Aegilops biuncialis is closely related to *Triticum* and represents a rich source of agronomically important traits including resistance to pests and diseases, grain dietary fibre content, abiotic stress tolerance and adaptability. The main goal of the project was to directly support the wheat breeding for protection against leaf rust and for increasing grain edible fiber content by providing genomic resources and molecular tools supporting the efficient transfer of *Aegilops* chromosome segments into wheat and for later physical mapping and cloning of resistance genes and by providing prebreeding material for the breeders.

The grant has been divided into 4 work packages:

(1) Production of *Ae. biuncialis* genetic map
(2) Design of gene-based markers
(3) Determination of genomic regions linked to leaf rust resistance and edible fiber content in *Ae. biuncialis*
(4) Production of new wheat-*Aegilops* introgression lines

(1) Production of *Ae. biuncialis* genetic map
The *Aegilops biuncialis* Vis. (2n=4x=28, $U^bU^bM^bM^b$) accession MvGB382 (ICAG401297) collected from Iran and its natural habitat characterized by 320m above sea level and 500mm per year annual precipitation. The accession MvGB642 (ICAG400940) collected from Syria from an 1160m above sea level habitat with annual precipitation of 1143mm per year. These *Ae. biuncialis* accessions were maintained by self-fertilization for more than twenty years in the ATK MGI. The accession MvGB642 have been crossed with MvGB382 as a pollinator and the F_1 plants were self-pollinated to obtain F_2 seeds. The *Ae. umbellulata* AE740/03 and *Ae. comosa* MvGB1039 accessions used to flow sort individual U- or M-genome chromosomes (1U-7U, 1M-7M) (Molnár et al. 2016) were also investigated.

Genotyping of the mapping population and designing the genetic map
Total genomic DNA was isolated from the parental *Ae. biuncialis* accessions MvGB642 and MvGB382, from 262 MvGB642 x MvGB382 F_2 genotypes, and from the *Ae. umbellulata* AE740/03 and *Ae. comosa* MvGB1039 accessions and used for genotyping together with DNA of individual chromosomes (1U-7U, 1M-7M) flow-sorted from the diploid *Ae. umbellulata* AE740/03 and *Ae. comosa* MvGB1039. DNA samples were sent to Diversity Arrays Technologies Pty. Ltd., Australia (http://www.diversityarrays.com) in order to determine their allele composition using 'wheat DArTseq™ 1.0' platform.
The generated 6273 co-dominant SNP-DArT and 47121 dominant Silico-DArT markers were filtered on the basis of individual marker-related statistics and the markers with <0.95 Call Rate, <5% Minor Allel Frequecy and <0.95 Reproducibility were removed. During the map construction the F_2 genotypes with more than 10% of missing data points were excluded from the analysis. The markers exhibited inappropriate segregation rate (co-dominant SNP markers: 1:2:1; dominant Silico-DArT markers: 1:3) were also excluded. Finally we used 224 F_2 genotypes, 5893 SNP-DArT and 22,180 Silico-DArT markers for constructing a genetic map of *Ae. biuncialis*.
For the map construction we used the MultiPoint software (www.multigtl.com) which constructs maps by using re-sampling techniques (jackknife) and detect markers causing neighborhood instabilities. As a first step, a skeleton map was constructed from the co-dominant markers. Non-polymorphic markers and markers with heterozygous genotype on at least one of the parental lines have been eliminated. The setting 'F2_population' with a maximum threshold rf_s value of 0.25 was used to initially group the markers into sixteen linkage groups (LGs). Multipoint linkage analysis of loci for each LG was carried out and marker order was further optimized by re-sampling for quality control through jack-knifing (Mester et al. 2003). The ordered markers with a jack-knife value ≥ 90% were included as ' skeleton ' markers, while the

remaining markers causing unstable neighborhoods were initially excluded from the map, including the redundant markers with the same map location.
As a second step, the skeleton map was saturated using the previously excluded dominant Silico-DArT markers (separately by dominant markers specific for the maternal and paternal genotypes) by inserting them to the most optimal intervals on the framework map and labelled as attached markers. The redundant markers labelled as delegates were also included on the final, complete map.

