Molecular cytogenetic analysis of *Triticum monococcum* gene bank accessions and their utilization in wheat improvement

Main points of the PhD thesis

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1 BACKGROUND AND AIMS

The primary aim of wheat breeding is to increase the quantity and security of wheat yield. The wheat yield could be improved by cultivars which are tolerant to biotic and abiotic stresses. Useful genes responsible for the wheat biotic and abiotic stresstolerance could be derived from wheat related species.

Species having homologous genomes with hexaploid wheat (*Triticum aestivum* L., 2n=6x=42, AABBDD), belongs to the primary gene pool of wheat (Friebe et al. 1996). Gene transfer from these species can be achieved by direct hybridization, homologous recombination, backcrossing, and selection. Einkorn (*T. monococcum* L., 2n=2x=14, A^mA^m) belongs to this group. Its A^m genome shows close homology with the A genome of hexaploid wheat. Einkorn wheat is one of the most ancient cultivated crops. Its great resistance to stem rust, leaf rust, stripe rust and powdery mildew has been transferred into hexaploid wheat (Monneveux et al., 2000).

The transfer of agronomically useful traits from alien species into the wheat could be achieved by means of interspecific crosses. The chromosome-mediated gene transfer via interspecific hybrids is the only allowed method by law in Hungary nowadays.

The aim of our research is the efficient use of *T. monococcum* chromatin in wheat breeding programmes. This aim will be implemented by the following tasks:

1. In the course of alien gene transfer it is need to follow the alien chromosomes and chromosome segments. DNA *in situ* hybridization is one of the most powerful techniques for detection and identification of the introgressed chromosome segments in the wheat background. Fluorescence *in situ* hybridization (FISH) with defined DNA sequences results in chromosome specific hybridization patterns making this technique an excellent tool for the karyotypic analysis of interspecific hybrids and their progenies (Rayburn and Gill 1985, Pedersen and Langridge 1997). Most frequently, molecular cytogenetic analysis of wheat chromosomes is carried out by repetitive DNA probes (Mukai et al. 1993). **Our aim is the modification and development of molecular cytogenetic methods, which permit the following of *T. monococcum* chromosomes in wheat background.** First we identify the *T. monococcum* chromosomes using repetitive DNA probes. For the better discrimination of einkorn and wheat chromosomes we elaborate new karyotypes of *T. monococcum* using microsatellite FISH probes. We analyse the differences among A chromosomes of di-, tetra- and hexaploid *Triticum* species on the basis of new karyotypes.

2. One of the most important requirement of chromosome-mediated gene transfer from einkorn into the wheat is the pairing and recombination of different A chromosomes during the meiosis. The information of meiotic chromosome pairing could be maximizing by means of DNA *in situ* hybridization techniques (Miller et al. 1994). **Our aim is the analysis of individual chromosome pairing in wheat–alien interspecific hybrids in meiosis metaphase I.** Analyzing of individual chromosome pairing is studied first in *T. aestivum* × *Secale cereanum* F_1 hybrids by means of FISH and GISH. The newly developed method is applied for evaluation of chromosome pairing in *T. turgidum* subsp. *durum* × *T. monococcum* F_1 hybrid.

3. The gene transfer from alien species is a very long process with several steps. The first step is the identification of the most appropriate genotypes as gene sources. The aim of prebreeding is to produce genetically stable genotypes with the desired traits. The developement of agronomically useful genotypes by using prebreeding material is a conventional method, usually the prebreeding material is crossed with modern wheat varieties. **In our *T. monococcum* based prebreeding programme firstly we studied the crossability of *T. monococcum* with tetra- and hexaploid *Triticum* species.** To produce *T. monococcum* recombinant lines, we develope two type of genetic material. *T. turgidum* subsp. *durum* × *T. monococcum* synthetic amphiploid are produce with ABA^m genome constitution, wich are use in wheat crossing programmes to produce wheat-einkorn recombinants. **A backcrossing programme is planned to use *T. monococcum* × *T. aestivum* hybrids. The aim is to produce genotypes with outstanding resitance of *T. monococcum* that could be use directly in wheat breeding programmes.**

2 MATERIALS AND METHODS

2.1 Plant materials and crossing programme

The following plant materials were used for interspecific crosses: *T. monococcum* subsp. *monococcum* L. '1T-1' semi-dwarf einkorn line and *T. monococcum* subsp. *monococcum* cv. 'Mv Alkor', traditional type einkorn variety. *T. aestivum* L.: Martonvásári 9 *kr1* (Mv9kr1) wheat genotype with recessive crossability allele *kr1* and Martonvásári 9 (Mv9) wheat variety. *T. turgidum* subsp. *durum* L.: MvTD14-04 durum wheat line.

