp) absent in three *Trititrigia* octoploids were detected by *Barc053* and *Barc172* located on the **7D** chromosomes, (Fig. 4 and Supplemental Fig. 4), respectively. The results for several specific markers, such as *Xwmc245*, *Xgdm35*, *Xgdm77*, *Xgdm107*, *Barc095*, *Barc168* and *Ppd-D1*, located on the **2D** chromosomes (Fig. 4 and Supplemental Fig. 4) demonstrated great variation, i.e., the absent of specific bands in the **2D** chromosomes in TE266-1, but the **2D** chromosomes of TE261-1 and TE346-1 remained unchanged.

The sequences of the primers used for these 16 chromosome-specific markers were employed to run local BLAST searches against the database of the *T. aestivum* (CS) whole-genome sequence. Eleven markers were screened according to screening criteria including the appropriate chromosomes, initial position, terminal position and degree of strict matching (Table 1). For example, the chromosomes mapped by BLAST should be matched the specific chromosomes detected by PCR; the higher matching degree means the better option, and the 3' of primer must be matched; the absolute value of margin between forward primer initial position and reverse primer terminal, should match the size of specific bands; and last, the appropriate position mapped by the makers were picked out. Compared BLAST with PCR results after screening, the size of specific bands amplified with majority makers were similar, but the **7D** specific band (~ 240 bp) detected by *Barc053* was different from BLAST result (295 bp), and difference exist in *Ppd-D1* (R1) result of PCR (250 bp) and BLAST (413 bp). The difference could be attributed to the errors occurring during *Triticum aestivum* (CS) whole genome sequencing. The screened markers were then labeled at the specific location of the corresponding chromosomes using MapGene2Chromosomes v2, according to their physical position on the chromosomes (Fig. 5). The positions of the variations detected with markers were consistent with the FISH signals on the **6A**, **6BL**, **2D** and **7D** chromosomes, while the variation at the **1AL** telomere was detected with markers, rather than FISH signals.

Table 1
Sequence alignment results for the screened wheat chromosome-specific molecular markers

Molecular marker	Forward/reverse	Sequence (5'-3')	Chromosome	Initial position	Terminal position	Degree of matching
Xmag3124	F	ACCTAGCCAGCACATCATCC	chr1A	593287178	593287161	94.444
	R	CGAGAAAGTGAGGAGGTCCA	chr1A	593286873	593286855	94.737
GPW4344	F	CCTGCAAGGTTTCAATTCGT	chr6A	389958799	389958780	100
	R	TGAGGACCGTTGGTGTCAT	chr6A	389958694	389958676	100
GPW7465	F	GAGAAGCCATTAAAGCCGC	chr6A	489511748	489511730	100
	R	CAGCTTGGAGACGATCAGC	chr6A	489511527	489511509	100
Xgwm219	F	GATGAGCGACACCTAGCCTC	chr6B	674843297	674843316	100
	R	GGGGTCCGAGTCCACAAC	chr6B	674843460	674843497	100
wmc245	F	GCTCAGATCATCCACCAACTTC	chr2D	186776793	186776772	100
	R	AGATGCTCTGGGAGAGTCCTTA	chr2D	186776665	186776644	100
Xgdm35	F	CCTGCTCTGCCCTAGATACG	chr2D	13754194	13754175	100
	R	ATGTGAATGTGATGCATGCA	chr2D	13753556	13753537	100
Xgdm77	F	GACACACAATAGCCAAAGCA	chr2D	11582137	11582118	100
	R	TGATGTCGGCACTATTTTGG	chr2D	11582020	11582001	100
BARC095	F	GGGGTGTGGTTGTTTGTAAGG	chr2D	14032404	14032424	100
	R	TGCGAATTCTATATACGATCTTGAGC	chr2D	14032568	14032593	100
Ppd-D1	F	ACGCCTCCCACTACACTG	chr2D	33957798	33957781	100
	R1	GTTGGTTCAAACAGAGAGC	chr2D	33957403	33957385	100
	R2	CACTGGTGGTAGCTGAGATT	chr2D	33955441	33955422	100
BARC053	F	GCGTCGTTCCCTTTGCTTGTACCAGTA	chr7D	613146874	613146852	100
	R	GCGCGTCCTTCCAATGCAGAGTAGA	chr7D	613146600	613146579	100
BARC172	F	GCGAAATGTGATGGGGTTTATCTA	chr7D	511064337	511064317	100
	R	GCGATTTGATTTAACTTTAGCAGTGAG	chr7D	511064181	511064158	100

Phenotypic evaluation of the three *Trititrigia octoploids*

The reactions to stripe rust, powdery mildew and aphids in TE261-1, TE266-1 and TE346-1 were evaluated at the seedling and adult plant stages using the wheat parents YN15 and *Th. intermedium* as controls (Table 2). At the seedling stage, TE261-1, TE266-1, and TE346-1 were immune to the stripe rust race CYR32 and resistant to the powdery mildew race E09. At the adult stage, all three *Trititrigia octoploids* showed good resistance to stripe rust and powdery mildew in the field. TE261-1 and TE346-1 also showed moderate resistance to aphids at the adult stage, while TE266-1 and YN15 were susceptible. As the common wheat parent YN15 is susceptible to stripe rust, powdery mildew and aphids, whereas *Th. intermedium* is immune to all three, we deduce that the resistance of *Trititrigia* is derived from *Th. intermedium*.

Table 2
Evaluation of stripe rust, powdery mildew and aphid resistances in TE261-1, TE266-1 and TE346-1

Materials	Seedling stage		Adult plant stage		
	Stripe rust CYR32	Powdery mildew E09	Stripe rust	Powdery mildew	Aphid
<i>Th. intermedium</i>	0	0	0	0	0;
YN15	4	4	4	4	5
TE261-1	0	2	0;	0;	2
TE266-1	0	1	0;	0;	4
TE346-1	0	1	0;	0;	3

Discussion

Th. intermedium is a valuable perennial species of Triticeae for wheat improvement due to its resistance to a number of wheat diseases and pests as well as its stress tolerance and high crossability with various *Triticum* species (Gupta 2016, Han et al. 2003, Jiang et al. 1993, Li and Wang 2009, Wang 2011). Partial wheat-*Th. intermedium* amphiploids showing high cross-compatibility with wheat are desirable 'bridge' materials for transferring desirable genes from *Th. intermedium* to common wheat (Kruppa et al. 2016, Wang and Wang 2016).

