FINAL REPORT (NKFI K119387)

Development and molecular genetic/cytogenetic identification of wheat-perennial rye (*Secale cereanum*) introgression lines as new genetic resources for wheat breeding

The perennial *S. cereanum* rye cultivar Kriszta originates from the cross between the *S. cereale* cv. Várda and the wild mountain rye *S. strictum* (syn. *S. montanum*) ssp. *anatolicum*, and was developed and registered in Hungary in 2001. Owing to its good nutritional parameters, winter hardiness, drought tolerance, and resistance to leaf rust, stem rust, yellow (stripe) rust, and powdery mildew, 'Kriszta' is a useful gene source for widening the genetic variability of the bread wheat. In the project, three main goals were set, which were successfully achieved.

Results

1. Cytogenetic and molecular genetic identification of the rye chromosomes or chromosome segments in the backcrossed and selfed progenies of the Mv9kr1 (wheat) × 'Kriszta' hybrid

In 2001, the hexaploid winter wheat line Martonvásári 9 kr1 (Mv9kr1) was crossed with 'Kriszta' rye to transfer its beneficial traits into wheat. Since then, numerous backcrossed and selfed progeny plants carrying rye introgression have been produced. For their future usability in different pre-breeding processes (e.g. tests for biotic and abiotic stresses or quality traits), determination of the precise composition of the introgression lines is essential. The present project aimed to set up a detailed karyotype for 'Kriszta' based on chromosome measurements and FISH (fluorescence *in situ* hybridization), as well as to determine individual chromosome arm landmarks using subtelomeric tandem repeat and synthetic microsatellite DNA sequences, which enable us to unambiguously identify 'Kriszta' chromosome arms in the wheat-perennial rye translocation lines.

The chromosome morphology analysis of 'Kriszta' and its parental rye species performed with the use of the freely available DRAWID software version 0.26 (Kirov et al. 2017) showed that all three rye genotypes have a symmetrical, 1A-type karyotype composed of only metacentric [1R (satellited), 2R, 3R, and 7R] and submetacentric (4R, 5R, and 6R) chromosomes of similar size. The measured short arm length (S), long arm length (L), total chromosome length (TL), relative length (RL), arm ratio (AR), and centromeric index (CI) values of 'Kriszta' were not statistically different from those of the parental genotypes except for the AR and CI values of chromosome 3R. Nevertheless, the S, L, TL, and RL values were statistically either equal to those of one of the parents or were the mean of them. The ideogram (Figure 1a) constructed from the measured data clearly shows that the morphology of the perennial rye 'Kriszta' chromosome set is not identical with either that of Várda or that of *S. strictum* ssp. *anatolicum*.

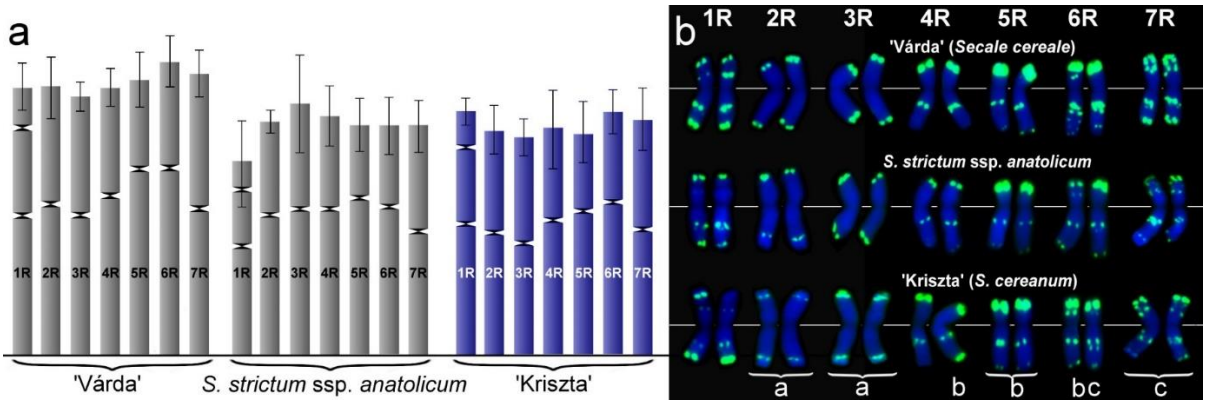

Figure 1 Ideogram of *S. cereale* 'Várda', *S. strictum* ssp. *anatolicum*, and *S. cereanum* 'Kriszta' chromosomes (a) based on measurements by DRAWID software, and the *in situ* hybridization signals with the repetitive DNA sequence pSc119.2 probe (green) of the same genotypes. The same lower-case letters indicate similar FISH patterns (b).