The plants were pollinated 3days after emasculation in the field nursery (durum × einkorn crosses) and in the phytotron (wheat × einkorn crosses) under controlled enviromental conditions (Tischner et al. 1997).

The genome duplication of durum × einkorn F_1 hybrid seedlings was achieved by colchicine treatment (0.04% colchicine, 4 h) and ABA^m amphiploids were developed.

FISH karyotype of A genome of different *Triticum* species were prepared on mitotic chromosome preparation of *Triticum* species which were used for crossings and *T. urartu* MVGB115 gene bank accession.

The meiotic chromosome paring were analysed in interspecific hybrids (*T. aestivum* × *Secale cereanum* and *T. turgidum* ssp. *durum* × *T. monococcum*).

2.2 Molecular cytogenetic analysis

Mitotic chromosome preparation: 1-1.5 cm long roots of germinating seeds were cut and store in ice-cold water for 24 h followed by a fixation in 1:3 (v/v %) acetic acid/ethanol and staining in acetocarmine. The root tip chromosome preparations with a satisfactory mitotic index were stored at –20°C until the *in situ* hybridization experiments.

Meiotic chromosome preparation: Anthers containing pollen mother cells (PMCs) at metaphase I of meiosis were fixed in 1:3 (v/v %) acetic acid/ethanol and squashed in 45% acetic acid.

Probe labelling

FISH probes: The repetitive DNA sequences pSc119.2 and Afa family were amplified from the genomic DNA of *S. cereale* and *Ae. tauschii*, respectively, using PCR. The Afa family probe was labelled with digoxigenin-11-dUTP according to Nagaki et al. (1995) and pSc119.2 with biotin-11-dUTP by nick translation. The $(GAA)_n$, $(CAG)_n$, $(AAC)_n$ and $(AGG)_n$ microsatellite probes were

amplified from the genomic DNA of *T. aestivum* by PCR (Vrána et al. 2000). The $(GAA)_n$ probe was labelled with biotin-11-dUTP and the $(CAG)_n$, $(AAC)_n$ and $(AGG)_n$ probes with digoxigenin-11-dUTP by nick translation. The 18S– 5.8S–26S rDNA clone pTa71 (Gerlach and Bedbrook, 1979) was labelled with 50% biotin-11- dUTP and 50% digoxigenin-11-dUTP.

GISH probes: Total genomic DNA was isolated from rye (*Secale cereale* L.), *T. monococcum* and *Ae. speltoides*. The DNA was labelled with biotin-16-dUTPby nick translation.

Fluorescence *in situ* hybridization (FISH)

FISH was carried out according to Molnár et al. (2011). After pretreatment and the stringency washing of the slides were hybridized using a hybridization solution (30 µl per slide) containing 50% v/v formamide, 2 × SSC, 10% w/v dextran sulphate, 50 ng blocking DNA, 0.1% w/v sodium dodecyl sulphate and 30 ng of labelled probe. After the DNA denaturation (10 min., 80 ^{o}C) of hybridization mixture and denaturation of chromosomes (6 min, 75 ^{o}C) the hybridization was carried out overnight at 37°C. Digoxigenin and biotin were detected by anti-digoxigenin rhodamine Fab fragments and streptavidin-fluorescein isothiocyanate (FITC), respectively. The chromosome preparations were counterstained with DAPI before chromosome observation.

Genomic *in situ* hybridization (GISH)

GISH was carried out on meiotic chromosome spreads of the interspecific hybrids according to Molnár-Láng et al. (2010).

The GISH of *T. aestivum* × *S. cereanum* F_1 hybrids was carried out using total genomic rye DNA as a probe, the blocking DNA was hexaploid wheat and salmon sperm DNA. The slides were hybridized using a hybridization solution (25 µl per slide) containing 50% v/v formamide, 2 × SSC, 5% w/v dextran sulphate, 70 ng of labelled rye genomic probe and 2.1 µg blocking DNA.

The genome constitution of *T. turgidum* subsp. *durum* × *T. monococcum* F_1 hybrids and amphiploids was also analyzed by GISH. The hybridization probes were total genomic DNA of *T. urartu* and *Ae. speltoides.* The blocking DNA was *Ae. speltoides* DNA in 50× concentration.

After *in situ* hybridization, the preparations were examined with a fluorescence microscope

Images were acquired with a Zeiss AxioCam MRm CCD camera and compiled with AxioVision 4.8.2 software.

2.3 Pairing analysis

The chromosome pairing of interspecific hybrids were studied by means of sequential GISH and FISH. The calculated pairing frequency represents the percentage of cells in which a given configuration was observed. The nuber of chromosome associations was evaluated on the basis of their meiotic configuration: one association per rod bivalent, two associations per ring bivalent and chain trivalent, and three per frying pan trivalent.

Chi-square was calculated for the comparison of the expected and observed pairing frequency of the wheat chromosome arms within and between the genotypes.

2.4 Artifical leaf rust inoculation in the greenhouse

In the greenhouse experiments, artificial leaf rust (*Puccinia triticina*) inoculation was performed with a uredospore suspension on *T. aestivum* × *T. monococcum* BC_3 plants. The plants in the 2-leaf stage were inoculated. The degree of infection was scored on the 10th day, according to Stakman et al. (1962).

2.5 Observations in the nursery

T. aestivum × *T. monococcum* BC_3 plants, *T. turgidum* subsp. *durum* T. *monococcum* amphiploids and *T. monococcum* gene bank accessions were phenotyped in the nursery for following characteristics: heading date, plant height, leaf rust resistance, powdery mildew resistance.

3 RESULTS

3.1 Identification of *Triticum monococcum* chromosomes by molecular cytogenetic methods

3.1.1 Karyotype of *T. monococcum* with standard repetitive DNA probes

FISH was carried out with the simultaneous hybridization of the repetitive DNA probes Afa family, pTa71 and pSc119.2 on mitotic metaphase cells of *T. monococcum.* All the chromosomes could be distinguished according to their FISH signals. Distinctive Afa family signals were observed on all chromosomes in the intercalary and distal regions with different intensity. The most complex Afa family hybridization patterns were detected on the $4A^m$ and $7A^m$, and the less on the $6A^m$ chromosomes. Strong fluorescent signals of pTa71 were observed on the telomeric region of short arms of $1A^m$ and $5A^m$ chromosomes. pSc119.2 signals were not detected on the chromosomes of *T. monococcum*. The FISH was also carried out on chromosome preparation of *T. urartu* (the A genome donor of hexaploid wheat) and similar hybridization pattern was observed as in *T. monococcum*.

Karyotypes of *T. monococcum* and *T. urartu* were created and compered with the karyotype of A genome of tetra- and hexaploid wheat species using the same hybridization probes. The chromosomes of *T. monococcum* and *T. urartu* have more complex Afa family hybridization pattern, than the A chromosomes of poliploid wheat species. A similar observation was done in case of ribosomal DNA probe (pTa71). On the other hand, the pSc119.2 signals located on the 4A and 5A chromosomes of tetra- and hexaploid wheat were not observed on the A chromosomes of diploid *Triticum* species. Our results showed that the A^m and the A chromosomes of wheat within the homoeologues group 1, 4, 5 and 7 can be clearly differentiated by the hybridization pattern of pTa71 and Afa family probes.

3.1.2 *In situ* hybridization with microsatellite probes

Two-step fluorescence *in situ* hybridization was used to identify the hybridization pattern of four microsatellite (SSR) probes on the chromosomes of *T. monococcum.* The first hybridization was carried out with the SSR probes $(GAA)_n$, $(CAG)_n$, $(AAC)_n$ and $(AGG)_n$. After the documentation of FISH sites, the slides were rehybridized with the standard repetitive DNA probes Afa family, pTa71 and pSc119.2.

The main distribution sites of the examined SSRs in *T. monococcum* are the follows (Fig. 2):

$(GAA)_n$: Distinctive telomeric signal on the short arm of $2A^m$ and strong centromeric signal on the $6A^m$ chromosomes.

$(CAG)_n$: Weak pericentromeric signal on $3A^m$, strong pericentromeric and intercalary signals of long arm of $6A^m$ and strong pericentromeric signals on $7A^m$ chromosomes.

$(AAC)_n$: Weak, disperse pericentromeric signals on $1A^m$, $2A^m$, $3A^m$, $4A^m$, $5A^m$ chromosomes; strong pericentromeric and intercalary signals on the long arm of $6A^m$ and strong pericentromeric signals on $7A^m$ chromosomes.

$(AGG)_n$: weak, intercalary signal on the short arm of $5A^m$ and strong pericentromeric signal on the $6A^m$ chromosomes.

FISH proved that GAA and CAG probes have the most specific hybridization pattern on *T. monococcum* chromosomes. All the SSR probes gave strong signal on the $6A^m$ chromosome, therefore SSRs represent additional landmarks for the identification of A^m chromosomes.

Comparing the SSR hybridization pattern of A chromosomes of *T. monococcum* and *T. aestivum,* we observed more complex hybridization pattern on the *T. aestivum* chromosomes. The GAA and CAG probes had significantly different hybridization patterns between the homologues of A and A^m chromosomes.

In conclusion, the fluorescence *in situ* hybridization using the repetitive DNA probes Afa family and pTa71 in combination with SSR probes, makes it possible to identify the A^m chromosomes of *T. monococcum* and to discriminate them from A^u chromosomes in the polyploid wheat background.

3.2 Chromosome pairing analysis of interspecific hybrids in meiosis metaphase I.

3.2.1 Investigation of chromosome pairing by GISH in wheat × rye F_1 hybrid

Application of fluorescent in situ hybridization in meiotic analysis was first studied in wheat × rye F_1 hybrid. The genetic distance between the two species and the relatively easy identification of wheat and rye chromosomes made this hybrid an ideal material for testing and refining this method in meiosis.

GISH resulted in strong fluorescent signal on the rye chromosomes, which confirmed the presence of seven rye chromosomes in the hybrid and allowed the discrimination of wheat and rye chromosomes. The GISH enabled the discrimination of intraspecific and interspecific chromosome associations. The number of chromosome associations was 194 in the examined 274 pollen mother cell, most of them (93.8%) were detected between the wheat chromosomes.

3.2.2 Molecular cytogenetic analysis of meiotic chromosomes by repetitive DNA probes in wheat × rye F1 hybrid

In order to identify the individual chromosome arm associations, the GISH signals were washed out and the slides were reprobed by fluorescent *in situ* hybridization (FISH) using Afa family, pSc119.2 and pTa71 probes. All wheat-wheat associations (182) were identified according to their FISH signals at genomic level, and 129 wheat-wheat associations were identified at chromosome arm level. The FISH discrimination of the constituent genomes showed that the intergenomic associations were more frequent than the intragenomic associations in the F_1 hybrid. Most of the wheat-wheat associations were found between the A and D genome (0.348 association/cell). The B-D chromosome associations were significantly lower, while the least pairing frequency were detected between A and B genome chromosomes. A more frequent pairing was observed between the homoeologous wheat chromosome arms.

3.2.3 Type and frequency of wheat-wheat chromosome arm associations

The different associations were compared on the basis of pairing frequency (number of chromosome arm association/cell). χ^2 were calculated to evaluate statistically the chromosome arm associations. The results confirmed the more frequent pairing between the chromosome arms of 3 homoeologous groups. The significantly highest pairing frequency was detected between the long arms of 3A and 3D and the short arms of 3D and 3B chromosomes.

3.2.4 Cytomolecular analysis of chromosomes of *Triticum turgidum* subsp. *durum* × *Triticum monococcum* F_1 hybrids in meiosis metaphase I.

Pairing of A and Am chromosomes was studied in *T. turgidum* subsp. *durum* × *T. monococcum* F_1 hybrids by means of sequential GISH and FISH. *Ae. speltoides* (B genome donor) and *T. urartu* (A genome donor) were used as hybridization probe. GISH resulted strong fluorescent signals on the chromosomes and allowed the discrimination of A and B chromosomes. The chromosome pairing frequency was high, the mean chromosome association nember per cell was 6.88 (total chromosome association /number of cells). The GISH made it possible to identification the paired chromosomes at genomic level. Most of the associations were identified between the A and A^m chromosomes (99.7%). A and B pairing was extremly rare (0.3%). The FISH was confirmed our results and allowed the identification of chromosomes with specific hybridization pattern.

3.3 Production of *Triticum monococcum* pre-breeding material

3.3.1 Evaluation of *Triticum monococcum* gene bank accessions

145 diploid *Triticum* gene bank accessions (domesticated and wild *T. monococcum* and *T. urartu*) were evaluated under field conditions in nursery in 2013. Remarkable powdery mildew infection was observed on *T. urartu* accesions, more than half of the accessions were susceptible to powdery mildew. In some cases leaf rust infection was also observed on *T. urartu* genotypes. Despite them, the studied *T. monococcum* accessions were resistant to the natural powdery mildew and laf rust infection. The largest diversity was observed in heading date. The earliest genotype headed in 13th May and the latest in 17th June.

The knowledge of heading date is important in the planning of T. *monococcum* × *T. aestivum* crossing programmes.

3.3.2 *Triticum aestivum* × *Triticum monococcum* crossings

The crossability of *T. monococcum* with wheat was studied between two wheat genotypes (Mv9kr1-wheat genotype with crossability allele *kr1* and Mv9-wheat variety without crossability allele) and two einkorn genotypes (Mv Alkor- a traditional type and 1T-1- a semi-dwarf type of einkorn). Pollinators were the einkorn genotypes.

F_1 seeds were get from all combination, but crossings with semi-dwarf einkorn were more effective (better seed set). The F_1 seeds derived from wheat × 1T-1 crossings had higher germination rate. Only 10 % of hybrid seeds of Mv9kr1 × Mv Alkor combination germinated, and the majority of plants died before heading. The seeds of Mv9kr1 × 1T-t germinated at 60-70% level.

The chromosome number of F_1 seeds were checked on mitotic chromosome preparation ($ABDA^m$; 2n=4x=28) and further FISH confirmed the presence of 14 A, 7B and 7D chromosomes.

The sterile F1 hybrids were backcrossed with wheat (Mv9kr1) through several generation, to produce fertile plants with similar chromosome number to hexaploid wheat. The first backcrossing is the hardest, not more than 0.86 % of the pollinated flowers were produced seed. At second and third backcrossing the seed set was better. Molecular cytogenetic evaluation was also performed in the progenies. FISH of BC_1 plants made it possible the identification of wheat and *T. monococcum* chromosomes. The mean number of *T. monococcum* chromosomes was 2.4 per plant. The number of identified *T. monococcum* chromosomes of BC_2 plants (1.8 A^m chromosome/BC_2 plant) was lower than in BC_1 plants. All the identified *T. monococcum* chromosomes have specific hybridization pattern, it is not impossible that further *T. monococcum* chromosomes and chromosome segments are in the progenies, which are unidentifiable using repetitive DNA probes.

3.3.3 Artificial leaf rust infection of BC_3 plants

The fertility of the backcrossed plants was increased in every backcrossed generation. After the third backcrossing we obtained sufficient number of seeds to test their resistance to leaf rust.

The leaf rust resistance of BC3 plant were evaluated in greenhouse by artificial leaf rust infection at seedling stage. The majority of BC_3 plants (85%) were susceptible to leaf rust, but we identified resistant and immune plants.

FISH was carried out on the mitotic chromosome preparation of immune BC_3 plants. Whole *T. monococcum* chromosome was not detected in the studied plants.

3.3.4 *Triticum turgidum* subsp. *durum* × *Triticum monococcum* crossings

Traditional (Mv Alkor) and semi-dwarf (1T-1) *T. monococcum* genotypes were crossed with tetraploid durum wheat, in order to produce synthetic amphiploid ABA^m genome constitution. The seed set was very low, significant difference was not observed between the *T. monococcum* pollinators.

Production of synthetic amphiploid

The chromosome number of F_1 seeds (2n=3x=21, ABA^m) was cytologically contolled, and FISH analysis made it possible the identification of 14 A and 7 B chromosomes. The genome duplication was carried out by colchicine treatment. 63 amphiploid seeds were produced (seed set: 16.3 %).

The C_3 genereation of *T. turgidum* subsp. *durum* – *T. monococcum* amphiploids are late heading type, as the *T. monococcum* parent. The morological characteristics of amphiploids are sinilar to the durum wheat parent.

The genetic stability of amphiploid was confirmed by *in situ* hybridization on mitotic chromosome preparation of C_3 generation plants. The ABA^m genome constitution was confirmed by means of GISH. *T. urartu* genomic DNA hybridization probe resulted strong fluorescent signal on the A chromosomes and allowed the discrimination of 28 A and 14 B chromosomes. The similarity of *T. monococcum* and *T. urartu* did not permit of the discrimination of A and A^m genomes. Sequential FISH on the same chromosome preparation confirmed our results and allowed the identification of chromosomes.