Assigning linkage groups to chromosomes
In order to assign linkage groups to chromosomes we used the allelic data of diploid *Ae. umbellulata* (UU) and *Ae. comosa* (MM) and the data of their flow sorted chromosomes. We also took into account the wheat-*Aegilops* homoeologous relationships previously reported for the chromosomes of *Ae. umbellulata* using biparental segregating genetic map (Zhang et al. 1998, Edae et al. 2017).
In order to obtain data for the synthenic relationships between the LGs of the preset map and the chromosomes of bread wheat, a sequence homology search (BLASTn) of trimmed sequences of the mapped markers of each linkage groups was performed on the wheat pseudomolecules (v.2.0) (IWGSC 2018) using the blastn package of the Blast Command Line Application 2.9.0 (ftp://ftp.ncbi.nlm.nih.gov/) with the following parameters: -task 'blastn'; -evalue 1e-5; -max_target_seqs 1; -max_hsps 1. Chromosome location and start position of the best hits were used to investigate collinearity between the LGs and wheat chromosomes. Finally, the linkage groups were assigned to *Ae. biuncialis* chromosomes, $1U^b$-$7U^b$ and $1M^b$-$7M^b$ according to recent nomenclature (Badaeva et al. 2004). The Kosambi (Kosambi 1944) mapping function was used to calculate the centimorgan (cM) values. The graphical representation of the map was drawn using Strudel software (https://ics.hutton.ac.uk/strudel/).
The present version of the genetic map contains 16 linkage groups (LGs), where the chromosomes 5U and 6U represented by two and two LGs, respectively. The total length of the *Ae. biuncialis* map is 2618.26 cM, which is comparable with the total map length of durum wheat (2317 cM; Theor. Appl. Genet. 117:103-115.), another allotetraploid Triticeae species. The present version of the map containing 975 individual loci. The average distance between two loci is 2.72 cM and the biggest gap is 23.94 cM. We mapped a total of 11317 markers.

The genetic map of *Ae. biuncialis* allowed us to investigate wheat-*Aegilops* synteny by sequence similarity search (BLASTn). The genetic map will also used to ordering the shot-gun sequences of chromosomes flow sorted from *Ae. umbellulata* and *Ae. comosa*, the diploid progenitors of U and M genomes, respectively. The production of recombinant inbreed lines from the F2 individuals of the *Ae. biuncialis* mapping population is also in progress, which will open the way for QTL mapping QTLs for leaf rust resistance, drought and salt tolerance, root architecture and edible fiber content.

Table 1. Descriptive statistics of the *Ae. biuncialis* F_2 segregating genetic map

	LG	No. of markers	No. of individual loci	Average gap (cM)	Bigest gap (cM)	Total map size (cM)
1M	LG16	636	50	0.67	2.04	33.58
1U	LG13	538	62	2.41	7.92	149.78
2M	LG7	982	85	2.02	11.73	172.44
2U	LG6	584	66	3.43	17.9	226.82
3M	LG4	1466	84	2.48	13.54	209.13
3U	LG5	518	58	2.51	14.64	145.59
4M	LG1	662	59	2.39	11.6	141.03
4U	LG11	454	43	3.67	23.94	158.13
5M	LG14	1292	88	2.56	12.02	225.49
5U_1	LG3	500	55	2.16	10.26	118.88
5U_2	LG9	257	48	2.94	17.26	138.59
6M	LG2	1023	66	2.26	9.2	149.61
6U_1	LG10	554	53	4.88	68.6	258.88
6U_2	LG12	353	51	3.22	8.21	164.62
7M	LG8	988	50	2.55	10.79	125.39
7U	LG15	510	57	3.51	10.72	200.3
Total:		11317	975	2.72	23.94	2618.26