According to the FISH hybridization patterns of the repetitive DNA sequence pSc119.2, changes also have occurred in the heterochromatin of 'Kriszta' chromosomes compared to those of 'Várda'

and *S. strictum* ssp. *anatolicum* (Figure 1b). As a result, the following long arms became indistinguishable (contrary to those in the parental species): 2RL and 3RL showing a single subterminal band, 5RL and one of the homologs of 4RL and 6RL having a terminal and an intercalary signal at the same position, as well as 7RL and the other homolog of 6RL. (The short arms remained unambiguously identifiable with this probe.) After FISH karyotyping carried out by using highly repetitive heterochromatic sequences pSc200 and pSc250, and 12 synthetic simple sequence repeat oligonucleotide (microsatellite) probes (AC)n, (AG)n, (AAC)n, (ACG)n, (ACT)n, (AGG)n, (ATT)n, (CAC)n, (CAG)n, (CAT)n, (GAA)n and (GCC)n, we found that $(AAC)_5$ gave a strong terminal signal on the chromosome arm 4RL and a faint subterminal signal on 3RL, but the detectability of this latter highly depended on the quality of the FISH (Figure 2a). Oligonucleotides (ACG)n, (CAC)n, and (CAG)n, apart from the differences in intensity, provided the same pattern as $(AAC)_5$. Oligonucleotides (AGG)n and (GAA)n showed a specific signal on the long arm of chromosome 5R at the terminal position, but only on one member of the chromosome pair. Probe (GAA)n also showed a single intercalary band on one of the homologs of 4RL (Fig. 2b). Probes (AC)n, (AG)n, (ACT)n, (ATT)n, (CAT)n, and (GCC)n did not hybridize to the 'Kriszta' chromosomes.

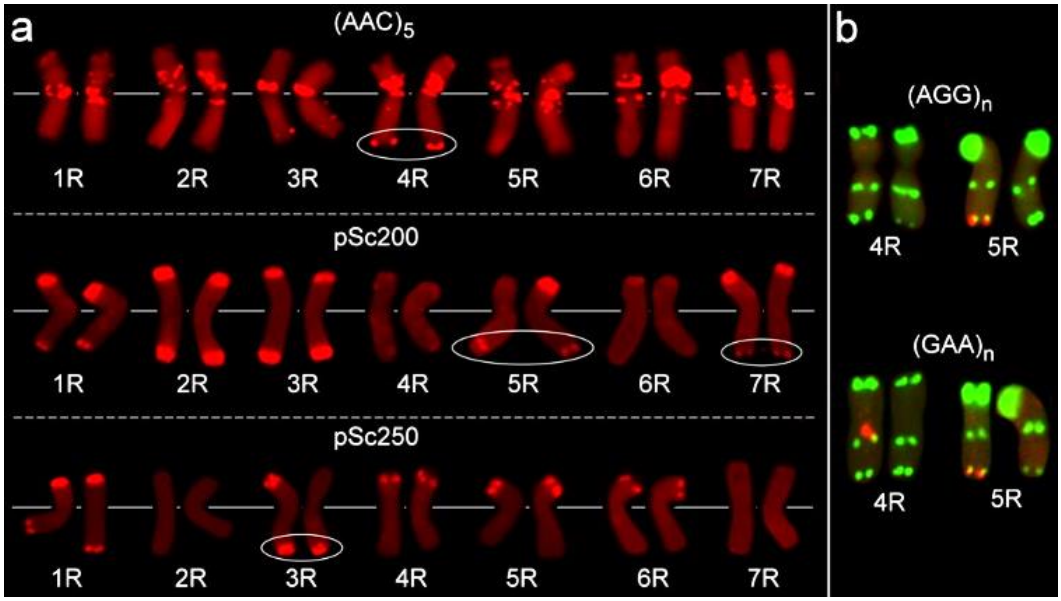

Figure 2 FISH pattern of 'Kriszta' rye with DNA probes $(AAC)_5$, pSc200, pSc250 (a), and (AGG)n, (GAA)n, and pSc199.2 (b). Except for pSc119.2 (green), hybridization sites are detected in red. Signals of diagnostic value are circled.

FISH with the repetitive sequence pSc200 located a specific subterminal hybridization site on the chromosome arm 5RL and a specific weak terminal signal on the chromosome arm 7RL, while pSc250 showed a weak fluorescence band on 1RL and a strong one on 3RL (Figure 2a).

The results presented above make us conclude that rearrangements may have occurred between (and maybe within) the parental rye chromosomes during the hybridization or the breeding of 'Kriszta', consequently, its chromosome complement is not a simple combination of the chromosomes of *S. cereale* and *S. strictum* ssp. *anatolicum*. The results also indicate, that an adequate pair-wise combination of the DNA sequences pSc119.2, pSc200, pSc250, and $(AAC)_5$ makes it possible to identify the long arms of *S. cereanum* chromosomes (e. g. in wheat-'Kriszta' translocations).

From wheat lines carrying 'Kriszta' chromosome arms with specific FISH hybridization patterns, the translocation chromosomes can be sorted by flow cytometry based on FISH in suspension and used in chromosome-based genomics. This makes it possible in the future to sequence chromosome arm-specific DNAs and to isolate genes originating from the exotic genome of 'Kriszta'.

From the results summarized above a manuscript has been prepared and submitted to the journal Cereal Research Communications (see Annex).

GISH (genomic *in situ* hybridization) experiments were also carried out to distinguish the *S. cereale* and *S. strictum* ssp. *anatolicum* chromatins in 'Kriszta'. As a first approach, isolated and digoxigenin-labeled genomic DNA of 'Várda' was used as a probe (producing red signals), while unlabeled genomic DNA of *S. strictum* ssp. *anatolicum*, added in an excess amount to the hybridization mix, was applied as a block. Three ratios (10:1, 50:1, and 150:1) of blocking DNA to probe, and two hybridization temperatures (37 and 42°C) were tested. Under each experimental condition, the same hybridization pattern was observed. All the 7 pairs of 'Kriszta' chromosomes were covered by speckled fluorescent signals. As the second approach, genomic DNA of Várda was labeled in the same way, while DNA of *S. strictum ssp. anatolicum* was labeled with biotin and was also used as a probe (producing green signals). In this case, all the 7 pairs of 'Kriszta' chromosomes fluoresced red, and green cross-hybridization signals were detected. Surprisingly, cross-hybridization patterns of the individual chromosome pairs were very similar to those obtained by using the $(AAC)_5$ microsatellite sequence in the FISH experiments. At present, we do not know the reason for this phenomenon. From these results, it seems that the R and R^m genomes of the two rye species, respectively, are so closely related that GISH is inappropriate to differentiate between them.