3.4 New scientific results

1. Karyotype of *Triticum monococcum* was developed by means of fluorescent in situ hybridization using repetitive DNA probes Afa family and pTa71, which allowed the discrimination of 1A, 4A, 5A and 7A homoeologues chromosomes of wheat and *T. monococcum.*
2. Hybridization patterns of GAA, CAG, AAC and AGG microsatellite probes were determined on the *T. monococcum* chromosomes. GAA and CAG probes are the most effective for identification of A^m chromosomes combined with repetitive DNA probes.
3. Wheat-wheat chromosome pairings and chromosome arm associations were identified in meiosis by sequential genomic and fluorescent *in situ* hybridization in wheat × rye F_1 hybrids. The method confirmed the preferential pairing of A and D chromosomes (53%). The most frequent pairings were observed between the long arms of 3A and 3D and between the short arms of 3D and 3B chromosomes.
4. Pairing of A and A^m chromosomes were detected by genomic *in situ* hybridization in *T. turgidum* subsp. *durum* × *T. monococcum* hybrid.
5. Crossing programme was started to transfer useful properties of T. monococcum into hexaploid wheat. Good crossability and leaf rust resistance of 1T-1 semi-dwarf einkorn line was detected.
6. *T. aestivum* (Mv9kr1) × *T. monococcum* (1T-1) BC3 plants were produced. Among the BC3 plant leaf rust resistant and immune plants were detected by artificial infection at seedling stage.
7. Synthetic amphiploid with was produced by crossing of *T. turgidum* subsp. *durum* and *T. monococcum*. The ABA^m genome constitution of amphiploid was confirmed by means of genomic and fluorescent *in situ* hybridization.

4 CONCLUSIONS AND RECOMMENDATIONS

Identifications of *Triticum monococcum* chromosomes by molecular cytogenetic methods

We identified significant differences between the FISH karyotypes of *Triticum monococcum* and A genome of hexaploid wheat using Afa family and pTa71 repetitive DNA probes and GAA, CAG, AAC and AGG microsatellite probes. The differences could be explained by sequence-elimination, mutation and intergenomic chromosome rearrangement which occurred during the evolution of hexaploid wheat. The A^m chromosomes of *T. monococcum* and the A chromosomes of tetra- and hexaploid wheat could not be discriminate according their repetitive DNA hybridization signals in all homoeologues group in the same genetic background. Microsatellites, as hybridization probes, increased the diagnostic bands on A chromosomes and allowed the discrimination of 6A and $6A^m$ chromosomes. Testing of further hybridization probes would be expedient for the better identification of A and A^m chromosomes.

According to our results the A and A^m genomes cannot be discriminate by means of genomic *in situ* hybridization. The use of molecular markers is recommended to follow the translocations in wheat-einkorn pre-breeding material. To achieve this, it is necessary to identify molecular markers linked to *T. monococcum* chromosomes.

Analysis of chromosome pairing

Sequential genomic and fluorescent *in situ* hybridization of interspecific hybrids in meiosis metaphase I. allowed the identification of chromosome pairings and furthermore the identification of chromosome arm associations.

The homology of chromosomes in wheat × rye hybrids did not studied before by sequential GISH and FISH. Our results confirmed the earlier results found in the literature. Analysing of paired chromosomes according their FISH signal enabled the identification of 3DS–3BS chromosome arm association, which was the most frequent among the observed associations and never referred in the literature earlier. Our results confirmed the possibility of use of FISH in meiosis. Therefore the sequential use of FISH and GISH is able to maximize the amount of information accessible from a cytological preparation also in meiosis.

We demonstrated the frequential pairing of A^m and A chromosomes in durum wheat × einkorn F_1 hybrids, which confirmed the good combination ability of A^m chromosomes of *T. monococcum* and the A chromosomes of hexaploid wheat.

Production of *Triticum monococcum* pre-breeding material

The successful gene transfer from *T. monococcum* into wheat is a circuitous and longer process, than a PhD project, but our initial results are promising.

We successful developed durum wheat × einkorn and hexaploid wheat × einkorn hybrids using semi-dwarf einkorn line as pollinator. Wheat × einkorn F_1 hybrids were backcrossed with wheat and BC_3 plant were produced, durum wheat× einkorn hybrids was the basis material to produce ABA^m amphiploids. The BC3 plants and the amphiploids are actually under testing their phenotype in the nursery, but their further use in wheat breeding is very promising, because leaf rust resistant genotypes were identified by artificial infection.

A large genetic diversity could be find among the *T. monococcum* accessions of Martonvásár Cereal Gene Bank. Good crossable einkorn genotypes enable the utilization of further einkorn accessions with good agronomic traits. The good crossable accession could be used as bridge between the wheat and einkorn genotypes with poor crossability.

The main requirement of use of further *T. monococcum* genotypes in pre-breeding is the better characterization of einkorn collection of Martonvásár Cereal Gene Bank.

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