Chromosome counting in metaphase spreads revealed that the chromosome number of these three octoploids of *Trititrigia* was consistently $2n = 56$. Molecular cytogenetic analysis then revealed that the three *Trititrigia* accessions all carry 42 wheat chromosomes and 14 *Th. intermedium* chromosomes and that the 14 alien (*Th. intermedium*) chromosomes are composed of a mixed genome consisting of **J**-, **J^S**- and **St**-genome chromosomes rather than a single **J**, **J^S** or **St** genome, similar to other reported of *Trititrigia* octoploids (Han et al. 2004, Bao et al. 2014, Yu et al. 2019). The mechanism underlying the formation of the mixed genome is uncertain, and further research will contribute to elucidating the homologous relationship and integrality between the **J**, **J^S** and **St** genomes and the origin and evolution of *Th. intermedium*.

Two types of structural variation in the alien chromosomes were detected: **J-St** translocated chromosomes, which were present in all three *Trititrigia* octoploids, and **J^S** acrocentric chromosomes in TE261-1 (Fig. 2), which might have formed via the centromere breakage of one pair of **J^S**-genome chromosomes. **J-St** translocated chromosomes also exist in many TE series of *Trititrigia* octoploids (Bao et al. 2009, Bao et al. 2014, Qi et al. 2017), but **J^S** acrocentric chromosomes were first discovered in the TE series of *Trititrigia* octoploids. The formation of these two types of variations might occur through chromosome breakage and fusion when F_1 hybrids obtained through distant hybridization are backcrossed with hexaploid wheat (Guo et al. 2015).

Although the alien chromosomal constitution of three *Trititrigia* octoploids was different, some of the alien chromosomes were probably identical according to the GISH and FISH signals. For example, the **J**-genome chromosomes with the satellite sequence and the **J-St** translocated chromosomes in these three *Trititrigia* octoploids were similar. The **St** chromosomes of TE261-1 were also similar to the **St-1** chromosomes of TE266-1, and the **St** chromosomes of TE346-1 were similar to the **St-2** chromosomes of TE266-1 (Fig. 2).

In addition to the structural variation in the alien chromosomes, structural variations, such as the variations detected in the **1A**, **6A**, **6B**, **2D** and **7D** chromosomes, occurred in the wheat chromosomes as well. The results indicated that during the formation of partial amphiploids, the wheat chromosomes underwent various types of chromosomal recombination due to chromosome segments introgressed from *Th. intermedium* into the common wheat chromosomes, although the introgressed segments were too small to detect via GISH. The structural variations may have been generated via recombination between different wheat chromosomes, such as homoeologous chromosomal recombination between **A**-, **B**-, and **D**-genome chromosomes, influenced by the *Th. intermedium* chromosomes. The variation in chromosomal patterns of repetitive sequences (e.g. chromosome **6B**) could be also caused by repetitive sequence reorganization (amplification/loss). After hybridization, genomes can undergo genetic and epigenetic changes (genomic shock) which can reorganize their structure.

Similar chromosomal variations were detected in the **1A** and **6A** chromosomes of the three *Trititrigia* and might have been derived from the same origin in the earlier generations of backcrosses. Additionally, there were distinct variations in the **6B**, **2D** and **7D** chromosomes

of these *Trititrigia* octoploids, such as pAs1 signals at different positions of 6BS in TE266-1 and TE346-1 and the 2A-2D translocation in TE266-1. The variations in the 7D chromosomes of the three *Trititrigia* octoploids were also different. McGISH indicated that the partial 7DS chromosomes of TE266-1 and TE346-1 were replaced with an A-genome chromosome segment, but the corresponding position in TE261-1 was on the 7DL chromosome. The original position replaced in 7DL or 7DS was difficult to discern via FISH because the signals on the two arms of the 7D chromosomes were similar. The results of molecular marker analyses also supported the results of FISH and McGISH in TE261-1. Additional work will be needed to obtain strong evidence indicating how these chromosomal recombinations occurred as well as the effect of these chromosomal variations.

The drastic and abundant chromosome variations were detected in these three *Trititrigia* octoploids, a kind of generic hybrid between wheat and *Th. intermedium*. However, we guess the more drastic variations might exist in early-generation of *Trititrigia* octoploids, and then backcrosses with YN15 resulted in reducing variability and increasing stability. We hypothesized that *Th. intermedium*, as a natural generic hybrid, possessed drastic chromosomal variation causing difficulty to confirm the progenitor diploid species.

Phenotypic evaluation indicated the successful transfer of stripe rust and powdery mildew resistance from *Th. intermedium* to these three *Trititrigia* octoploids and that TE261-1 also exhibited moderate resistance to aphids. In addition, the chromosomal constitution of TE261-1, TE266-1 and TE346-1 is different from that of other TE series of *Trititrigia* octoploids reported previously (Bao et al. 2009, Bao et al. 2014, Qi et al. 2017). Therefore, these accessions could be employed as bridge parents for crossing with wheat to develop addition, substitution or translocation lines to generate new rust-, powdery mildew- and aphid-resistant germplasms for wheat breeding.

Conclusions

Three octoploid *Trititrigia* accessions (TE261-1, TE266-1, and TE346-1) with good resistances to stripe rust, powdery mildew and aphids were characterized by GISH, FISH, McGISH and molecular marker analyses. Molecular cytogenetic analysis revealed that three *Trititrigia* accessions all carry 42 wheat chromosomes and 14 *Th. intermedium* chromosomes, the 14 alien (*Th. intermedium*) chromosomes are composed of a mixed genome consisting of J-, J^S- and St-genome chromosomes rather than a single J, J^S or St genome. Different structural variations occurred in the wheat chromosomes, such as the variations detected in the 1A, 6A, 6B, 2D and 7D chromosomes as well. Results indicated that the wheat-*Th. intermedium* partial amphiploids possess 14 alien chromosomes which belong to a mixed genome consisting of J-, J^S- and St- chromosomes, and 42 wheat chromosomes with different structural variations.

Methods

Plant materials

Three *Trititrigia* octoploid accessions (TE261-1, TE266-1, and TE346-1) were selected from BC₁F₈ of common wheat Yannong15 crossed with *Th. intermedium*, and selfed for some generations. *Th. intermedium* from the original accession at the Northwest Institute of Botany, Chinese Academy of Sciences, was provided by the academician Li ZS; *Pseudoroegneria strigosa* was provided by the researcher Li LH from the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences; *Triticum urartu* Thum. ex Gandil. (2n = 2x = 14, AA); *Aegilops speltoides* Tausch. (2n = 2x = 14, BB); *Aegilops tauschii* Coss. (2n = 2x = 14, DD); the CS nulli-tetrasomic accessions N1AT1B, N6AT6B, N2DT2A, N7DT7A; and the common wheat variety YN15 were also used in this study.