The FISH technique has two drawbacks. As its sensitivity and spatial resolution on chromosome spreads are limited, the cryptic or micro-introgressions remain undetected, furthermore, it is laborious and time-consuming thus hampering the extensive screening of large Mv9kr1/'Kriszta' progeny populations for rye chromatin introgressions. The publicly available, sequence-independent, high-throughput, and cost-effective DArTseq technology (Diversity Arrays Technology Pty Ltd., Canberra, Australia) provides a huge number of high-quality SNP (single nucleotide polymorphism) markers allowing the simultaneous detection of several thousands of polymorphic loci spread over a given genome. DArTseq markers have been successfully used by us for studying the genetic diversity and population structure of another wild gene source of wheat, *Aegilops biuncialis* (Ivanizs et al., 2019). To generate and identify *S. cereanum* ('Kriszta')-specific DArTseq markers, genomic DNA was extracted from fresh young leaves of the rye varieties Várda, Petkus, Lovászpatonai, and Imperial, *S. strictum ssp. anatolicum*, 'Kriszta', Chinese Spring-Imperial disomic addition series (1R-7R), Mv9kr1-'Lovászpatonai' disomic 4R addition line, wheat cultivars Mv Magdaléna and Mv Matador carrying 1RS.1BL translocation, Chinese Spring (CS), wheat line Mv9kr1, and several Mv9kr1/'Kriszta' BC_2F_8 progeny plants containing rye chromatin. 30-µl aliquots of the samples (70 ng/µl each) were sent to Diversity Arrays Technology Pty Ltd to perform the DArT assay.

The DArTseq genotyping resulted in a total of 329,267 high-quality reads. For sequence similarity searches, the trimmed (69 bp) sequences of the 258,090 SilicoDArTseq and 71,177 DArTseq SNP markers were blasted against chromosome-specific rye pseudo-molecules. Markers having alignment with less than 70% identity score, and those specific for several loci were discarded. The further filtering of the obtained 35 694 SilicoDArTseq and 27,321 SNP markers was carried out differently. To select rye-specific sequences, SilicoDArTseq markers with alleles also present in wheat (Mv9kr1 and CS) were thrown out. From the selected 31,684 markers, those absent from both the cultivated and wild ryes were also discarded. Out of the 30,702 markers obtained, after retaining only one marker from those that had the same start position during the sequence alignment, 27,822 markers remained. In the case of the DArTseq SNPs, after discarding those having at least one wheat (Mv9kr1 or CS) allele identical to one of the rye ('Imperial', 'Petkus', 'Lovászpatonai', 'Várda', *S. strictum* ssp. *anatolicum* or 'Kriszta') alleles, and those absent from the rye genotypes, out of the 71,177 markers 14,042 rye-specific ones were selected. Some of these SNP markers also had common match sequences at the start position. These markers, contrary to the SilicoDArTseq markers, were used to create new, so-called consensus markers by combining their sequences. In total, 8,842 SNP markers (including 3,634 consensus ones) were produced.

These genotyping data were then analyzed to select putative 1R-7R specific markers. The newly available genomic DNA reference sequence set (pseudo-molecules) of *S. cereale* (Rabanus-Wallace et al., 2019) was retrieved and used to identify the chromosomal location of these markers by sequence similarity search performed using BLASTn. The distribution of the generated chromosome-specific SilicoDArTseq and DArTseq SNP markers among the rye chromosomes (pseudo-molecules) 1R to 7R is shown in Table 1.

Table 1 Distribution among the *S. cereale* chromosomes of the selected rye-specific SilicoDArTseq and SNP markers.

Marker type	Chromosome							Total number
	1R	2R	3R	4R	5R	6R	7R	
SilicoDArTseq	3,381	4,373	3,512	4,359	4,103	4,467	3,627	27,822
DArTseq SNP	1,208	1,504	1,241	1,159	1,417	1,074	1,239	8,842

Knowing the size in base pair (bp) of the rye pseudo-molecules and the starting points of the aligned 27,822 SilicoDArTseq and 8,842 DArTseq SNP markers, we were able to determine their linear order and visualize them (using MeV 4.9.0. software) on the chromosomes 1R to 7R as heat maps. The two heat maps showed similarities in the distribution of the markers. In the absence of space, only the heat map of the SNP markers on chromosomes 3R, 6R, and 7R is shown here (Figure 3). The explanation of the code numbers and the abbreviations in the figure are summarized in Table 2.