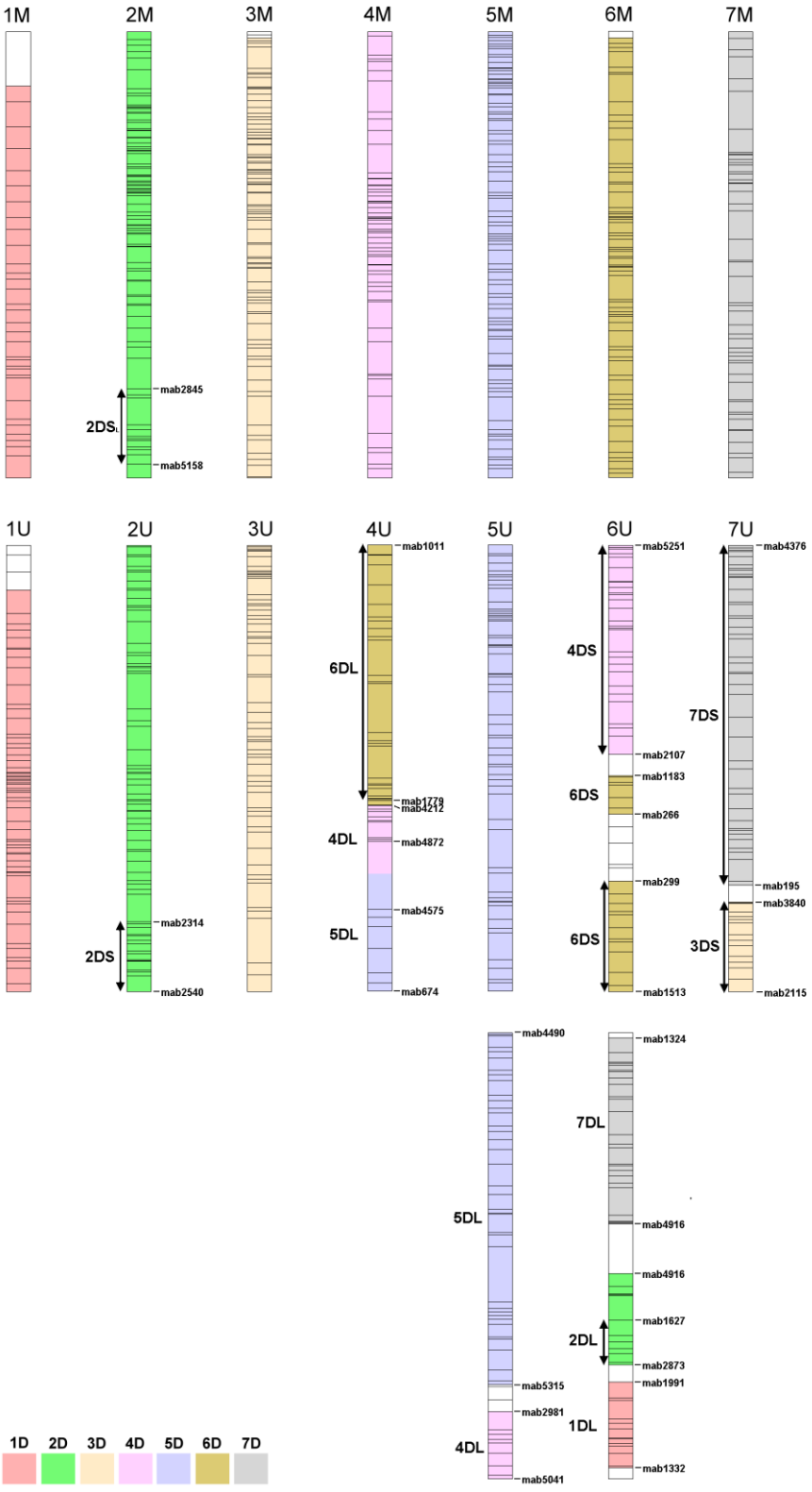

Fig. 1. Graphical representation of the *Ae. biuncialis* skeleton map. Colour code shows the wheat-*Aegilops* homeologous relationships. Arows indicate inversions of the specific chromosomal region relative to the corresponding wheat (D-genome) chromosome.

(2) Design of gene-based markers

As a second task within the project, we designed gene-based molecular markers specific for the *Aegilops* chromosomes.
As a first step, conserved ortholog set (COS) markers with known chromosome deletion bin map positions on wheat were tested on the flow sorted chromosomes 1U-7U of *Ae. umbellulata* and 1M-7M of *Ae. comosa*. One hundred markers resulted in 137 and 131 PCR products in *Ae. umbellulata* and *Ae. comosa*, respectively (Molnár et al. 2016). Out of 118 loci assigned to U-genome chromosomes of diploid *Ae. umbellulata*, 63 loci (53.38%) were polymorphic (≥5 bp) relative to wheat cv. GK Öthalom. In *Ae. comosa*, where 114 loci were mapped to M-genome chromosomes, 53 loci (46.49%) were polymorphic. **We found 21 COS markers specific for the chromosomes of *Ae. umbellulata*** (1U:3; 2U:4; 3U:3; 4U:1; 5U:4; 6U:4; 7U:2) and **14 for *Ae. comosa*** (2M:1; 3M:9; 4M:3; 7M:1), while 29 markers were specific for both U and M genomes.
Because of the (COS) markers have known positions on the deletion bin map of A,B and D genomes of wheat, these COS markers have been used to study the homoeologous relationships of wheat and *Aegilops* chromosomes (Molnár et al. 2016). This approach developed by the project have also been applied to study the genome relationships between wheat and other wild gene source species used in the introgression breeding programs in Martonvásár, such as *Thinopyrum elongatum* (Gaál et al. 2018) and *Agropyron cristatum* (Said et al. 2019ab).