DNA extraction, PCR amplification and marker analysis

The CTAB method was used to extract total genomic DNA from the tender leaves (Li 2001) of *Pseudoroegneria strigosa* (St genome), *Triticum urartu* (A genome), *Aegilops speltoides* (B genome) and *Aegilops tauschii* (D genome). In total, 176 pairs of SSR primers (Röder et al. 1998, Song et al. 2005) (<https://wheat.pw.usda.gov>) were used to identify chromosomal variations. PCR amplification was performed according to Beales et al. (2007), and marker screening was conducted by means of polyacrylamide gel electrophoresis (PAGE). The selected markers were analyzed with BLAST (Basic Local Alignment Search Tool) based on the database containing the *Triticum aestivum* (CS) whole genome sequence (IWGSC RefSeq v1.0, <https://wheat-urgi.versailles.inra.fr/morgoth/Seq-Repository/BLAST>) and then labeled on the corresponding chromosomes using MapGene2Chromosomes v2 (http://mg2c.iask.in/mg2c_v2.0/).

Chromosome slide preparation, meiotic preparation, GISH, FISH and McGISH

Fresh root tips were collected from germinating seeds treated with nitrous oxide (N₂O) for 2 h (Kato 2009) and then immersed in 90% glacial acetic acid to fix cell division. The preparation of root tip cell chromosome slides and analysis was performed referring to the

method of Han (Han et al. 2004). When the flag leaf of wheat was spread, young spikes were sampled and anthers at metaphase I (MI) of meiosis were fixed in Carnoy's solution. The meiotic chromosomes were prepared from pollen mother cells (PMCs) by knocking and pressing anthers in 45% acetic acid, after which the chromosome configuration in PMC metaphase I (MI) was observed.

St-genome DNA and **D**-genome DNA were labeled with Texas-Red-5-dCTP. The clone pAs1 (GenBank: D30736.1) is a repetitive DNA sequence (1015 bp) from *Aegilops tauschii* (Nagaki et al. 1995, Rayburn and Gill 1986b). The pAs1 clone and **A**-genome DNA were labeled with fluorescein-12-dUTP using the nick translation method. Oligonucleotides (GAA)₈ with 5' TAMRA were synthesized by Sangon Biotech (Shanghai, China). The procedures for GISH, FISH and McGISH; signal detection; and image collection were done according to Han et al. (2009) and He et al. (2017).

The reaction volume for *in situ* hybridization was 10 µL per slide. GISH was conducted using a probe of 50 ng **St**-genome DNA, with a block of 6,000 ng YN15 DNA; FISH was performed with 5 ng (GAA)₈ and 200 ng pAs1; and McGISH was carried out with probes of 50 ng **A**-genome DNA and 100 ng **D**-genome DNA and a block of 8,000 ng **B**-genome DNA diluted to the desired total volume with ddH₂O. The *in situ* hybridization program consisted of denaturation at 95 °C for 5 min and hybridization at 55 °C for 6 h. The fluorescent signals of FITC (Excitation Filter EX465-495, Dichroic Mirror DM505, Barrier Filter BA515-555), TRITC (Excitation Filter EX540/25, Dichroic Mirror DM565, Barrier Filter BA605/55) and DAPI (Excitation Filter EX340-380, Dichroic Mirror DM400, Barrier Filter BA435-485) were detected, and images were collected using a NIKON eclipse Ni-U fluorescence microscope; the images were processed using NIS-Elements BR 4.00.12 software. After rinsing with 2X SSC solution, the slides were used for *in situ* hybridization again.

Evaluation of resistance to powdery mildew, stripe rust and aphids

The powdery mildew and stripe rust resistance of plants in the seedling stage were tested in a plant incubator. The wheat variety Huixianhong was employed for inoculation of the *Blumeria graminis* f. sp. *tritici* (*Bgt*) pathotype E09 and the *Puccinia striiformis* f. sp. *tritici* (*Pst*) pathotype CYR32. Thirty seeds of the test materials were grown in seedling plates and placed in a plant incubator under 12 h light (25,000 lx) at 25 °C and 12 h of darkness at 17 °C, with 70% relative humidity. Huixianhong and YN15 were used as controls. Inoculation with E09 and CYR32 was achieved by smearing at the one-leaf stage, and the gradient of infection was investigated when Huixianhong had been completely infected; these experiments were repeated three times. The powdery mildew and stripe rust resistances of adult-stage plants were tested via natural infection in an open field. The resistance evaluation criteria for powdery mildew and stripe rust followed the methods of Sheng (1988) and Li et al. (2006). In the scale of powdery mildew resistance, 0 is immune; 0 is nearly immune with necrotic flecks; 1 is highly resistant with small lesions (< 1 mm) and thin mycelium; 2 is moderately resistant with small lesions (< 1 mm) and a slightly thick mycelium; 3 is moderately susceptible with lesions (> 1 mm) and a thick mycelium; and 4 is highly susceptible with lesions (> 1 mm) and a consecutive thick mycelium. In the scale of stripe rust resistance, 0 is immune; 0 is nearly immune with tiny flecks; 1 is highly resistant with yellowish-white flecks; 2 is moderately resistant with necrotic plaques around small spore stripes; 3 is moderately susceptible with fading around spore stripes; and 4 is highly susceptible with large spore stripes.

For the identification of aphid resistance, natural aphids were employed, and the evaluation was performed during the wheat grain-filling stage. The test materials were sown on two 1.4-meter-long rows with YN15 as CK. The investigation of aphid injury and the classification criteria for aphid resistance followed the method of Liu et al. (2006). The number of aphids on 10 seriously aphid-damaged stems was determined for all materials; then, using this number as the numerator and the average number of aphids on YN15, CS and Lumai5 as the denominator, the ratio was computed as the evaluation criterion: 0 is immune with no aphids; 1 is highly resistant with a ratio between 0.01 and 0.30; 2 is moderately resistant with a ratio between 0.31 and 0.6; 3 is slightly resistant with a ratio between 0.61 and 0.90; 4 is slightly susceptible with a ratio between 0.91 and 1.2; 5 is moderately susceptible with a ratio between 1.21 and 1.5; and 6 is highly susceptible, with a ratio > 1.5.

Abbreviations

CS

Chinese Spring

FISH

Fluorescence *in situ* hybridization

GISH

Genomic *in situ* hybridization

McGISH

Multicolor GISH

YN15

Declarations

Ethics approval and consent to participate

There is no ethics approval and consent to participate in this manuscript.

Consent for publication:

Not applicable.

Availability of data and materials:

The datasets supporting the conclusions of this article are included within the article and its additional files.

Competing interests:

The authors declare that they have no conflict of interests.

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Author Contributions:

YC, PX performed the experiments, data analysis and manuscript writing. XQ, YB, HW participated in some experiments and data analysis. RRCW helped in the manuscript preparation and data analysis. XL designed the project and contributed to the writing of the manuscript and approved the final manuscript.