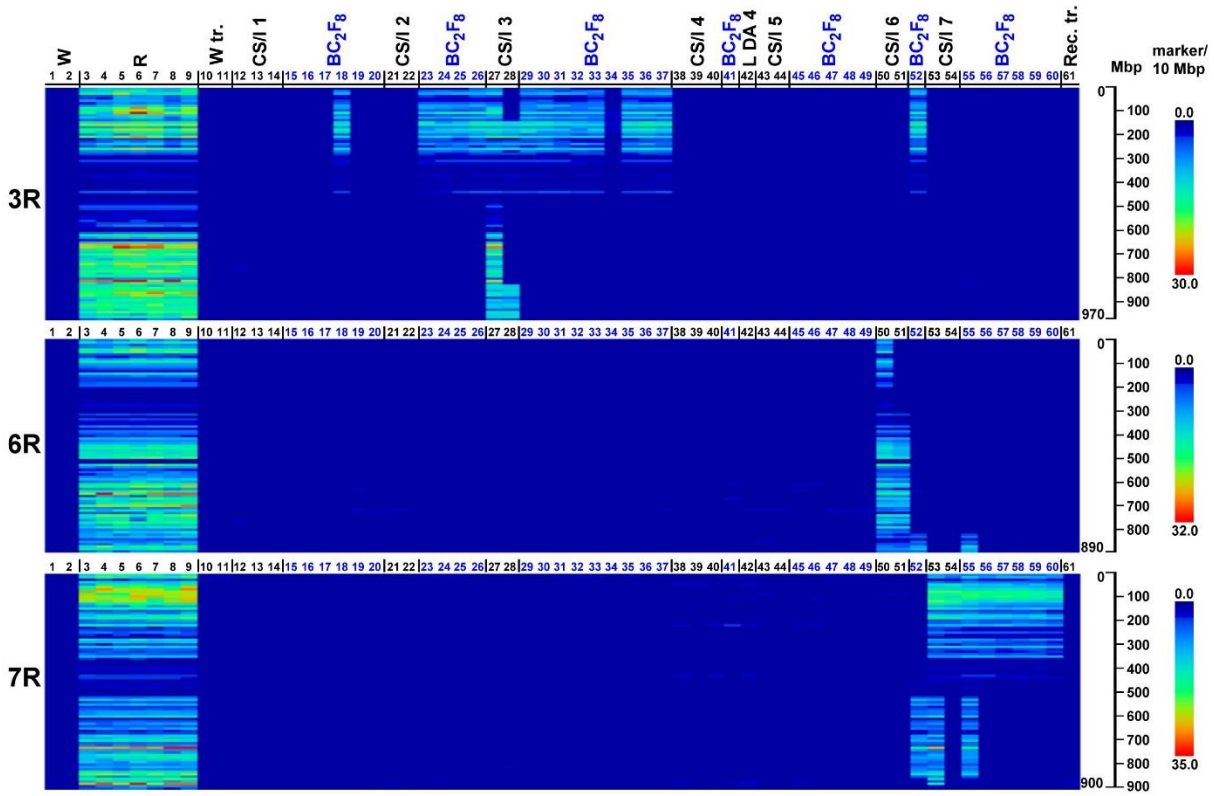

Figure 3 Heat map of the DArTseq SNP markers on the 3R, 6R, 7R rye pseudo-molecules. The numbered scales represent chromosome lengths in megabase pair (Mbp), the colored scales represent marker densities (number of markers per 10 Mbp).

In general, the marker analysis confirmed the genetic constitution of the plants from which the DNA samples were isolated, however, discrepancies have also been revealed. For example, a genebank-originated genetic material presented in lane 28 (Figure 3) has been believed to be ditelosomic 3RS, but according to the DArTseq markers, the telocentric chromosomes contain a proximal segment (including the centromere) of 3RS ($3RS^{del}$) and a distal segment (including the telomeric region) of 3RL ($3RL^{del}$).

Table 2 Explanation of the code numbers and abbreviations of the genotypes analyzed by DArTseq markers. DA: disomic addition, DT: ditelosomic addition, T: translocation

Codes in the heat maps		Genotype	Codes in the heat maps		Genotype
1	W	*T. aestivum* Mv9kr1	**21**	CS/I 2	CS/'Imperial' DA 2R
2		*T. aestivum* CS	**22**		CS/'Imperial' DT 2RL
3	S	*S. strictum ssp. anatolicum*	**27**	CS/I 3	CS/'Imperial' DA 3R
4-5		*S. cereale* ''Várda'	**28**		CS/'Imperial' DT 3RS
6		*S. cereanum* 'Kriszta'	**38**	CS/I 4	CS/'Imperial' DA 4R
7		*S. cereale* 'Imperial'	**39**		CS/'Imperial' DT 4RS
8		*S. cereale* 'Petkus'	**40**		CS/'Imperial' DT 4RL
9		*S. cereale* 'Lovászpatonai'	**42**		Mv9kr1/Lovászpatonai DA 4R
10	W tr.	T1BL.1RS wheat 'Mv Magdaléna'	**43**	CS/I 5	CS/'Imperial' DA 5R
11		T1BL.1RS wheat 'Mv Matador'	**44**		CS/'Imperial' DT 5RL
12	CS/I 1	CS/Imperial DA 1R	**50**	CS/I 6	CS/'Imperial' DA 6R
13		CS/Imperial DT 1RS	**51**		CS/'Imperial' DT 6RL
14		CS/Imperial DT 1RL	**53**	CS/I 7	CS/'Imperial' DA 7R
15-20, 23-26	BC_2F_8	Mv9kr1/'Kriszta' BC_2F_8 generation	**55**		CS/'Imperial' DT 7RL
29-37, 41			**61**	Rec. tr.	'Mv Magdaléna'/Mv9kr1/'Kriszta' recombined T1BL.1RS
45-49, 52					
55-60					

Our results indicate that the generated markers specific for rye chromosome arms are suitable for detecting and identifying not only translocations between wheat and rye (Figures 4c, e, h) and rye chromosome arms (Figures 4a, b, d) but also small (cryptic) rearrangements between rye chromosomes (Figures 4a, b, d, f, g), [which are undetectable by either FISH or GISH. For example, the chromosome shown in Figure 4f can be identified as 6R based on the hybridization pattern of the pSc119.2 probe but actually, it carries a 3R short arm and a long arm composed of a larger 7RL and a shorter 6RL chromosome segment (Figure 3, lane 52 and Figure 4f).