In order to produce new gene specific markers, we also aligned wheat EST (Expressed Sequence Tag) sequences covering the group I-VII chromosomes of wheat (Sum of aligned ESTs=4456; mean no. of ESTs / chromosome: 636.5) to chromosome contigs of *Ae. umbellulata.* Using pairwise alignment, we identified polymorphic (>5bp INDELs) hits with 399 ESTs on *Ae. umbellulata* chromosomes (1U:48, 2U:90, 3U:56, 4U:44, 5U:32, 6U:82, 7U:47). Out of the **313 primer pairs designed for the polymorphic regions of 204 ESTs**, 124 primer pairs were tested by PCR on the wheat (Mv9kr1), *Ae. biuncialis* (MvGB642, MvGB382, MvGB1112 and MvGB470) and on *Ae. umbellulata* (AE740/03). Fifty markers (40.3%) produced polymorphic amplicons between wheat and *Aegilops* accessions. We wanted to know the chromosome location of the polymorphic markers, so we assigned the markers to *Aegilops* chromosomes using wheat-*Aegilops* chromosome addition lines representing the chromosomes 1U-7U and 1M-7M. Out of the 34 tested markers, we assigned 23 markers to single chromosomes (1U: 6, 2U:1, 4U:3, 5U:6, 6U:4, 7U: 1, 1M: 1, 5M:1). These markers will be suitable to select wheat lines with the corresponding *Aegilops* chromosomes. PCR validation of the remaining markers is going on which will provide additional markers for the *Aegilops* chromosomes.

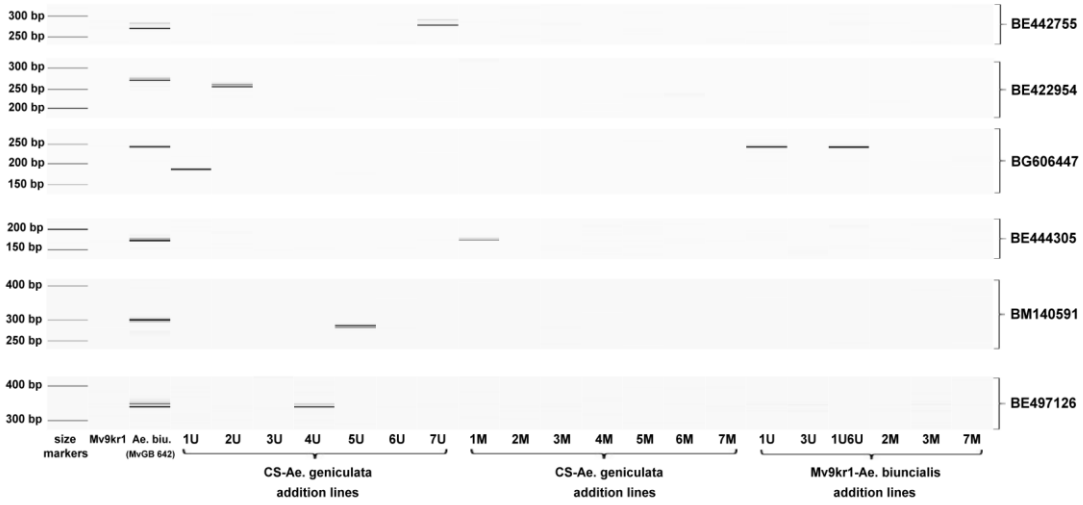

Fig. 2. Assignment of molecular markers to chromosomes of *Aegilops*. Representative electrophoretic patterns of DNA fragments amplified by PCR using total genomic DNA of wheat cultivar Mv9kr1, *Ae. biuncialis* MvGB642 and wheat (Mv9kr1)-*Ae. biuncialis* (MvGB642) and wheat (Chinese Spring) –*Ae. geniculata* addition lines. A 35-500 bp DNA ladder was used as size marker to estimate the fragment size.