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References

1. Banks P, Larkin P, Bariana H, Lagudah E, Appels R, Waterhouse P, Brettell R, Chen X, Xu HJ, Xin ZY. The use of cell culture for subchromosomal introgressions of barley yellow dwarf virus resistance from *Thinopyrum intermedium* to wheat. *Genome*. 1995;38:395–405. <https://doi.org/10.1139/g95-051>.
2. Bao Y, Li X, Liu S, Cui F, Wang H. Molecular cytogenetic characterization of a new wheat-*Thinopyrum intermedium* partial amphiploid resistant to powdery mildew and stripe rust. *Cytogenet Genome Res*. 2009;126:390–5. <https://doi.org/10.1159/000266169>.
3. Beales J, Turner A, Griffiths S, Snape JW, Laurie DA. A Pseudo-Response Regulator is misexpressed in the photoperiod insensitive *Ppd-D1a* mutant of wheat (*Triticum aestivum* L.). *Theor Appl Genet*. 2007;115:721–33. <https://doi.org/10.1007/s00122-007-0603-4>.
4. Cabrera A, Martin A, Barro F. (2002) *In-situ* comparative mapping (ISCM) of Glu-1 loci in *Triticum* and *Hordeum*. *Chromosome Res*. 10: 49–54. <https://doi.org/10.1023/A:1014270227360>.
5. Cai X, Jones S, Murray T, Weber W. Molecular cytogenetic characterization of *Thinopyrum* genomes conferring perennial growth habit in wheat-*Thinopyrum* amphiploids. *Plant Breeding*. 2001;120:21–6. <https://doi.org/10.1046/j.1439-0523.2001.00560.x>.
6. Cauderon Y, Saigne B, Bonhomme H. (1966) Étude cytogénétique de l'évolution du matériel issu de croisement entre *Triticum aestivum* et *Agropyron intermedium*: Création de types d'addition stables. Institut national de la recherche agronomique.
7. Chen Q, Conner RL, Laroche A, Thomas JB. Genome analysis of *Thinopyrum intermedium* and *Thinopyrum ponticum* using genomic in situ hybridization. *Genome*. 1998;41:580–6.

8. Chen Q, Friebe B, Conner R, Laroche A, Thomas J, Gill B. Molecular cytogenetic characterization of *Thinopyrum intermedium*-derived wheat germplasm specifying resistance to wheat streak mosaic virus. *Theor Appl Genet.* 1998;96:1–7. <https://doi.org/10.1139/g98-055>.
9. Chen Q, Conner RL, Li H, Sun S, Ahmad F, Laroche A, Graf R. Molecular cytogenetic discrimination and reaction to wheat streak mosaic virus and the wheat curl mite in Zhong series of wheat *Thinopyrum intermedium* partial amphiploids. *Genome.* 2003;46:135–45. <https://doi.org/10.1139/g02-109>.
10. Cui Y, Zhang YP, Qi J, Wang HG, Wang RRC, Bao YG, Li XF. Identification of chromosomes in *Thinopyrum intermedium* and wheat-*Th. intermedium* amphiploids based on multiplex oligonucleotide probes. *Genome.* 61(7): 515–521. <https://doi.org/10.1139/gen-2018-0019>.
11. Du P, Zhuang L, Wang Y, Yuan L, Wang Q, Wang D, Dawadondup, Tan L, Shen J, Xu H, Zhao H, Chu CG, Qi ZJ. (2017) Development of oligonucleotides and multiplex probes for quick and accurate identification of wheat and *Thinopyrum bessarabicum* chromosomes. *Genome* 60: 93–103. <https://doi.org/10.1139/gen-2016-0095>.
12. Friebe B, Zeller FJ, Mukai Y, Forster BP, Bartos P, McIntosh RA. Characterization of rust-resistant wheat- *Agropyron intermedium* derivatives by C-banding, in situ hybridization and isozyme analysis. *Theor Appl Genet.* 1992;83:775. <https://doi.org/10.1007/BF00226697>.
13. Friebe B, Jiang J, Raupp WJ, McIntosh RA, Gill BS. Characterization of wheat-alien translocations conferring resistance to diseases and pests: current status. *Euphytica.* 1996;91:59–87. <https://doi.org/10.1007/BF00035277>.
14. Guo X, Shi Q, Wang J, Hou Y, Wang Y, Han F. Characterization and genome changes of new amphiploids from wheat wide hybridization. *J Genet Genomics.* 2015;42:459–61. <https://doi.org/10.1016/j.jgg.2015.06.006>.
15. Gupta P. (2016) Use of alien genetic variation for wheat improvement. Springer International Publishing.
16. Han F, Fedak G, Benabdelmouna A, Armstrong K, Ouellet T. Characterization of six wheat x *Thinopyrum intermedium* derivatives by GISH, RFLP, and multicolor GISH. *Genome.* 2003;46:490. <https://doi.org/10.1139/g03-032>.
17. Han F, Liu B, Fedak G, Liu Z. Genomic constitution and variation in five partial amphiploids of wheat-*Thinopyrum intermedium* as revealed by GISH, multicolor GISH and seed storage protein analysis. *Theor Appl Genet.* 2004;109:1070–6. <https://doi.org/10.1007/s00122-004-1720-y>.
18. Han F, Gao Z, Birchler JA. Reactivation of an inactive centromere reveals epigenetic and structural components for centromere specification in maize. *Plant Cell.* 2009;21:1929–39. <https://doi.org/10.1105/tpc.109.066662>.
19. He F, Xing P, Bao Y, Ren M, Liu S, Wang Y, Li X, Wang H. (2017) Chromosome pairing in hybrid progeny between *Triticum aestivum* and *Elytrigia elongata*. *Front Plant Sci* 8. <https://doi.org/10.3389/fpls.2017.02161>.
20. Hu L, Li G, Zhan H, Liu C, Yang Z. New St-chromosome-specific molecular markers for identifying wheat-*Thinopyrum intermedium* derivative lines. *J Genet.* 2014;93:69–74.
21. Ji W, Xue X, Wang Q. GISH analysis of *Thinopyrum intermedium*. *Acta Bot Boreal-Occident Sin.* 2001;21:401–5.
22. Jiang J, Friebe B, Gill BS. Recent advances in alien gene transfer in wheat. *Euphytica.* 1993;73:199–212. <https://doi.org/10.1007/BF00036700>.
23. Joppa LR. (1987) Aneuploid analysis in tetraploid wheat. *Wheat Wheat Improvement:* 255–67. <https://doi.org/10.2134/agronmonogr13.2ed.c11>.
24. Kato A. Air drying method using nitrous oxide for chromosome counting in maize. *Biotech Histochem.* 2009;74:160–6. <https://doi.org/10.3109/10520299909047968>.
25. Komuro S, Endo R, Shikata K, Kato A. Genomic and chromosomal distribution patterns of various repeated DNA sequences in wheat revealed by a fluorescence *in situ* hybridization procedure. *Genome.* 2013;56:131–7. <https://doi.org/10.1139/gen-2013-0003>.
26. Kruppa K, Turkosi E, Mayer M, Toth V, Vida G, Szakacs E, Molnar-Lang M. McGISH identification and phenotypic description of leaf rust and yellow rust resistant partial amphiploids originating from a wheat x *Thinopyrum* synthetic hybrid cross. *J Appl Genet.* 2016;57:427–37. <https://doi.org/10.1007/s13353-016-0343-8>.
27. Le H, Armstrong K, Miki B. Detection of rye DNA in wheat-rye hybrids and wheat translocation stocks using total genomic DNA as a probe. *Plant Mol Biol Rep.* 1989;7:150–8.
28. Li G, Quiros CF. Sequence-related amplified poly-morphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. *Theor Appl Genet.* 2001;103:445–61. <https://doi.org/10.1007/s001220100570>.

29. Li H, Wang X. *Thinopyrum ponticum* and *Th. intermedium*: the promising source of resistance to fungal and viral diseases of wheat. J Genet Genomics. 2009;36:557–65. [https://doi.org/10.1016/S1673-8527\(08\)60147-2](https://doi.org/10.1016/S1673-8527(08)60147-2).
30. Li Z, Zheng T, He Z, Li G, Xu S, Li X, Yang G, Singh R, Xia X. Molecular tagging of stripe rust resistance gene YrZH84 in Chinese wheat line Zhou 8425B. Theor Appl Genet. 2006;112:1098–103. <https://doi.org/10.1007/s00122-006-0211-8>.
31. Lin Z, Huang D, Du L, Ye X, Xin Z. Identification of wheat–*Thinopyrum intermedium* 2Ai-2 ditelosomic addition and substitution lines with resistance to barley yellow dwarf virus. Plant Breeding. 2010;125:114–9. <https://doi.org/10.1111/j.1439-0523.2006.01167.x>.
32. Liu J, Chang Z, Zhang X, Yang Z, Li X, Jia J, Zhan H, Guo H, Wang J. Putative *Thinopyrum intermedium*-derived stripe rust resistance gene Yr50 maps on wheat chromosome arm 4BL. Theor. Appl Genet. 2013;126:265. <https://doi.org/10.1007/s00122-012-1979-3>.
33. Liu S, Wang H, Zhang X, Li X, Li D, Duan X, Zhou Y. Molecular cytogenetic identification of a wheat-*Thinopyrum intermedium* (Host) Barkworth & DR Dewey partial amphiploid resistant to powdery mildew. J Integr Plant Biol. 2005;47:726–33. <https://doi.org/10.1111/j.1744-7909.2005.00051.x>.
34. Liu X, Wang Y, Sang L, Xiang J, Ji W. Relationship between morphological characters of wheat germplasm and their resistance to sitobion avenae (F.). J Trit Crops. 2006;26:24–8.
35. Mahelka V, Kopecky D, Pastova L. On the genome constitution and evolution of intermediate wheatgrass (*Thinopyrum intermedium*: Poaceae, Triticeae). BMC Evol Biol. 2011;11:127. <https://doi.org/10.1186/1471-2148-11-127>.
36. Mukai Y, Nakahara Y, Yamamoto M. Simultaneous discrimination of the three genomes in hexaploid wheat by multicolor fluorescence in situ hybridization using total genomic and highly repeated DNA probes. Genome. 1993;36:489–94. <https://doi.org/10.1139/g93-067>.
37. Nagaki K, Tsujimoto H, Isono K, Sasakuma T. Molecular characterization of a tandem repeat, Afa family, and its distribution among Triticeae. Genome. 1995;38:479. <https://doi.org/10.1139/g95-063>.
38. Pedersen C, Rasmussen S, Linde-Laursen I. Genome and chromosome identification in cultivated barley and related species of the Triticeae (Poaceae) by *in situ* hybridization with the GAA-satellite sequence. Genome. 1996;39:93–104. <https://doi.org/10.1139/g96-013>.
39. Pedersen C, Langridge P. Identification of the entire chromosome complement of bread wheat by two-colour FISH. Genome. 1997;40:589–93. <https://doi.org/10.1139/g97-077>.
40. Qi X, Bao Y, Li X, Qian Z, Wang R, Wu K, Wang H. Cytological identification and chromosome constitution analyses of ten Octoploid *Triticigia* accessions. Acta Agron Sin. 2017;43:967–73.
41. Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier M, Leroy P, Ganal MW. (1998) A microsatellite map of wheat. Genetics 149: 2007.
42. Rayburn A, Gill B. Molecular identification of the D-genome chromosomes of wheat. J Hered. 1986;77:253–5. <https://doi.org/10.1093/oxfordjournals.jhered.a110231>.
43. Rayburn A, Gill B. Isolation of a D-genome specific repeated DNA sequence from *Aegilops squarrosa*. Plant Mol Biol Rep. 1986;4:102–9.
44. Sharma H, Ohm H, Goulart L, Lister R, Appels R, Benlhabib O. Introgression and characterization of barley yellow dwarf virus resistance from *Thinopyrum intermedium* into wheat. Genome. 1995;38:406–13. <https://doi.org/10.1139/g95-052>.
45. Sheng B. Using infection type records the wheat powdery mildew at seedling stage. Plant Protectino. 1988;1:49.
46. Song QJ, Shi JR, Singh S, Fickus EW, Costa JM, Lewis J, Gill BS, Ward R, Cregan PB. Development and mapping of microsatellite (SSR) markers in wheat. Theor Appl Genet. 2005;110:550–60. <http://dx.doi.org/10.1007/s00122-004-1871-x>.
47. Sun S. Research on *Triticum Agropyron* form and species formation. Acta Agron Sin. 1980;6(1):1–14.
48. Tang S, Li Z, Jia X, Larkin PJ. Genomic *in situ* hybridization (GISH) analyses of *Thinopyrum intermedium*, its partial amphiploid Zhong 5, and disease-resistant derivatives in wheat. Theor Appl Genet. 2000;100:344–52.
49. Tang Z, Yang Z, Fu S. Oligonucleotides replacing the roles of repetitive sequences pAs1, pSc119.2, pTa-535, pTa71, CCS1, and pAWRC.1 for FISH analysis. J Appl Genet. 2014;55:313–8. <https://doi.org/10.1007/s13353-014-0215-z>.
50. Wang HG, Liu SB, Li XF, Gao JR, Feng DS, Chen DH. Breeding and identification of six octoploid *Triticigia*. J Trit Crops. 2006;26:6–10.

51. Wang RC, Zhang XY. Characterization of the translocated chromosome using fluorescence in situ hybridization and random amplified polymorphic DNA on two *Triticum aestivum*-*Thinopyrum intermedium* translocation lines resistant to wheat streak mosaic or barley yellow dwarf virus. *Chromosome Res.* 1996;4:583–7. <https://doi.org/10.1007/BF02261721>.
52. Wang RR, Larson SR, Jensen KB, Bushman BS, DeHaan LR, Wang S, Yan X. Genome evolution of *intermediate wheatgrass* as revealed by EST-SSR markers developed from its three progenitor diploid species. *Genome.* 2015;58:63–70. <https://doi.org/10.1139/gen-2014-0186>.
53. Wang RRC. (2011) *Agropyron* and *Psathyrostachys*. p. 77–108. https://doi.org/10.1007/978-3-642-14228-4_2.
54. Yu Z, Wang H, Xu Y, Li Y, Lang T, Yang Z, Li G. Characterization of chromosomal rearrangement in new wheat-*Thinopyrum intermedium* addition lines carrying *Thinopyrum*-specific grain hardness genes. *Agronomy.* 2019;9:18. <https://doi.org/10.3390/agronomy9010018>.
55. Wang Y, WangH. Characterization of three novel wheat-*Thinopyrum intermedium* addition lines with novel storage protein subunits and resistance to both powdery mildew and stripe rust. *J Genet Genomics.* 2016;43:45–8. <https://doi.org/10.1016/j.jgg.2015.10.004>.
56. Zhang X, Koul A, Petroski R, Ouellet T, Fedak G, Dong Y. Molecular verification and characterization of BYDV-resistant germ plasms derived from hybrids of wheat with *Thinopyrum ponticum* and *Th. intermedium*. *Theor Appl Genet.* 1996;93:1033–9. <https://doi.org/10.1007/BF00230121>.
57. Zheng Q, Luo Q, Niu Z, Li H, Li B, Xu SS, Li Z. Variation in chromosome constitution of the Xiaoyan series partial amphiploids and its relationship to stripe rust and stem rust resistance. *J Genet Genomics.* 2015;42:657–60. <https://doi.org/10.1016/j.jgg.2015.08.004>.

Figures

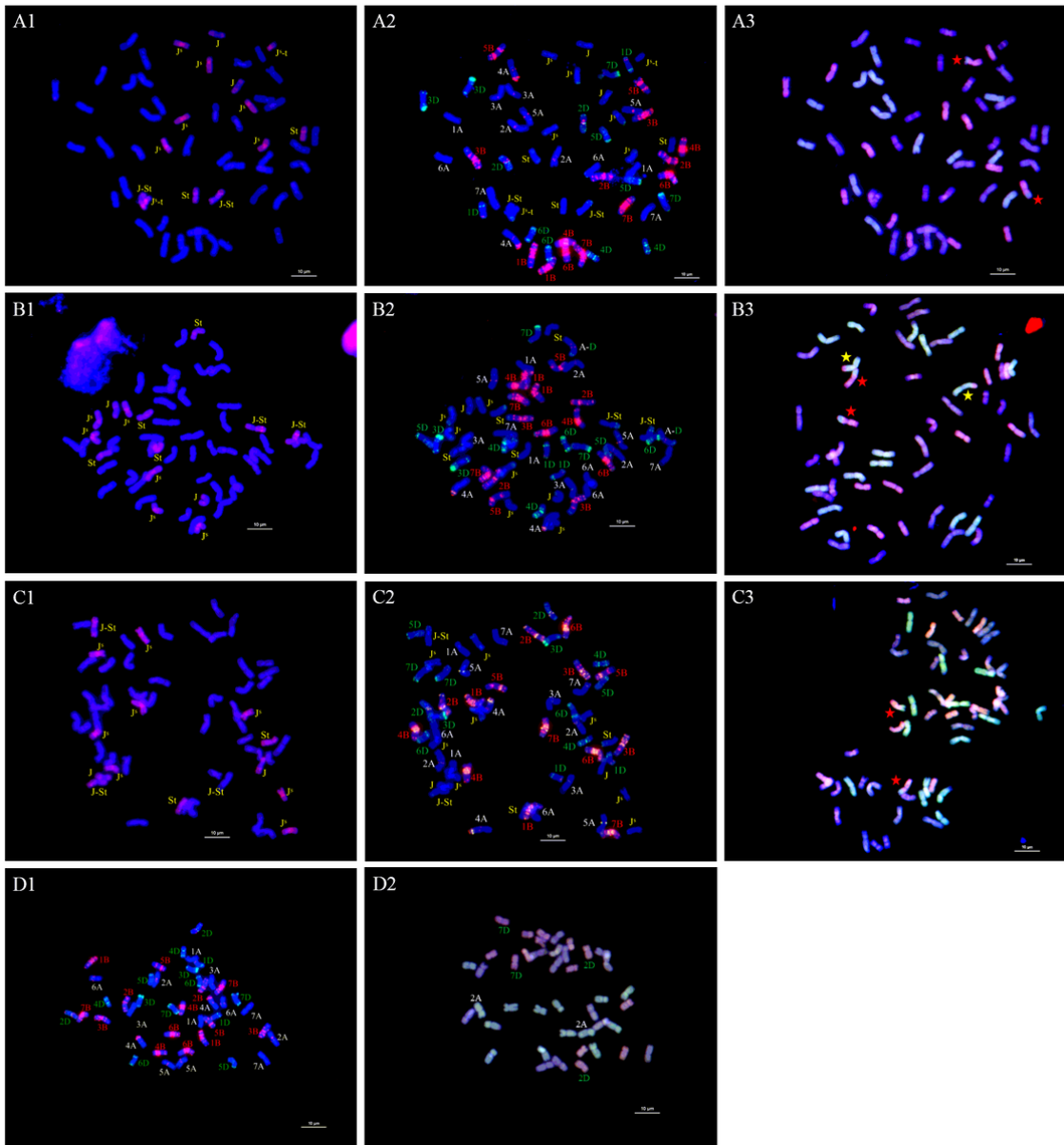


Figure 1

GISH, FISH and McGISH patterns of TE261-1, TE266-1, TE346-1 and YN15. GISH patterns of TE261-1 (A1), TE266-1 (B1) and TE346-1 (C1): St (*Ps. strigosa*)-genome DNA labeled with Texas-Red-5-dCTP was used as the probe, and YN15 genome DNA was employed as the block. FISH patterns of TE261-1 (A2), TE266-1 (B2), TE346-1 (C2) and YN15 (D1): red signals represent (GAA)₈ labeled with 5' TAMRA, and green signals represent pAs1 labeled with fluorescein-12-dUTP. McGISH patterns of TE261-1 (A3), TE266-1 (B3), TE346-1 (C3) and YN15 (D2): A genomes are labeled with fluorescein-12-dUTP; D genomes are labeled with Texas-Red-5-dCTP; and B-genome DNA (gray) is used for blocking. A-D (7D) translocation are indicated by red asterisks in McGISH patterns (A3, B3 and C3); A-D (2A) translocation are indicated by yellow asterisks in TE266-1 (B3); and 2A, 2D and 7D are marked in the McGISH patterns of YN15 (D2).

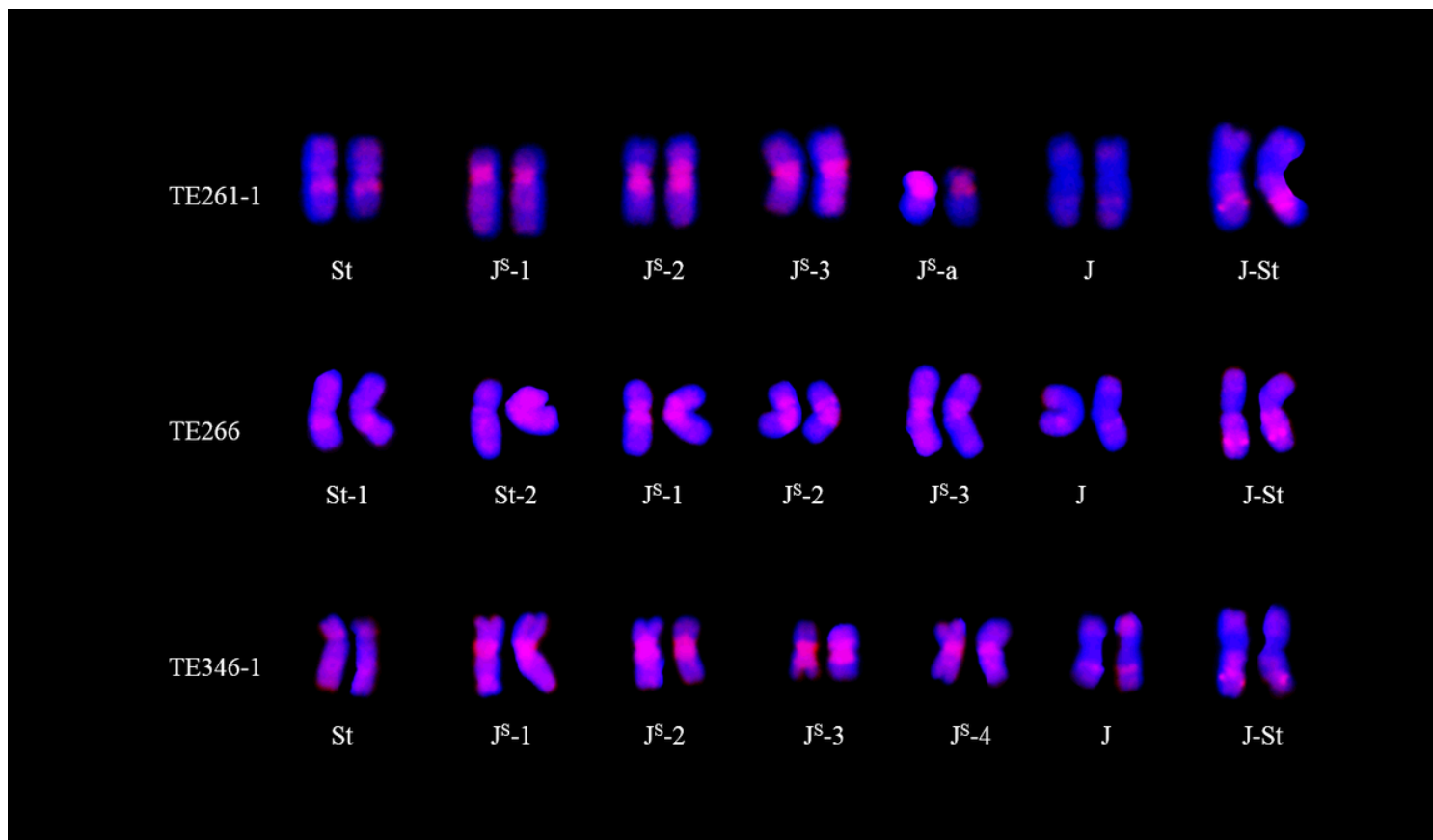


Figure 2

Alien chromosome GISH patterns of the three Trititrigia octoploids. St-genome DNA labeled with Texas-Red-5-dCTP was used as the probe, and YN15 genome DNA was employed as the block, JS-a represents acrocentric chromosomes from the JS genome.