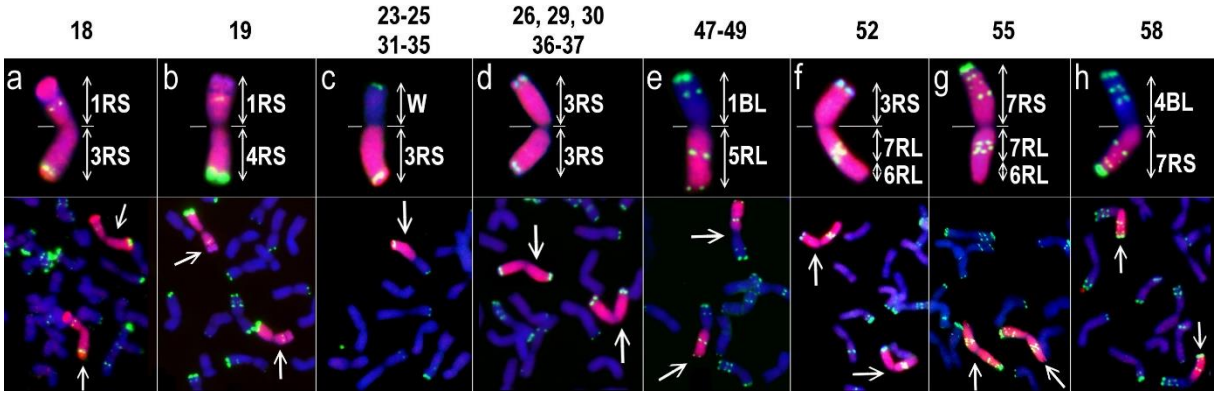

Figure 4 Composition of the translocated chromosomes identified by DArTseq markers (upper row), and the partial chromosome sets of the Mv9kr1/'Kriszta' BC_2F_8 plants in which the translocations (arrowed) occur (lower row). The GISH (rye total genomic DNA) and FISH (pSc119.2) signals are red and green, respectively. The unlabeled wheat chromosomes or chromosome arms are blue (counterstaining with DAPI). The numbers above the photos are the lane numbers in Figure 3.

After selecting the most reliable DArTseq markers, they can be converted into KASP (Kompetitive allele-specific PCR) markers (commonly used in marker-assisted plant breeding) that make possible the effective and rapid screening, as well as the identification of 'Kriszta' chromatin in the Mv9kr1/'Kriszta' progeny populations.

2. Production and identification of Mv9kr1/'Kriszta' chromosome translocation lines

To induce wheat-rye homoeologous recombinations, 90 plants of the stripe rust-resistant Mv9kr1/'Kriszta' 6R disomic addition line identified earlier (Schneider et al. 2016), as maternal

parent, and 140 plants of the Mv9kr1*ph1b* mutant wheat line (recently developed in our research group), as paternal genotype, were planted in phytotron climatic chambers in December of 2017. 189 spikes of the maternal genotype were emasculated and pollinated, and 1077 F_1 grains were harvested. As the *ph1b* gene expresses its effect in the next generation, 250 plants (the capacity of a GB 40 chamber) were planted at the end of September of 2018. From the isolated main spikes, a total of 5318 F_2 grains were harvested in January of 2019. So far, 500 F_2 plants were checked for translocation events. By using simultaneous GISH with total rye genomic DNA and FISH with DNA probe pSc119.2 we found that 393 plants did not contain rye chromatin (detectable by GISH), 22 were disomic and 81 were monosomic for the 6R chromosome, and 4 carried a translocation chromosome (monosomic centric fusion). The pSc119.2 FISH pattern of the translocated rye chromosome arm proved that the 6RL chromosome arm was present in all four plants (Figure 5). Unfortunately, we have so far failed to produce selfed progeny plants disomic for this translocation.