The first strategy to produce gene-specific markers was very time consuming. Therefore we applied a second strategy to produce intron targeting markers for *Aegilops* chromosomes. During this approach first we mapped *Ae. umbellulata* genes (gene models and chromosomal sequences) to the reference sequences of wheat (wheat pseudomolecules: RefSeq v1.0, IWGSC 2018) and compared the wheat and *Aegilops* sequences of first exon and intron. When we found wheat-*Aegilops* polymorphism a primer pair were designed targeted to the last 100 bp region of the exon (forward primer) and to the first 500 bp region of the intron (as reverse primer). Finally, 3095 polymorphic *Ae. umbellulata* contigs were used for designing 15,475 primer pairs (5 primer pairs per contig). For PCR validation on wheat Mv9kr1 and *Ae. biuncialis* MvGB642 genotypes (the parental lines of the BC population) 84 primer pairs (12 markers / chromosome) were randomly selected and 31 (36.9%) showed significant polymorphism (22 produced yes/no results on *Aegilops* and wheat, while 9 markers showed size polymorphism).

Summarizing the results, **we designed 15,788** (313 + 15,475) **primer pairs** as potentially new gene specific markers for the chromosomes of *Aegilops*. The PCR validation of these markers is in progress. **We assigned 58** (35 COS and 23 intron targeted) **markers to single U- or M-genome chromosomes by PCR**, which were started to use for the marker-assisted screening of wheat-*Ae. biuncialis* prebreeding population.

(3) Determination of genomic regions linked to edible fiber content and leaf rust resistance in *Ae. biuncialis*

To provide information about the chromosomal location of genes responsible for the grain edible fiber content we investigated the grain protein, arabinoxylan and β-glucan content of Mv9kr1-*Ae. biuncialis* addition lines $1U^b$, $1U^b$/$6U^b$, $3U^b$, $2M^b$, $3M^b$, $7M^b$ and Chinese Spring-*Ae. geniculata* addition lines $1U^g$-$7U^g$, $1M^g$-$7M^g$. The growing conditions and determination of the nutritional parameters were described in the publications of the results (Rakszegi et al. 2017; 2019). Briefly, we observed that *Aegilops* chromosomes $2U^g$, $4U^g$, $5U^g$, $7U^g$, $2M^g$, $5M^g$ and $7M^g$ and $3U^b$, $2M^b$, $3M^b$ and $7M^b$ increased the protein content of wheat. Both ***Aegilops* species had higher ratios of β-glucan to arabinoxylan than wheat lines, indicating that β-glucan is the dominant form in this Aegilops species**. We observed that **5U, 7U and 7M chromosomes** of both Aegilops species **were able to increase the β-glucan content of wheat**. Total arabinoxylan in wheat was increased by the addition of chromosome $5U^g$, $7U^g$, $1U^b$, while **water-soluble arabinoxylan was increased by the $5U^g$, $5M^g$, 7M**, and further chromosomes 3U, 4U,

6U^{g} and 2M^{b} with smaller effects (Rakszegi et al. 2017, 2019). These results will help to map genomic regions responsible for edible fiber content in *Aegilops* and will facilitate the gene transfer to obtain wheat varieties with improved health benefits.

We investigated the genetic diversity and population structure of an *Ae. biuncialis* collection (86 genotypes representing the geographical distribution of the species) by 32,700 DArT markers with simultaneous application of three statistical methods - Neigbour Joining clustering, Principal Coordinate Analysis (PCA) and the Bayesian approach. The collection was divided into five sunpopulations (A-E) that correlated well with their eco-geographic habitat: A- 6 genotypes from North Africa, B - 14 genotypes mainly from Balkan and North Greece, C -3 mixed genotypes from Near East, D – 31 genotypes from South-Peloponnese, Asia-minor and Crimea and E - 29 genotypes from the Levant region (Syria, Jordan, Israel) and Azerbaijan (Ivanizs et al. 2019). We also phenotyped of this collection for the heading time, which is the major component for the adaptation of plants to different eco-geographical environments, and the presence of leaf rust resistance over three years and the grain quality traits (protein-, β-glucan and arabinoxylan content) over two years.
ANOVA and Principal Component Analysis of the collection identified four phenotypic heading time categories associated with the genetic structure and geographic distribution, except for minor differences (Ivanizs et al. 2019). Subpopulations A and E, originating from North Africa and the Middle East, showed earliest heading time, whereas subpopulation D, mainly from Asia Minor, characterized by medium heading phenotype. Subpopulation B, derived from the Balkans, exhibited the latest heading time.
The variation in the grain protein, β-glucan, total- and water-extractable arabinoxylan content in the *Ae. biuncialis* collecion were also investigated in two years and the mean values varied in the range of 21.2-33.25 mg/g, 21.13-54.91 mg/g, 33.2-48.69 mg/g and 7.47-15.44 mg/g, respectively. The same quality parameters in the control wheat genotype (Mv9kr1) exhibited 12.9 mg/g, 9.44 mg/g, 40.13 mg/g and 10.82 mg/g values, respectively. These results indicate that the Ae. biuncialis population has enough genetic variation for improving the edible fiber, especially the β-glucan and water-extractable arabinoxylan content of wheat.
Of each quality traits, only the storage protein content correlated with the geographic distribution of the *Ae. biuncialis* accessions. The accessions from the Balkan Penninsula, West Turkey and Crimea showed generally low protein content. The genotypes collected in Azerbaijan, South Turkey and Syria had middle-rated protein content, whereas accessions from Jordan, Israel and North Africa exhibited higher grain protein content.

