Molecular cytogenetic characterisation of a leaf-rust resistant wheat-*Thinopyrum ponticum* partial amphiploid

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**Main points of the Doctoral Dissertation**

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1. Introduction

The loss of genetic diversity in wheat (*Triticum aestivum* L.), mainly caused by the introduction of modern high-yielding cultivars, resulted in increased vulnerability to biotic and abiotic stresses. An increasing global effort is underway to transfer desirable genes into bread wheat through genetic manipulation techniques or classical methods, such as including ancient wheat varieties in modern breeding programs (Bedő et al. 1998). There is also renewed interest in exploiting the rich genetic basis of the wild species of *Triticeae* by wide hybridisation. The production of wheat-alien hybrids makes the wild genes involved in biotic and abiotic stress resistance more accessible for wheat breeding programs.

Modern cytogenetic techniques enable complex intergeneric hybrids to be produced (Molnár-Láng and Sutka 1994). However, due to their unbalanced genetic background, $F_1$ hybrids are generally highly self-sterile, and backcrossing with wheat is extremely difficult. The use of colchicine, an amitotic agent, led to the production of a number of wheat-alien amphiploids which are stable and highly fertile, thus representing an important intermediate step in wheat breeding programs (Jiang et al. 1994).

The production of fertile hybrids alone is not sufficient to improve the biotic or abiotic stress resistance of wheat. To achieve effective gene transfer it is essential to understand the cytogenetic background of hybrid plants carrying the desired traits. The establishment of the genomic *in situ* hybridisation (GISH) technique for plants by Schwarzacher et al. (1989) opened up the opportunity to visualise relatively small introgressions of alien chromatin in interspecific hybrids (Molnár-Láng et al. 2000).

Wheat-alien hybrids, in addition to the alien genome, carry homoeologous genomes (ABD genomes) from hexaploid wheat. The high number of homoeologous genomes present make genome analysis difficult. Multicolour GISH (mcGISH) is a powerful tool for the analysis of such hybrids, as it enables the simultaneous visualisation of two or more homoeologous genomes (Mukai et al. 1993, Molnár et al. 2009). Fluorescence *in situ* hybridization (FISH) allows the visualisation of major repeat clusters, which results in a specific banding pattern making chromosome identification possible (Rayburn and Gill 1985). Moreover, FISH reveals and identifies intergenomic chromosome rearrangements in polyploid species (Linc et al. 1999). However, minor rearrangements, or the rearrangement of chromosomal regions without a characteristic FISH pattern, cannot be identified by FISH. The availability of molecular markers makes it possible to overcome such difficulties. Intergenomic rearrangements visualized by GISH or mcGISH and identified by FISH using
repetitive DNA sequences represent excellent genetic materials for cytogenetically based physical mapping of genes or molecular markers (Nagy et al. 2002).

*Thinopyrum ponticum* (Podp.) Z.W. Liu & R. R. -C. Wang [syn *Agropyron elongatum* (Host) Beauvoir ssp *ruthenicum* Beldie] (2n=10x=70), a wild relative of wheat, was frequently used in wheat improvement as a donor of various disease resistance genes, in particular for leaf rust (*Puccinia triticina*) and wheat streak mosaic virus (Friebe et al. 1996). Its genomic composition was revealed by GISH to be J'JJIJ. The J genome is homologous to the J genome of the diploid *Thinopyrum bessarabicum*, whereas the J' genome is a modified J genome of unknown origin characterized by the presence of S genome-specific hybridization signals in the pericentromeres (Chen et al. 1998).

The line BE-1 was a result of the first Hungarian wheat–*Thinopyrum ponticum* hybridisation program carried out by T. Rajháthy and Á. Kiss in Martonvásár in 1953 (Belea 1964). BE-1 was selected by D. Szalay in 1955 from the F$_3$ generation of the cross for its high protein content and resistance to leaf rust and powdery mildew (*Blumeria graminis f. sp. tritici*) (Szalay 1979). Besides its favourable characteristics, the line BE-1 was found to be stable and highly fertile, and was thus considered suitable genetic material for increasing the protein content and transferring disease resistance into wheat. Although its general appearance was intermediate between wheat and *Th. ponticum*, in the absence of appropriate cytogenetic techniques alien chromatin could not be detected in the line.

The present study aimed to use GISH, with genomic probes originating from diploid and decaploid *Thinopyrum* and *Pseudoroegneria* species, in order to detect alien chromatin in line BE-1 and to determine its precise chromosome composition. In addition, it was planned to use FISH with various repetitive DNA probes in order to identify the wheat chromosomes and any intergenomic rearrangements. Further analysis of BE-1 was planned by exploiting chromosome-specific SSR markers, with the objective of precisely defining minor intergenomic rearrangements.
1.1 Objectives

The aim of the present study:

- to improve the GISH technique to make it suitable for the routine detection of \textit{Thinopyrum} chromatin in wheat-\textit{Thinopyrum} hybrids, thus facilitating the incorporation of desired traits into the wheat genome
- to determine the number of chromosomes originating from the \textit{Th. ponticum} parent in BE-1
- to identify all the wheat chromosomes present in the partial amphiploid in order to reveal substitutions or additions in the wheat genome, if any
- to detect and identify wheat-\textit{Thinopyrum} translocations and intergenomic rearrangements among the A, B and D genomes of wheat
- to use the detected translocation chromosomes in the physical mapping of molecular markers
- to start a backcross program using the partial amphiploid BE-1 in order to transfer its leaf rust resistance to wheat