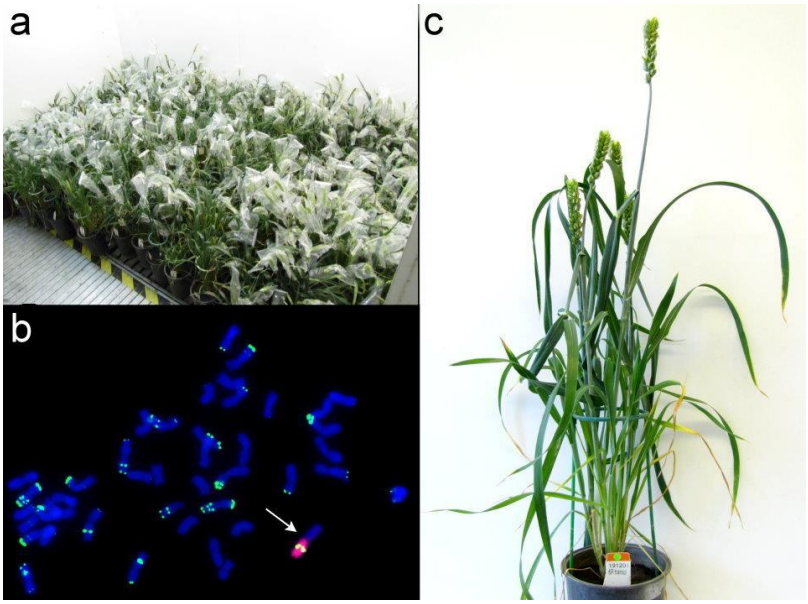

Figure 5 Isolated spikes of the Mv9kr1/'Kriszta' 6R disomic addition line crossed with the Mv9kr1*ph1b* mutant wheat line in a phytotron climatic chamber (a), the complete chromosome set (n=43) (b), and the plant and spike morphology of the 6RL monosomic translocation line. The translocated chromosome is arrowed. GISH and FISH signals (pSc119.2) are red and green, respectively.

Wheat-rye 1BL.1RS translocation has been widespread worldwide owing to the genes on the 1RS arm positively affecting stress resistance, grain yield, and adaptation ability of wheat. Nowadays, the 1BL.1RS wheat cultivars have become susceptible to rust diseases because of the monophyletic ('Petkus') origin of 1RS.

From the Mv9kr1/'Kriszta' backcross progenies, a wheat line (named '179' after the plot number) had been previously selected in the field, which showed resistance against stripe (yellow) rust in three consecutive years (2014, 2015, and 2016). During the present project, GISH and FISH were carried out to determine the chromosomal composition of this line. Hybridization pattern with the repetitive DNA sequence pSc119.2 revealed that this line carries a pair of 1BL.1RS translocation chromosomes. Morphology of the 1RS chromosome arms of *S. cereale* ('Várda), *S. strictum* ssp. *anatolicum*, 'Kriszta', the resistant 1BL.1RS line '179', and the wheat cultivar Mv Magdaléna containing 1RS of 'Petkus' rye origin (harboring the stripe rust resistance gene *Yr9* that is no longer effective), as well as results from DNA fragment analyses with 1RS-specific molecular markers, suggested that the 1RS arm of the resistant '179' was recombinant carrying segments from both *S. cereale* and *S. strictum*. This suggestion was proved by DArTseq marker analysis (Figure 6). After filtering 258,090 SilicoDArTseq and 71,177 SNP markers obtained from sequencing of wheat line Mv9kr1, *S. cereale* ('Petkus', 'Lovászpatonai', and 'Várda'), *S. strictum* ssp. *anatolicum*, *S. cereanum* ('Kriszta'), genotypes susceptible to stripe rust ('Mv Magdaléna', and a

Mv9kr1/'Kriszta' addition line disomic for 1R), and the resistant translocation line '179', 5312 putative 1RS-specific Silico-DArT and 1755 putative 1RS-specific SNP markers were selected.

