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A new long terminal repeat (LTR) sequence allows to identify J genome from J^S and St genomes of *Thinopyrum intermedium*

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Abstract A repetitive sequence of 491 bp, named pMD232-500, was isolated from *S. cereale* cv. Kustro using wheat SSR marker X*gwm*232. GenBank BLAST search revealed that the sequence of pMD232-500 was highly similar to a part of retrotransposon Nusif-1. Fluorescence *in situ* hybridization (FISH) analysis using pMD232-500 as probe indicated that only 14 *Thinopyrum intermedium* chromosomes and all the chromosomes of *S. cereale* cv. Kustro bear FISH signals, however, no FISH signals were observed on *Dasypyrum villosum* chromosomes. In addition, the FISH signals were distributed on whole arms except their terminal regions. Further genomic *in situ* hybridization (GISH) analysis using genomic DNA from *Pseudoroegneria spicata* indicated that the 14 *Th. intermedium* chromosomes bearing FISH signals should belong to J genome. Thereafter, the repetitive elements pMD232-500 showed the unambiguous features of genomic constitution of *Th. intermedium*. In addition, the results in the present study have indicated the similarity of genomes from *Th. intermedium* and *S. cereale*.

Keywords FISH · GISH · LTR-retrotransposon · *Secale cereale* · *Thinopyrum intermedium*

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The tribe Triticeae provides a vast gene pool for most agronomically important traits including some that do not exist in wheat. The wild wheatgrass, *Thinopyrum intermedium* (Host) Barkworth and Dewey (= *Agropyron intermedium* (Host) P. B.) is a hexaploid species ($2n=6x=42$) with genomes E_1E_2St (Wang et al. 1994) or JJ^SSt (Chen et al. 1998). The species has been hybridized extensively with wheat and proved to be a valuable source of genes for disease resistance (Larkin et al. 1995; Chen et al. 2003; Yang et al. 2006; Luo et al. 2009) and tolerance to salt stress (Dvořák 1985). The correct identification of *Th. intermedium* chromosomes involved in these gene transfers is important for cytogenetic investigations. Genomic *in situ* hybridization (GISH) has been used to examine the genomic constitution of *Th. intermedium* (Chen et al. 1998) and the results indicated that the S genomic DNA probe from the diploid species *Pseudoroegneria spicata* can effectively separate the *Th. intermedium* chromosomes into J, J^S and S (S=St) genomes (Chen et al. 1998; Chen 2005). However, S specific genomic DNA probe cannot divide J and J^S genomes into two obvious groups, which contain the basic 14 chromosomes in Triticeae species (Chen et al. 1998). That is, the exact genomic constitution of *Th. intermedium* is very complex and still unsettled, and has showed ambiguous features (Kishii et al. 2005). A new *Sabrina*-like long terminal repeat (LTR) pDbH12 has been isolated from *Dasypyrum breviaristatum* and this repetitive element could serve as a cytogenetic marker for tracing J^S genome in *Th. intermedium* (Liu et al. 2009). As so far, no repetitive elements have been obtained for tracing J genome of *Th. intermedium*. In the present study, a new repetitive DNA sequence belonging to a LTR-retrotransposon Nusif-1 family was isolated from *S. cereal*e cv. Kustro, and its chromosomal distribution was identified by FISH. A wheat SSR marker X*gwm*232 (Röder et al. 1998) was screened for

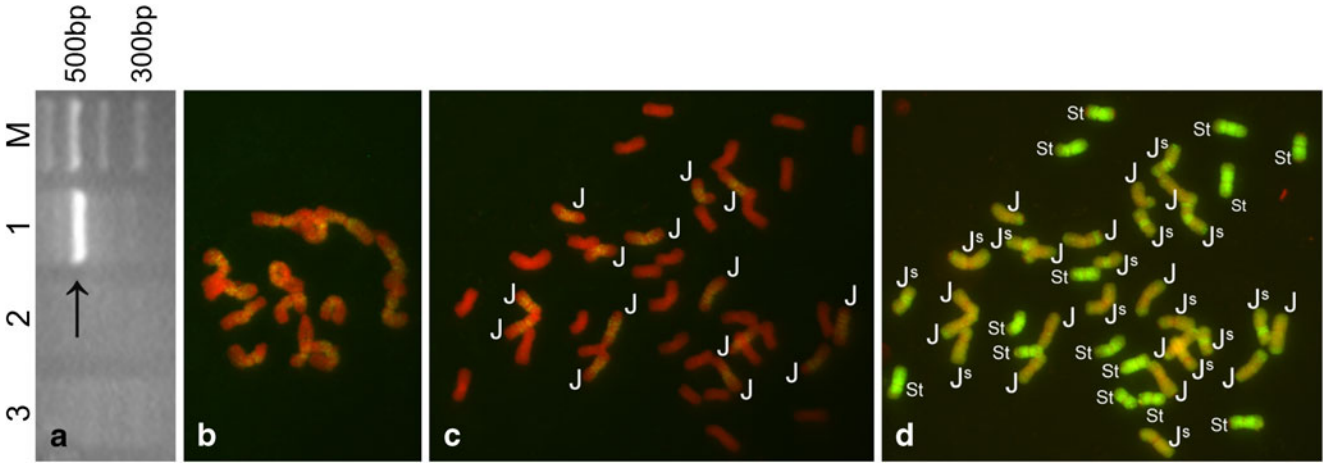

Fig. 1 PCR, FISH patterns of pMD232-500, and GISH pattern of the genomic DNA from *Pseudoroegneria spicata*: **a** PCR analysis with primer pair X*gwm*232 in wheat and rye: 1=*S. cereale* cv. Kustro; 2=*T. aestivum* cv. Chinese Spring; 3=*T. aestivum* cv. Mianyang11; M=DNA marker; *arrow* indicates the target band, **b** FISH pattern of metaphase chromosomes in *S. cereale* cv. Kustro, using pMD232-500 as probe, **c** and **d** Sequential FISH and GISH on the same spread of metaphase chromosomes of *Th. intermedium*, using pMD232-500 and genomic DNA from *Ps. spicata* as probes, respectively. The J, J^S and St genomes have been marked

amplification in *T. aestivum* cv. Mianyang11, *T. aestivum* cv. Chinese Spring (CS) and *S. cereale* cv. Kustro. This SSR marker gave rise to a specific DNA band of about 500 bp from *S. cereale* cv. Kustro and this band was not amplified from the two wheat cultivars (Fig. 1a). Subsequently, the rye-specific band was cloned and sequenced. The full length of the 491-bp sequence was obtained and designated as pMD232-500 (GenBank accession No. EF535858). BLAST search in the NCBI GenBank revealed that the 278-482 bp nucleotide sequence of pMD232-500 shows 77% identity to a LTR-retrotransposon Nusif-1 of *T. aestivum* (GenBank accession No. DQ537335). This shows that wheat SSR markers can be used to isolate the retrotransposon sequences in Triticeae plants enriching with repetitive elements. In order to determine the chromosomal distribution of the isolated LTR-retrotransposon like sequence, pMD232-500 was labeled with digoxigenin-11-dUTP by nick translocation, and the probe was used to hybridize the mitotic metaphases cells of *S. cereale* cv. Kustro, CS, *Th. intermedium* and *D. villosum* (PI 598390, supported by National Plant Germplasm System, USDA-ARS, Aberdeen, Idaho, USA) by FISH analysis with no block. The process was according to the protocols from Tang et al. (2008). All the chromosomes of *S. cereale* cv. Kustro and only 14 *Th. intermedium* chromosomes bear FISH signals (Fig. 1b-c), however, no FISH signals were observed on CS and *D. villosum* chromosomes (data not shown). In addition, the FISH signals were distributed on whole arms except their terminal regions (Fig. 1b-c). For determining the genome affiliations of the chromosomes bearing FISH signals in *Th. intermedium*, total genomic DNA from *Ps. spicata* was also labeled with digoxigenin-11-dUTP by nick translation, and the probe was used to hybridize the same mitotic metaphases cells of *Th. intermedium*, which have already been used for FISH analysis. The sheared genomic DNA of CS was used as blocking DNA. The process was according to the protocols described by Yang et al. (2006). In the *Th. intermedium*, the GISH analysis indicated that 14 chromosomes were strongly and uniformly hybridized along the entire chromosome length, eight chromosomes showed strong signals around the centromeres and weaker signals at the telomeres