The leaf rust resistance of the collection was also evaluated (inoculation with a mixture of leaf rust uredospores collected from varieties with various genetic backgrounds at seedling stage, under greenhouse conditions) in three years (2016, 2018 and 2020). **Twenty-one genotypes out of 86 (24.4%) showed resistance (hypersensitive/immune reaction) to leaf rust in all three years**, while fourty genotypes (46.5%) were higly susceptible (with 3-5 Stakman value). The subpopulations B and D contained resistant genotypes (5/13 and 10/31, respectively) in the highest percent (35.7% and 32.2%, respectively) while the subpopulation E contained significantly less resistant genotypes (3/29; 10.3%). On the other hand, the percent of highly susceptible genotypes in subpopulations B, D and E was 7.1%, 35.4% and 75.8%, respectively. These results indicate that *Ae. biuncialis* accessions collected from the balkanian countries (subpopulation B) contains effective resistance genes in the highest percent against the hungarian pathotypes of *P. triticina*.
In order to transfer suitable genetic variation of *Ae. biuncialis* for **leaf rust resistance into wheat**, **we selected six accessions** from different subpopulations (**MvGB1723; D: MvGB1733, E: MvGB1987, B: MvGB1714, MvGB380, MvGB1745**) **for the production of F_1 hybrids with Mv9kr1*ph1b* genotypes**.

The majority of disease resistance genes that have been identified so far encode for proteins with a nucleotide binding site and leucine rich repeats (NLRs). We investigated the repertoire of these NLR genes in the genome of *Ae. umbellulata* (accession AE740/03) the U genome progenitor of *Ae. biuncialis*. Because NLR genes are generally difficult to annotate with standard gene annotation pipelines, we used the *ab initio* tool NLR-Annotator to directly look for signatures of NLRs (highly

conserved amino acid motifs) within the genome sequence (Steuernagel et al. 2018, https://github.com/steuernb/NLR-Annotator). Default parameters were used for the analysis. If needed, scaffolds were fragmented in 20kb sub-sequences, overlapping by 5kb. Highly conserved amino acid motifs of each locus were extracted as a multiple alignment using -a option of NLR-Annotator. Motifs of cloned R genes (CZT14023.1, ADH04488.1, AGY30894.1, ABS29034.1, AAQ01784.1, ACO53397.1, CUM44200.1, AGQ17382.1, AGP75918.1, CUM44213.1, AYV61514.1, ALO61074.1, ACG63518.1, AAQ55541.1, ADH59445.1 were added to the multiple alignment.

Totally, we found 1,223 NLR loci, of which **601 were supposed to represent complete genes**. The others either lacked motifs associated with LRRs or the p-loop of the nucleotide binding site. Sixty-four NLRs with complete structure represented most likely pseudo-genes due to a stop-codon present in one of the highly conserved motifs. The NLRs are fairly distributed among the chromosomes, ranging from 90 NLRs on chromosome 5U to 308 NLRs on chromosome 6U (Table 2).

Table 2. Annotated NLRs per chromosome

	NLR	**complete**	**complete (pseudogene)**	**partial**	**partial (pseudogene)**
1U	136	73 (53.6%)	6 (4.6%)	48 (35.2%)	9 (6.6%)
2U	202	87 (43.0%)	8 (3.9%)	100 (49.5%)	7(3.6%)
3U	139	49 (35.2%)	5 (3.5%)	72 (51.7%)	13 (9.6%)
4U	94	28 (29.7%)	2 (2.3%)	57 (60.6%)	7 (7.4%)
5U	90	34 (37.7%)	4 (4.4%)	49 (54.4%)	3 (3.5%)
6U	308	133 (43.1%)	20 (6.4%)	131 (42.5%)	24 (0.8%)
7U	254	133 (52.3%)	19 (7.4%)	90 (35.4%)	12 (4.9%)

The annotated scaffolds of *Ae. umbellulata* chromosomes have been used for fine mapping of *Lr76* and *Yr70* resistance genes in a wheat-*Ae. umbellulata* T5D-5U introgression line (Bansal et al. 2020). Within an international cooperation, we found that the ***Ae. umbellulata* 5U segment carrying NLRs as candidates for *Lr76* and *Yr70* was located on a 9.47 Mb region on chromosome arm 5DS** (Bansal et al. 2020).

(4) Production of new wheat-*Aegilops* introgression lines

In order to select new wheat-*Ae. biuncialis* introgression lines, which was the main goal of WP4, we investigated the karyotypic constitution of a wheat (Mv9kr1) x *Ae. biuncialis* prebreeding population containing amfiploids, and backcrossed (BC) progenies. We determined the karyotypes by the molecular cytogenetic methods, sequential FISH with probes pSc119.2, Afa family and pTa71 and multicolour GISH with U- and M-genomic probes to identify new *Aegilops* chromosomes in the wheat genetic background. The following *Aegilops* chromosomes, not represented before in the addition lines, were identified in the 216 BC2-3 progenies of wheat (Mv9kr1) x *Ae. biuncialis* hybrid combinations with different *Ae. biuncialis* accessions, MvGB642: 2U, 4U, 5U, 6U and 6M; MvGB382: 1U-4U, 2M, 3M, 4M, 6M and 7M; MvGB1112: 5U, 7U, 1M-7M. The selection of disomic addition lines containing these chromosomes is in progress.

We also obtained wheat-*Ae. biuncialis* MvGB642 translocations (Fig. 8.) which was the most important goal of the project. A terminal translocation T1DL.1DS-U was detected in the genotype 181584 and selected the line 191922 containing this translocation in disomic form. A disomic T2DS.2DL-U translocation was also identified in eight genotypes. We identified the T4DL.4DS-M and T5MS.5ML-

D monosomic translocations in the line 181663. In the genotype 191977 a disomic T4DS.4DL-M translocation was also identified. Moreover, we also identified a disomic T5DS.5DL-M translocation in 191891 genotype. We also detected reciprocal wheat-*Aegilops* translocations involving the wheat chromosome 6B and an M chromosome (T6BS.6BL-M, TML.MS-6BL) in the genotype 192048 and another genotype (192003) contained the translocations 1US.2BL and 1UL.D translocations.

In 2018, 48 wheat (Mv9kr1) x Ae. biuncialis (MvGB642) BC progenies and translocations were tested for leaf rust resistance using the artificial leaf rust resistance test in the glasshouse as described before. We found 3 BC_1, 1 BC_2 and 9 BC_3 resistant plants (no pustules on upper surface of leaves). Out of the 10 plants containing 1DL.1DS-U translocation 8 were resistant (0-3 pustules) and 2 were susceptible (12-31 pustules), while all of the three tested genotypes containing 5DS.5DL-M translocation were resistant (0-1 pustules).