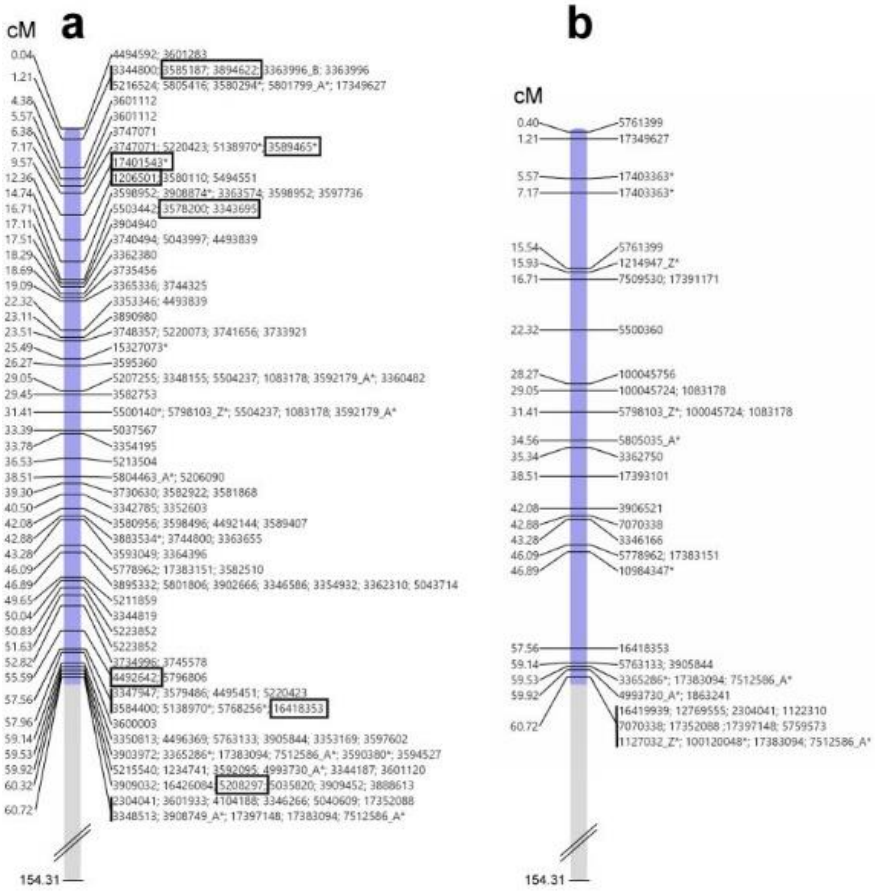

Figure 6 Genetic linkage maps for the rye chromosome arm 1RS of line '179'. (**a**) Distribution of the Silico and SNP-DArT markers specific for the stripe rust resistant '179'. Markers linked to LRR domain are enclosed by black rectangles. (**b**) Distribution of 1RS-specific markers exhibiting allelic variation between 'Kriszta' and 'Petkus' (present in 'Mv Magdaléna') 1RS. 1RS and 1RL (lacking from the T1BL.1RS translocation) chromosome arms are indicated in blue and gray, respectively. The marker distance is expressed in centimorgans (cM) from the telomere of 1RS (0 cM) to the putative centromere (60.72 cM) of chromosome 1R. *: SNP-DArT markers.

To confirm that these markers are specific for rye chromosome 1R, they were used for BLASTn against the rye Lo7 genomic sequences. Out of the 5312 Silico-DArT markers 679, while out of the 1755 SNP markers 168 gave hits against the Lo7 WGS 1R contigs. From these 1R-specific markers, 233 (27.5%) were located on 1RS, out of which 129 (111 Silico-DArT + 18 SNP; 55.36%) were specific for the stripe rust-resistant genotype '179' (Figure 6a) and 104 markers (44.64%) were specific for the susceptible DA1R line. This pronounced difference in the allelic composition proves that the 1RS arm fixed in the stripe rust-resistant line '179' is different from that of DA1R. We also compared the allelic composition of the 1RS arm of line '179' with the 'Petkus'-derived 1RS of 'Mv Magdaléna'. Out of the above-mentioned 233 1RS-specific markers, 225 gave unambiguous allelic results in both 1BL.1RS wheat genotypes. Thirty-seven (16.4%) of the 225 markers showed different alleles in '179' relative to 'Mv Magdaléna' (Figure 6b).

After functional annotation to Gene Ontology (GO) terms, we found 10 markers polymorphic between the susceptible (DA1R) and the stripe rust-resistant line '179', and, at the same time, associated with genes coding 'Leucine-Rich Repeat' (LRR) domains, which were found to be typical of most disease resistance proteins in the rye. These markers were located on the subtelomeric or the pericentromeric regions homologous to the 1.21–16.71 cM and 55.59–60.32 cM intervals, respectively, of the 1RS chromosome arm (Figure 6a).

These data, together with other results concerning the line '179' only briefly summarized in Figure 7, are published in Szakács et al. 2020 (Scientific Reports, 10:1792).

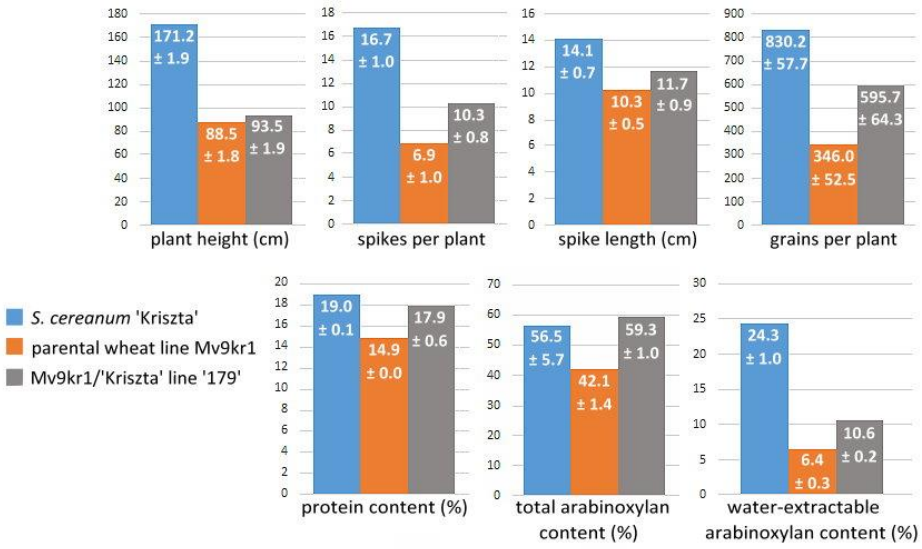

Figure 7 Morphological features and quality properties of the parental perennial rye (*S. cereanum*) 'Kriszta', the wheat line Mv9kr1, and the Mv9kr1/'Kriszta' 1BL.1RS line '179'. *: Significantly different from Mv9kr1 at the $P < 0.01$ level.

3. Incorporation of new disease resistance genes into cultivated wheat

The stripe rust-resistant line '179' (Figure 8a-d) was artificially infected with stem rust (*Puccinia graminis* f. sp. *tritici*) in the Martonvásár rust trap nursery in 2020 and 2021. In both years, this translocation line showed resistance to this disease (Figure 8e, f). From the Mv9kr1/'Kriszta' BC_2F_8 generation another 1BL.1RS translocation line was also selected in 2019, which showed sensitivity to stripe rust, but the artificial inoculation of 10-day-old seedlings under greenhouse conditions with a pathotype population virulent on differentials *Lr1*, *2a*, *2b*, *2c*, *3*, *3bg*, *3ka*, *10*, *11*, *12*, *13*, *14a*, *14b*, *15*, *16*, *17*, *18*, *20*, *21*, *22a*, *22b*, *23*, *26*, *30*, *32*, *33*, *34*, *35*, *37*, *38*, *44*, and *51* revealed, that it was resistant to leaf rust (Figure 8g-j)