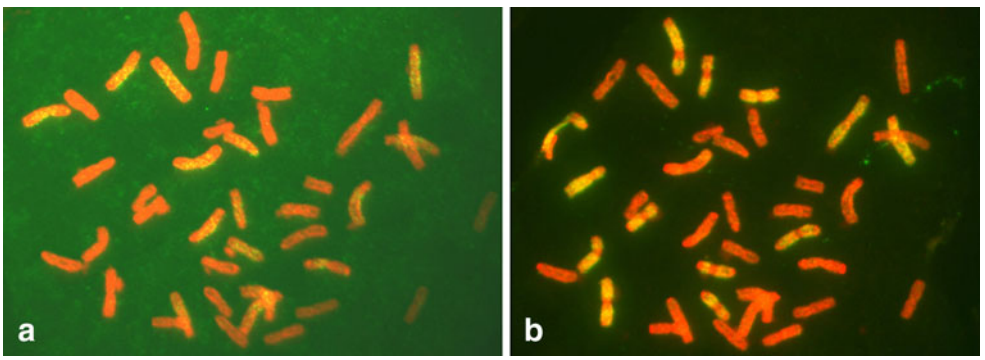

Fig. 2 **a** and **b** Sequential FISH of pMD232-500 and pDbH12 on the same spread of metaphase chromosomes of *Th. intermedium*

(Fig. 1d). According to the genomic formula of *Th. intermedium* created by Chen et al. (1998), the 14 chromosomes should belong to the St genome, the eight chromosomes belong to the J^S genome, and the remaining 20 chromosomes belong to the J genome. Apparently, the pMD232-500 probe only hybridized to 14 of the 20 chromosomes. This result indicated that the 14 chromosomes bearing FISH signals of pMD232-500 probe should be an integrated set of genome chromosomes and they should be different from the other six of the 20 chromosomes. In the present study, six of the 20 chromosomes were putatively included into J^S genome (Fig. 1d) and the 14 chromosomes bearing FISH signals were designated as J genome. Liu et al. (2009) have isolated a repetitive element pDbH12, which gives rise to hybridization signals on 14 *Th. intermedium* chromosomes and can be used to identify the J^S genome. In addition, pMD232-500 probe was used to hybridize another root-tip preparation of *Th. intermedium*. After rinsing the root-tip preparation, FISH analysis using pDbH12 as a probe was carried out on the same slide. Results apparently indicated that pMD232-500 probe and pDbH12 probe hybridized to different groups of chromosome of *Th. intermedium* (Fig. 2). The pDbH12 sequence must be different from the pMD232-500 sequence because it was isolated from *D. villosum* and could hybridize to *D. villosum* chromosomes, however, the pMD232-500 was isolated from rye and could not hybridize to *D. villosum* chromosomes. Thereafter, pDbH12, pMD232-500 and genomic DNA from *Ps. spicata* can be used to identify J^S, J and St genomes of *Th. intermedium*, respectively. Using these two repetitive elements and the genomic DNA from *Ps. spicata*, the exact genomic constitution of *Th. intermedium* has been settled and has shown unambiguous features.

In the present study, we distinguished the J genome in *Th. intermedium* using FISH of pMD232-500 sequence. The hybridization pattern of the J genome was similar to R genome chromosomes of *S. cereale*. This result indicated similarity of genomes from *Th. intermedium* and *S. cereale*. Kishii et al. (2005) have tentatively re-designated the genomic formula of *Th. intermedium* as $StJ^S(V\text{-}J\text{-}R)^S$, superscripted s stands for St genome signals in E/J and V-J-R genomes, and V-J-R stands for derivative genome involved in the evolution of the three genomes. This formula also implies that *Th. intermedium* has a close relationship with *S. cereale*. Liu et al. (2009) have modified the genome formula of *Th. intermedium* as StJ(V-J-H) ^{S}, superscripted s stands for St genome signals in V-J-H genomes, and V-J-H stands for derivative genome involved in the evolution of the three genomes. Thereafter, as that indicated by Liu et al. (2009), it is possible that an ancestral genome was (V-J-R-H) that evolved to (V-J-R) and (V-J-H), which in turn diverged further into (V-J), (V-R), (J-R), (V-H), and (J-H), prior to evolving to the diploids V, J, R and H.

In conclusion, the new LTR-retrotransposon-like sequence pMD232-500 can be used to identify J genome of *Th. intermedium*, and the further investigation of its distribution on the chromosomes of other Triticeae species is worth doing.

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