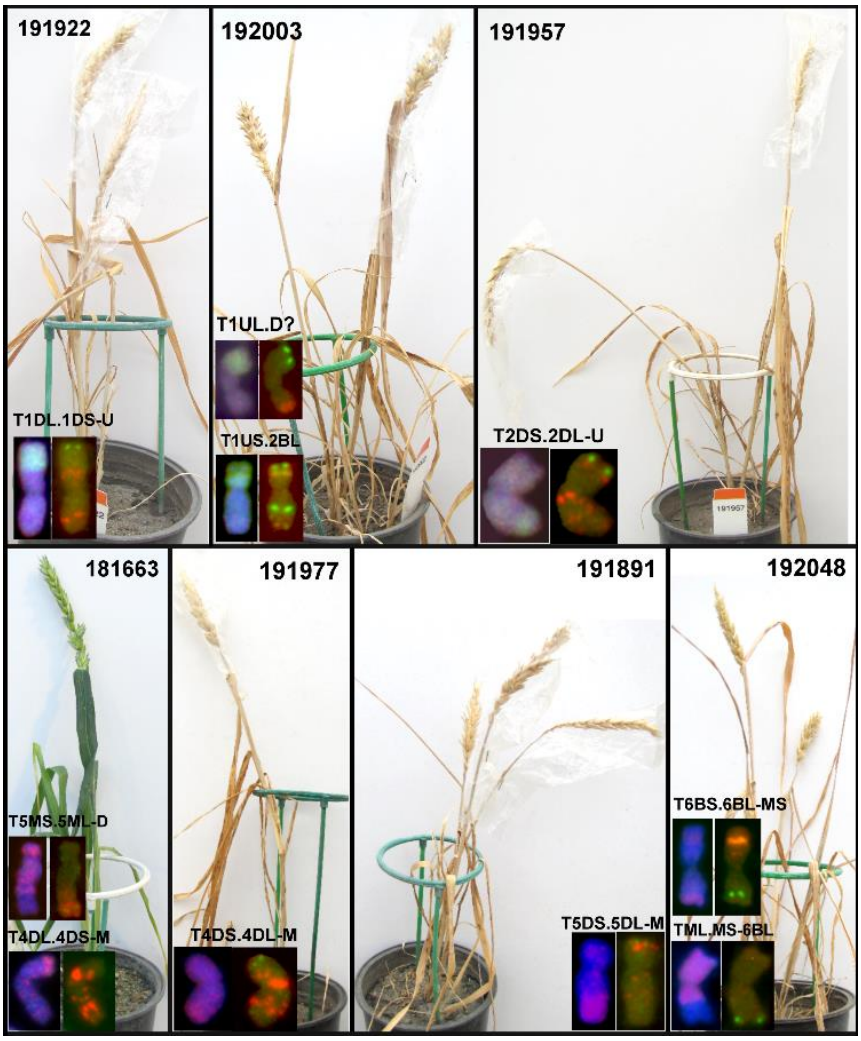

Fig. 3. The wheat (Mv9kr1) x Ae. biuncialis introgression lines developed by the project. Inserts: GISH and FISH pictures of the wheat-Aegilops translocation chromosomes.

Table 3. Morphological traits and yield components of the wheat-*Ae. biuncialis* introgression lines.

Genotype	Plant height (cm)	Tillering (spikes/plant)	Length of main spike (cm)	Spikelets/main spike	Seeds/main spike	Seeds/plant
191922	28	3	9.5	21	25	25
192003	35	1	7.5	18	26	26
191957	42	3	9	19	39	59
181663	n.d.	1	n.d.	n.d.	4	4
191977	44	1	11.5	20	8	8
191891	34	3	8	12	3	3
192048	36	3	5.5	9	18	39

In order to transfer different leaf rust resistance genes from *Ae. biuncialis* into wheat, six *Ae. biuncialis* accessions selected in WP3 for the presence of leaf rust resistance during three years, were used as crossing partners with the wheat genotype Mv9kr1*ph1b*. We produced a total of 2232 F_1 hybrid seeds, 180 of them were treated with colchicine **resulting in 56 amphiploid seeds with five *Ae. biuncialis* accessions** (Table 4). The wheat genotype Mv9kr1*ph1b* has a good agronomical performance and lacking the *Ph1* locus, the major locus responsible for homolog chromosome pairing in wheat. Therefore, it will be realistic to select new wheat-*Aegilops* homoeolog recombinations in these Mv9kr1*ph1b* x *Ae. biuncialis* hybrid progenies.

Table. 4. The number of Mv9kr1*ph1b* x *Ae. biuncialis* F_1 hybrids, of the colchicine treated plants and the obtained amphiploid seeds

Crossing combination	F_1 hybrids	No. of F_1 plants treated with colchicine	No. of amphiploid seeds
Mv9Kr1ph1b x MvGB 1723	166	10	4
Mv9Kr1ph1b x MvGB 380	346	40	1
Mv9Kr1ph1b x MvGB 1733	442	40	14
Mv9Kr1ph1b x MvGB 1987	611	50	31
Mv9Kr1ph1b x MvGB 1714	381	40	6
Mv9Kr1ph1b x MvGB 1745	286	-	-
Total	2232	180	56

The results of the project K116277 were published in 9 scientific papers (Sum IF: 34.469) and disseminated in 8 (4 international and 4 national) scientific conferences.