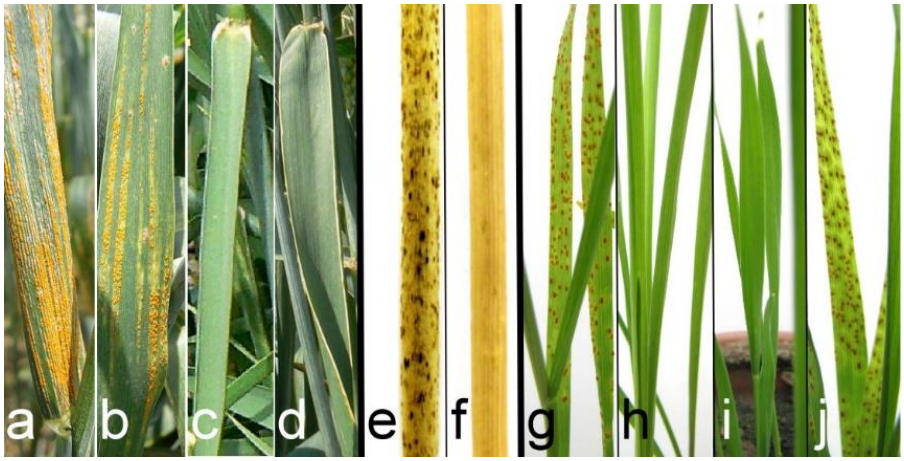

Figure 8 Stripe rust (*Puccinia striiformis* f. sp. *tritici*) infection in the field (a–d), artificial stem rust (*Puccinia graminis* f. sp. *tritici*) infection in the rust trap nursery (e, f), and leaf rust (*Puccinia triticina*) resistance tests in the greenhouse (g-h). Stripe rust-sensitive leaves of the parental wheat genotype Mv9kr1 (a) and the susceptible 'Mv Magdaléna' (carrying 1RS from 'Petkus' rye) (b), and the resistant leaves of the parental perennial rye 'Kriszta' (c) and the 1BL.1RS translocation line '179' (d). The infected stem of the stem rust-susceptible Mv9kr1 (e), and the asymptomatic stem of the resistant line '179' (f). Leaf rust-sensitive leaves of Mv9kr1 (g) and 'Mv Magdaléna' (j), and the resistant leaves of 'Kriszta' (h) and the new, stripe rust-sensitive 1BL.1RS translocation line (i).

For the time being, we are uncertain whether the genes providing resistance against stripe rust and stem rust are variants of *Yr9* and *Sr31*, respectively, or they are new resistance genes. Genetic mapping of resistance using an M9kr1-'Kriszta' 1BL.1RS line '179' (resistant) x M9kr1-'Kriszta'

DA1R (susceptible) bi-parental mapping population will help to identify the position of the resistance genes in the line ‘179’. Later, production of knockout mutants in ‘179’, sequencing of 1BL.1RS chromosomes flow-sorted from the resistant genotype ‘179’ and its susceptible mutants (MutChromSeq), and combination of the sequences with the mapping data will open the way for the positional cloning of the resistance genes introgressed into the line ‘179.
The line ‘179’, as a promising gene source for wheat breeding has been involved in a Martonvásár breeding program aiming the transfer of the new 1BL.1RS translocation into modern winter wheat cultivars.

References

Ivanizs, L., Monostori, I., Farkas, A., Megyeri, M., Mikó, P., Türkösi, E., Gaál, E., Lenykó-Thegze, A., Szőke-Pázsi, K.,; Szakács, É., et al. (2019). Unlocking the genetic diversity and population structure of a wild gene source of wheat, *Aegilops biuncialis* Vis., and its relationship with the heading time. *Front. Plant Sci.* 10 Paper: 1531. doi: 10.3389/fpls.2019.01531

Kirov, I., Khrustaleva, L., van Laere, K., Soloviev, A., Sofie, M., Romanov, D., Fesenko, I. (2017). DRAWID: user-friendly java software for chromosome measurements and idiogram drawing. *Comp. Cytogenet.* 11:747–757. doi: 10.3897/CompCytogen.v11i4.20830

Rabanus-Wallace, M. T., Hackauf, B., Mascher, M., Lux, T., Wicker, T., Gundlach, H., et al. (2019). Chromosome-scale genome assembly provides insights into rye biology, evolution, and agronomic potential. *bioRxiv*, 2019.12.11.869693. doi: 10.1101/2019.12.11.869693

Schneider, A., Rakszegi, M., Molnár-Láng, M., and Szakács, É. (2016). Production and cytomolecular identification of new wheat-perennial rye (*Secale cereanum*) disomic addition lines with yellow rust resistance (6R) and increased arabinoxylan and protein content (1R, 4R, 6R). *Theor. Appl. Genet.* 129, 1045–1059. doi: 10.1007/s00122-016-2682-6

Szakács, É., Szőke-Pázsi, K., Kalapos, B., Schneider, A., Ivanizs, L., Rakszegi, M., et al. (2020). 1RS arm of *Secale cereanum* 'Kriszta' confers resistance to stripe rust, improved yield components and high arabinoxylan content in wheat. *Sci. Rep.* 10, 1792. doi: 10.1038/s41598-020-58419-3

Annex