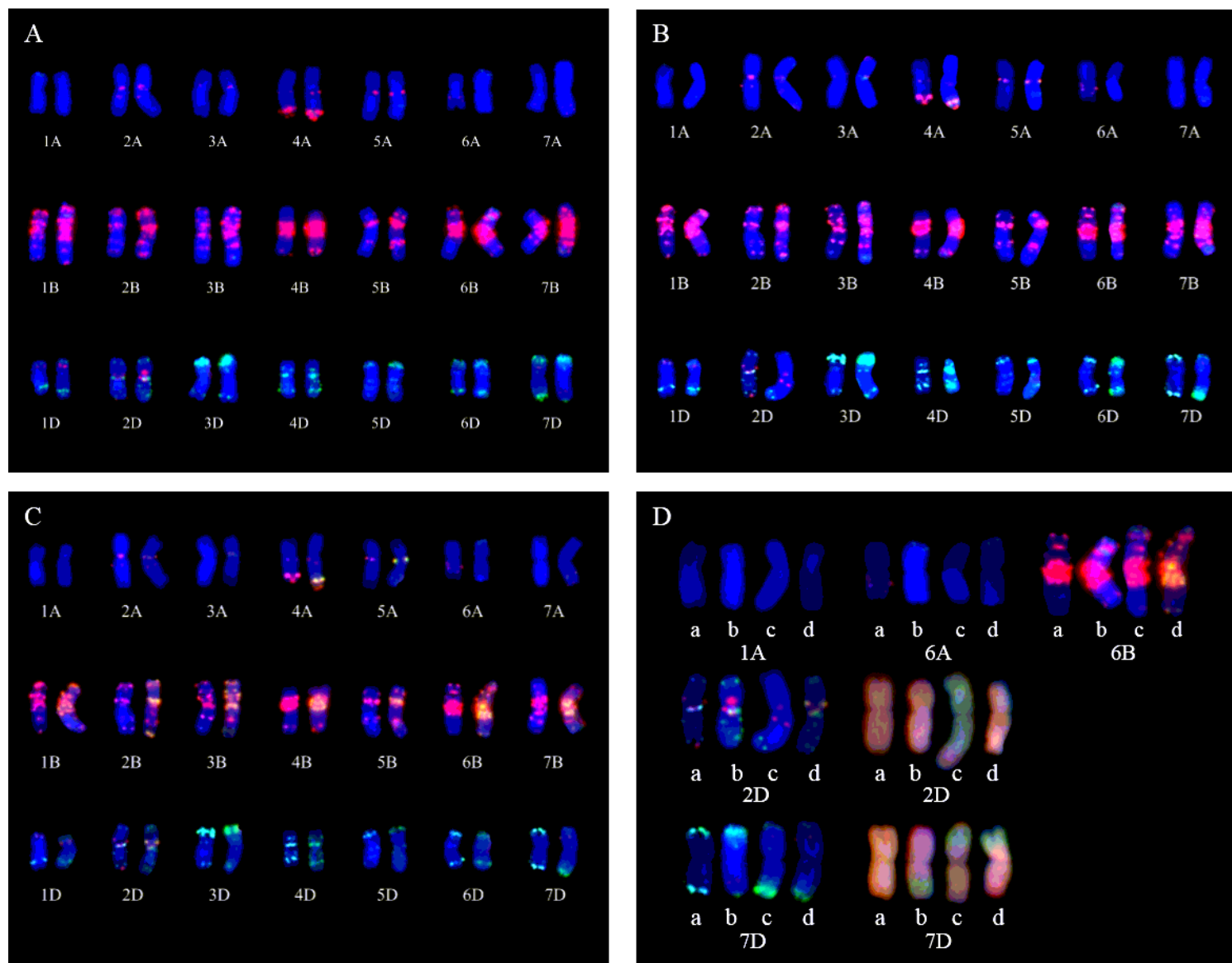


Figure 3

FISH patterns for comparison of YN15 with the three Trititrigia octoploids. In the FISH patterns comparing YN15 with TE261-1 (A), TE266-1 (B), and TE346-1 (C), the results for one pair of chromosomes for YN15 are on the left, and those for the Trititrigia octoploids are on the right. D: FISH and McGISH patterns for the comparison of YN15 with three Trititrigia octoploids on various chromosomes. The patterns in a, b, c and d indicate the corresponding chromosomes of YN15, TE261-1, TE266-1 and TE346-1, respectively. The lower right corner of D shows the 7 chromosome D McGISH patterns of YN15 and the three Trititrigia octoploids.

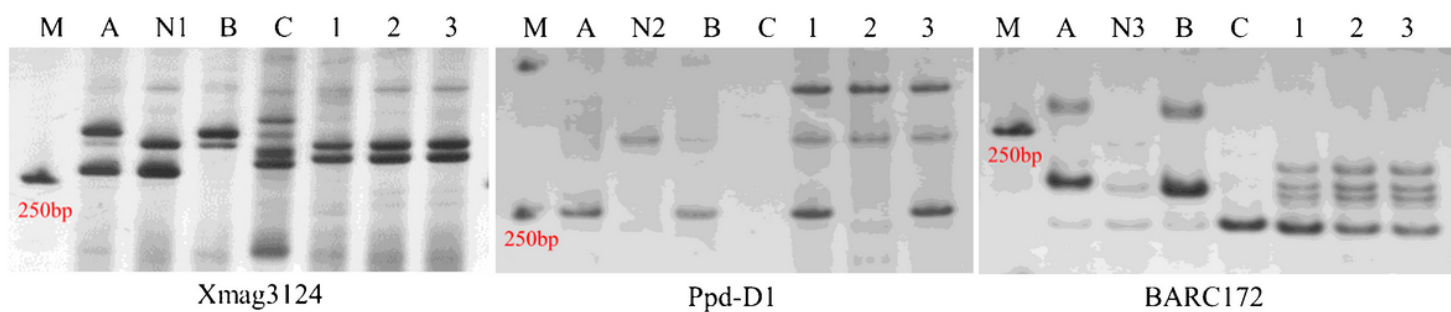


Figure 4

Results of amplification of the wheat chromosome-specific markers Xmag3124 (1A), Ppd-D1 (2D) and BARC172 (7D). M: Marker DL2000, A: CS, N1: CS Nulli-tetrasomes N1AT1D, N2: N2DT2B, N3: N7DT7B, B: YN15, C: Th. intermedium, 1: TE261-1, 2: TE266-1, 3: TE346-1.

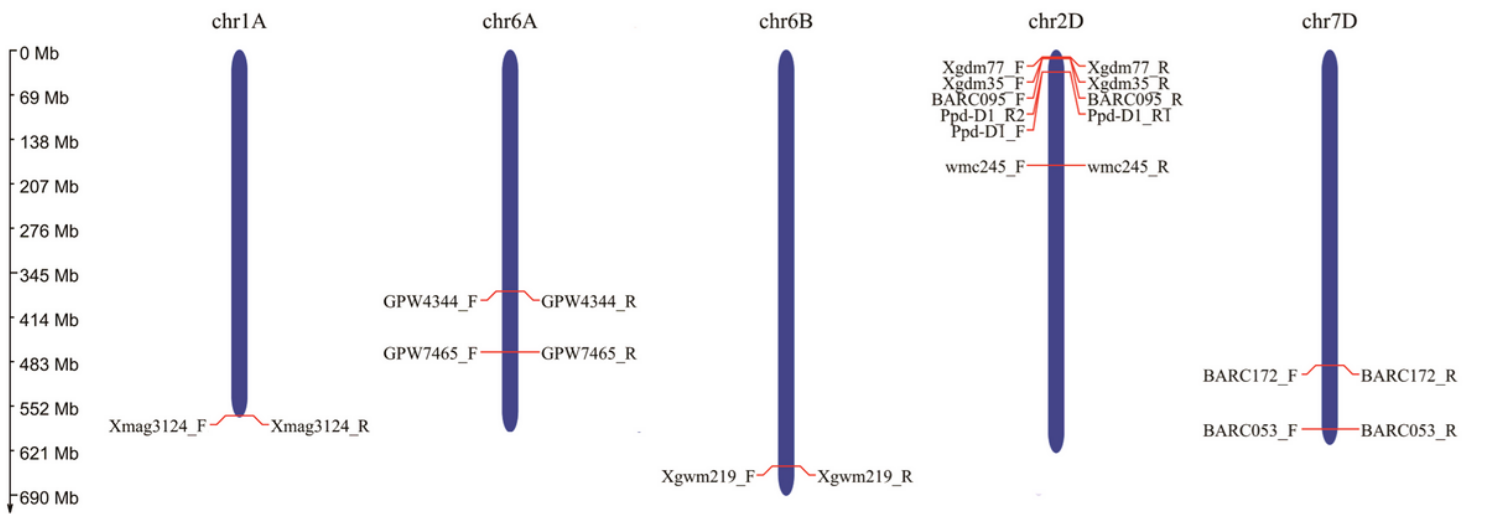


Figure 5

Specific molecular marker map indicating chromosomal variation; chr indicates the corresponding chromosome. The length of the chromosome reflects chromosome size; and positional variation was labeled with molecular markers.

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