2. Materials and Methods

2.1 Plant material

The following plant genotypes were used in the present study: the partial amphiploid BE-1, \textit{Triticum urartu} Thum. (2n=2x=14, AA), \textit{Aegilops bicornis} Forsk. (2n=2x, SS=BB), \textit{Ae. tauschii} Coss. (2n=2x=14, DD), \textit{Triticum durum} Desf. (2n=2x=28, AABB), \textit{T. aestivum} L. (2n=6x=42, AABBDD) cv. Bánkúti, cv. Mv Suba, Mv9kr1, Chinese Spring ph mutant, \textit{Elytrigia elongata} (Host) Nevski (2n=2x=14, EE), \textit{Thinopyrum bessarabicum} Savul. & Rayss (2n=2x=14, JJ), \textit{Pseudoroegneria strigosa} subsp. \textit{aegilopoides} (2n=2x=14, SS), \textit{Thinopyrum ponticum} (2n=10x=70, JJJJsJs).
2.2 Genomic in situ hybridisation

Total genomic DNA was extracted following the phenol-chloroform method (Anderson et al. 1992). Labelling was carried out by nick-translation using digoxigenin-16-dUTP and biotin-11-dUTP. The chromosomes were incubated in the presence of the hybridization solution at 42°C overnight. Biotinylated and digoxigenated sequences were detected using streptavidin-FITC (fluorescein isothiocyanate) and anti-digoxigenin-rhodamine. Fluorescent signals were visualized with a Zeiss Axioscope 2 epifluorescence microscope equipped with a filter for detecting DAPI (Zeiss, Filterset 01) and a dual band filter set (Zeiss, Filterset 24) for the observation of FITC and rhodamine signals simultaneously. Photographs were taken with a Spot CCD camera (Diagnostic Instruments, Inc., USA). The image processing was carried out using Image-Pro Plus 5.1 (Media Cybernetics, USA) software.

2.3 Fluorescence in situ hybridisation

FISH was applied by simultaneously hybridizing three labelled repetitive DNA sequences (pSc119.2, the Afa-family and pTa71). The pSc119.2 and Afa-family sequences were amplified and labelled by PCR with biotin-11-dUTP and digoxigenin-16-dUTP, respectively (Contento et al. 2005; Nagaki et al. 1995). The clone pTa71 was labelled combinatorially with 50% biotin-11-dUTP and 50% dig-11-dUTP.

After washing the GISH hybridization signals off the slides in 4× SSC Tween at 25°C overnight, they were used for three-colour FISH according to a protocol similar to GISH, except that the hybridization temperature was 37°C in this case.

2.4 SSR marker analysis

Total genomic DNA was extracted according to Anderson et al. (1992). Twenty-five 7D-specific and eleven 7A-specific wheat microsatellite markers were selected from the GrainGenes 2.0 database (http://wheat.pw.usda.gov/GG2/index.shtml). PCR reactions were performed as described in Sepsi et al. 2008.
3. **Results**

- Among the 56 chromosomes of the amphiploid, 16 *Thinopyrum* and 40 wheat chromosomes were detected, revealing the absence (substitution) of one wheat chromosome pair. Alien chromosome segments were detected in the pericentromeres of six *Thinopyrum* chromosomes using the J genome probe, which suggested that they were involved in intergenomic translocations. Among these translocation chromosomes, four (two pairs) were identified as J° chromosomes, as the S genome probe hybridized strongly to their pericentromeres.

- The simultaneous hybridization of J- and A-genomic probes labelled with different fluorochromes, and subsequently the hybridization of J- and D-genomic probes detected 14 A genome, 14 B genome and 12 D genome chromosomes in addition to the 16 *Thinopyrum* chromosomes. The substituted wheat chromosome pair thus arosed from the D genome.

- All the wheat chromosomes were unequivocally identified with FISH and the missing chromosome pair was identified as 7D.

- The FISH banding pattern of the *Thinopyrum* chromosomes was described, making chromosome identification possible in the progenies.

- A new intergenomic rearrangement involving the A genome and a short D genome segment was discovered. FISH identified the A chromosome involved in an intergenomic translocation with a short D genome segment as 7A.

- SSR marker analysis revealed the identity of the short translocated D chromosome segment. The A-D translocation was thus identified as 7AL.7DL.

- A minor deletion involving 7AL was also detected by 7A-specific SSR markers, suggesting that the translocation event was preceded by the elimination of a short terminal segment of 7AL.

- The position of the translocation breakpoint within 7AL and 7DL was different from that of known deletion lines, so new physical landmarks were discovered in the terminal regions of both chromosome arms (7AL and 7DL).

- The new translocation and its breakpoint position made it possible to allocate a 7D-specific marker with previously unknown position to the 7DL terminal region, demonstrating the usefulness of the translocation in the physical mapping of molecular markers.
4. **Conclusions**

- The detailed description of the various alien chromosomes in the partial amphiploid BE-1 reported in the present study makes it possible to trace the transfer of *Th. ponticum* chromosomes from this amphiploid into wheat.
- The present study demonstrated that *in situ* hybridization techniques, combined with SSR marker analysis, are extremely useful in detecting and identifying intergenomic rearrangements in the wheat genome, leading to the selection of genetic materials useful for future mapping studies. The fine mapping of the 7AL.7DL translocation chromosome opens up the possibility of more precise physical mapping of the terminal regions of 7DL and 7AL.

5. **References**


6. Publications

6.1 Peer-reviewed scientific papers


6.2 Conference proceedings:


