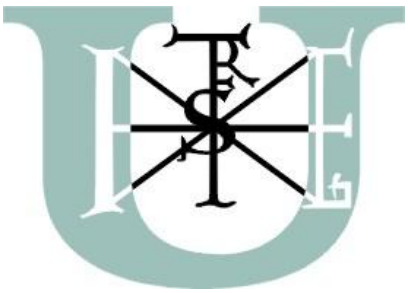

SZENT ISTVÁN UNIVERSITY

IDENTIFICATION OF ALIEN CHROMOSOMES IN INTROGRESSION LINES DERIVED FROM CROSSES BETWEEN WHEAT (*TRITICUM AESTIVUM*) AND RELATED SPECIES (*HORDEUM VULGARE*, *AGROPYRON GLAEL*) USING MOLECULAR CYTOGENETIC METHODS

Doctoral thesis

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1. BACKGROUND AND OBJECTIVES

The most important cereal in Hungary is the bread wheat (*Triticum aestivum* L.), but there is a challenge to realize its yield stability. In order to mitigate this problem, wheat can be crossed with its cultivated or wild relatives characterized by good adaptation capabilities. Incorporation of alien chromosome segment into wheat can be achieved by the selection of genetically stable translocation lines from wheat/alien hybrid progenies through partial amphiploids, additions and substitutions. Translocations can occur spontaneously, but it is more appropriate to apply translocation inducing agents such as ionizing radiation, genetic methods, etc.

Transfer of favourable nutritional parameters of barley (dietary fiber, essential amino acids) into cultivated wheat is an objective of wheat/barley crosses. Wheatgrass species of the *Thinoyprum* genus can be found even under the most extreme climatic conditions due to their significant genetic diversity. Several leaf rust (*Lr19, Lr24, Lr29, Lr38*) and stem rust (*Sr24, Sr25, Sr26, Sr43, Sr44*) resistance genes have been incorporated into wheat from them (Wang et al. 2011). *Agropyron glael*, a synthetic hybrid of *Th. intermedium* and *Th. ponticum* produced in the former Soviet Union in the 1930's has been involved in the wheat/alien crossing programmes in Hungary since 2001. Several self-pollinated BC_1, BC_2 and BC_3 derivatives have been produced from the Mv9kr1 (wheat)/ *Agropyron glael* cross.

Molecular cytogenetic techniques for detection and identification of the alien chromatin in the wheat genetic background are required to follow up the successful wheat/alien transfer. With the help of advanced fluorescence *in situ* hybridization techniques (mcGISH, mcFISH), specific hybridization patterns were described in several species. Karyotypes of the majority of wild wheatgrasses (e.g. *Thinopyrum*) have not been published yet.

The aim of the present study:

- to describe three-colour FISH pattern of different barley (*Hordeum vulgare*) genotpyes and to use the FISH karyotype for molecular cytogenetic analysis of wheat/barley addition lines and the 4BS.7HL translocation line developed in Martonvásár
- to identify translocations in the progenies of 'Chinese Spring'/'Betzes' 4H(4D) substitution line treated with irradiation (^{60}Co) or crossed with the 'Chinese Spring'*ph1b* mutant genotpye
- to characterize the spontaneous 5HS-7DS.7DL chromosome translocation in the progenies of the Mv9kr1/'Igri' wheat/barley hybrid using mcGISH and FISH, and to physically localize SSR markers to the telomeric region of the 7DS chromosome arm
- to improve the mcGISH technique in the parent species of *Agropyron glael* (*Th. intermedium* and *Th. ponticum*) to make it suitable for the routine detection of *Thinopyrum* chromatin in wheat/*A. glael* hybrids

- to select leaf rust resistant plants from the backcrossed progenies of wheat (Mv9kr1)/*A. glael* cross, and to identify the chromosome composition of these plants using mcGISH and FISH techniques
- to backcross wheat/*A. glael* BC_1 progenies which carry several wheatgrass chromosomes

2. MATERIALS AND METHODS

2.1. Plant material

The following plant genotypes were used: *Hordeum vulgare* L. ('Manas', 'Betzes', 'GK Sztáromega', 'Olte's', WI2291, AZ-8501, CNE-16, CNE-73, CNE-91, 'Golden Promise'); *Triticum aestivum* L. (Mv9kr1, 'Asakazekomugi', 'Chinese Spring', 'Chinese Spring' *ph1b* mutant, 'MvKarizma'); wheat/barley 4H(4D) substitution line; wheat/barley translocation lines (4BS.7HL and 7D.5HS); perennial wheatgrasses (*Thinopyrum bessarabicum, Th. intermedium, Th. ponticum, Pseudoroegneria spicata)*; *Agropyron glael* (*Thinopyrum intermedium/Th. ponticum* synthetic hybrid); Mv9kr1/*Agropyron glael* BC_1-BC_2 selfed progenies produced in Martonvásár, Hungary.

2.2. Genomic *in situ* hybridization

High concentration (>1000μg/mL) total genomic DNA was extracted from the diploid species *Ae. tauschii* (D), *H. vulgare* (H), *Th. bessarabicum* (J) and *Ps. spicata* (St) carrying the detectable genomes of wheat/alien introgressions. Genomic DNA was labelled by digoxigenin-11-dUTP or biotin-11-dUTP using nick translation or random priming. The chromosomes were incubated in the presence of the hybridization solution (contains probe or probes –mcGISH– DNA and blocking DNA) at 42°C. Biotinylated and digoxigenated sequences were detected using streptavidin-FITC (green) and anti-digoxigenin-rhodamine (red) (Roche).

2.3. Fluorescence *in situ* hybridization

The repetitive DNA sequences were amplified and labelled by PCR and/or nick translation with biotin (green) and digoxigenin (red). The third colour of the three-colour FISH was produced combinatorially with 50% biotin and 50% digoxigenin resulting yellow colour during the detection phase. Different combinations of HvT01, $(GAA)_7$, pTa71, Afa-family, $(AGGGAG)_n$ repetitive DNA probes were used for identification of barley chromosomes. Afa-family, pSc119.2 and pTa71 DNA sequences were used for identification of wheat chromosomes and characterization of *Agropyron glael* chromosomes. The hybridization temperature was 37°C.

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 (MediaCybernetics, USA) software.

2.4. SSR marker analysis

Fortyfive 7D specific SSR markers were used for the physical mapping of 7D chromosome. Total genomic DNA was extracted from the 7D.5HS translocation line and its parental genotypes (Mv9kr1, 'Igri'). PCR reactions and PCR product separations were performed as described in Kruppa et al. (2013a).

Four primer pairs were used to detect the absence or presence of certain *Thinopyrum*-derived leaf rust and stem rust resistance genes in wheat/*A.glael* progeny plants: STSLr19$_{130,}$ J09-STS (*Lr24*), Lr29F18-Lr29R18, and Sr26#43.

2.5. Phenotypic characterizations, crossings

The cytogenetically analysed plants were grown up in phytotron chambers. Wheat/barley and wheat/*Agropyron glael* introgression lines and their plant materials were sown and self-pollinated in the nursery every year. Mv9kr1 and 'Mv Karizma' wheat genotypes were used as pollen donors during backcrossings of the BC_1 wheat/*A. glael* derivatives.

Ten plants were randomly selected from each genotype foranalysis. The morphological traits of the introgression lines were compared with threir wheat parent using the Student’st test for paired data at the P = 0.05 significance levels.

The degree of spontaneous leaf rust and yellow rust infection in the organic nursery was scored from 0 to 4 according to Stakman et al. (1962).

3.1. Results of the wheat/barley crosses

Translocation-inducing methods were used for incorporation of barley DNA segments into wheat genetic background. Several wheat/barley translocations (interstitial and terminal) were observed in the M_0 generation of irradiated 'Chinese Spring'/'Betzes' 4H(4D) substitution line produced in Martonvásár. In the next generation (M_1), all analysed plants lost their interspecific rearrangements. Translocations between the wheat chromosomes could have been present, but their detection was not objective of the experiment.

Barley FISH karyotype was constructed using three different DNA probes simoultaneously. There were detectable polymorphism in the hybridization pattern of the analysed barley genotypes and varietes. The extent of polymorphism was restricted to the long chromosome arms with differences between the chromosomes. 1H and 3H proved to be the most variable chromosomes and 4H and 6H the most conserved (Szakács et al. 2013).

The 4H(4D) wheat/barley substitution line was crossed with the 'Chinese Spring' *ph1b* mutant genotype in order to induce wheat/barley homoeologous recombinations. A Robertsonian translocation was detected and identified as 4HL.5DL using FISH and SSR markers. This line exhibited supernumerary spikelet character, but the number of seeds/plant did not increase showing the negative effects of non-compensating translocations (Kruppa et al. 2013b).

Spontaneous interspecific Robertsonian translocation was revealed by genomic *in situ* hybridization (GISH) in the progenies of a monosomic 7H addition line originating from a wheat 'Asakazekomugi'/barley 'Manas' hybrid. The translocated chromosome carried a barley centromere which was identified by $(AGGGAG)_n$ barley centromere-specific repetitive sequences during FISH (Cseh et al. 2011).

A spontaneous wheat-barley translocation line was previously detected in the progenies of the Mv9kr1/'Igri' wheat-barley hybrid; the translocation was identified as 5HS-7DS.7DL. The breakpoint was more distal than that of reported deletion lines which provides new physical landmark for future deletion mapping studies. From among the analysed 45 microsatellite markers, ten (*Xbarc0184, Xwmc0506, Xgdm0130, Xgwm0735, Xgwm1258, Xgwm1123, Xgwm1250, Xgwm1055, Xgwm1220,* and *Xgwm0635*) failed to amplify any 7DS-specific fragments, signalling the elimination of a short chromosome segment in the telomeric region (Kruppa et al. 2013a).

3.2. Results of the wheat/*Agropyronglael* crosses

McGISH was used to detect wheatgrass chromosomes in Mv9kr1/*Agropyron glael* hybrid progenies. This technique was optimized in the parental species of *Agropyron glael* (*Th.*

intermedium and *Th. ponticum*). Genome composition of these materials was characterized. Hexaploid *Th. intermedium* contained 19 J, 9 J^S and 14 St chromosomes. The three analysed *Th. ponticum* accession showed different chromosome compositions: 43 J + 27 J^S (PI531737), 40 J + 30 J^S (VIR-44486) and 38 J + 32 J^S (D-3494).

A. glael carried J, J^S, St chromosomes including J-St, J^S-St translocations and/or decreased fluorescent intensity, resulting in unique hybridization patterns. Chromosome composition of the wheat/*A.glael* F_1 hybrid was 21 wheat + 28 *Agropyron glael* (11J + 14J^S + 3S).

Different lines with leaf rust and yellow rust resistance were selected from the hybrid derivatives. These progeny plants carried 51-62 chromosomes. Elimination of St chromosomes and presence of intergenomic translocations between J-St genomes were described in each lines. Partial amphiploids were identified among them. Chromosome counting on these lines revealed 58 chromosomes (40 wheat + 18 wheatgrass) in line 194, 56 (42 wheat + 14 wheatgrass) in line 195 with the elimination of 3D wheat chromosomes. Molecular marker analysis showed the presence of the *Lr24* leaf rust resistance gene in line 195 which was a 4D tetrasomic-3D nullisomic line.

Reduced alien chromosome number (2-7) was detected in BC_2 self-pollinated progenies produced in 2005, but the loss of leaf-rust resistance was also identified.

Leaf rust resistant BC_1 self-pollinated lines were not only maintained in the nursery, but were used after successful propagation in new crossing programmes with modern, high-yielding wheat varietes in order to decrease the number of wheatgrass chromosomes and to incorporate leaf rust and yellow rust resistance through wheat/*Agropyron glael* translocations. The selection and identification of resistant progenies is now in progress.

3.3. New scientific results

1. Karyotyping based on the mcFISH pattern of the rDNAclone pTa71, the barley-specific subtelomeric tandem repeat HvT01 and repetitive DNA GAA revealed intraspecific polymorphism between theten *H. vulgare* cultivars and lines studied.
2. A new wheat/barley translocation line was identified containing the long arm of 4H and the long arm of 5D chromosome arms (4HL.5DL).
3. Barley centromere was identified with the help of AGGGAG barley centromere-specific repetitive FISH probe in 'Asakazekomugi'/'Manas' (wheat/barley) 4BS.7HL centric fusion line.
4. Position of 7D short arm-specific SSR markers was clarified. The breakpoint of the 5HS-7DS.7DL translocation (FL 0.76 +/- 0.04) identified to be more distal than that of reported deletion lines which provides a new physical landmark for future deletion mapping studies.
5. Genome composition and simoultaneous visualization of different genomes (J, J^S and St) in *Thinopyrum intermedium* and *Thinopyrum ponticum* were described by multicolour genomic *in situ* hybridization (mcGISH).
6. Different lines with leaf rust and yellow rust resistance were selected from the wheat/*Agropyron glael* hybrid derivatives. Two different partial amphiploid lines (containing 56 and 58 chromosomes) with leaf rust resistance were selected by multicolour genomic *in situ* hybridization.
7. Introgression of leaf rust and yellow rust resistance of wheat/*Agropyron glael* self-pollinated progenies into high yielded, modern wheat varietes has begun through backcrossing with 'MvKarizma' wheat varieties.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1. Wheat/barley hybridization

Genetic stability of wheat/alien introgression lines can be different, thus cytogenetic control of the used lines is necessery in every case.

Crossing with 'Chinese Spring' *ph1b* mutant induces homoeologous pairing, results compensating translocation and avoids the negative effects of non-compensating translocations (reduced viability, yield components), thus this is the most powerful method for producing new wheat/barley translocation lines. However, introgression lines containing translocation between non-homoeologous chromosomes can carry other advantageous traits which make them valuable genetic materials. With the help of appropriate backcrossing, these lines can be transferred into compansating translocations. Undirected chromosome breakages can be induced by irradiation, but if the dosage of treatment is not optimal, the number of chromosome breaks will be too high or too low. In this case, more generations should be analysed. This method can generate sterility problems and morphological defects in plants, thus this technique is not the most appropriate to induce translocations. Genetic transformation, which uses more and more backgroung knowledge, is a possible method for precise introgression of DNA segments into wheat. Advantages and disadvantages of this technique have been discussed for several years however the main profile of our department (Department of Plant Genetic Resources) prefers traditional breeding methods (crosses).

The aim of the wheat/barley crosses is to produce introgression lines with beneficial agronomic traits. Sometimes these lines can not been utilized in plant breeding because of the presence of non-homoeologous translocations. Genotypes with these disadvantageous agronomic traits can be used in fundamental research, physical mapping or keeping up alien chromosome segments (which are eliminating fast anyway) in wheat genetic background. Therefore, it is recommended to preserve these genetic materials.

4.2. Wheat/*Agropyron glael* hybridization

Species belonging to the tertiary gene pool of bread wheat are important genetic materials for wheat improvement. Thus, species with known origin, resistance and genome composition are valuable sources and supplies the need for preserving plants and seeds in perennial nurseries and in genebanks. Genome composition and evolution of most of the wheatgrasses are insufficient or have not been analysed yet. The open-pollinating nature of these species, the heterogeneity and the large number of spontaneous hybrids in the nature make the identification process difficult. Different chromosome numbers, unknown DNA sequences can be found in the same species. Difficulties of identifying are in parallel with the success of perennial wheatgrasses. Utilization of these materials

is based on their excellent biotic and abiotic stress resistance. Natural populations are thus valuable genetic sources in wheat breeding. The synthetic hybrid, *Agropyron glael*, is also a unique material as it contains advantageous traits of its parental wheatgrass species, the *Th. intermedium* and *Th. ponticum*.

The number of progeny lines deriving from wheat/*Agropyron glael* hybrid is increasing exponentially. Derivatives analysed in the dissertation are only parts of a larger whole. Our further aim is to select and identify more lines carrying advantageous traits based on observations in the nursery together with molecular cytogenetic techniques and molecular marker analysis.

Among the ABD genomes of wheat, the D genome shows the closest relationship with the wheatgrass genomes which has been supported by a large number of changes (elimination or translocation) of the D genomebecause of the presence of *A.glael* chromosomes.

In the future, it is planned to analyse the quality parameters of the resistant progeny lines, primarily in case of protein, dietary fibre and mineral composition.

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