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)

Annamária Schneider, Marianna Rakszegi, Márta Molnár-Láng, Éva Szakács
Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Brunszvik u. 2, H‑2462 Martonvásár, Hungary
corresponding author e-mail: szakacs.eva@agrar.mta.hu
telephone: 00 36 22 569 500, fax: 00 36 22 569 576

Abstract

Perennial rye (*Secale cereanum*, 2n=2x=14, RR) cultivar Kriszta has a large gene pool that can be exploited in wheat breeding. It has high protein and dietary fibre content, carries several resistance genes, tolerant to frost and drought, and adapts well to disadvantageous soil and weather conditions. In order to incorporate agronomically useful features from this perennial rye into cultivated wheat, backcross progenies derived from a cross between the wheat line Mv9kr1 and perennial rye 'Kriszta' have been produced, and addition lines disomic for 1R, 4R and 6R chromosomes have been selected using GISH, FISH and SSR markers. Quality measurements showed that addition of 'Kriszta' chromosomes 4R and 6R to the wheat genome had increased the total protein content. The 4R addition line contained slightly, while 1R and 6R additions significantly higher amount of arabinoxylan than the parental wheat line. Besides this, the 6R addition line appeared to be resistant to yellow rust in highly infected nurseries, consequently it may carry a new effective gene different from that harboured in the 1RS.1BL translocation for resistance to this disease.

Keywords

perennial rye (*Secale cereanum*), addition lines, fluorescence in situ hybridisation, SSR markers, dietary fibre, yellow rust

Acknowledgements

This work was financed by the Hungarian National Scientific Research Fund (OTKA - No. K104382). Dietary fibre measurements were supported by OTKA 112169 project. The authors are grateful to I. Molnár for his advice regarding molecular marker analysis. The authors thank the colleagues working in the Wheat Quality Laboratory to measure the quality parameters. The technical assistance of F. Tóth, J. Bucsi and the Organic Breeding Team is gratefully acknowledged. Thanks are due to A. Bacskovszky for revising the manuscript linguistically.

Conflict of interest

The authors declare that they have no conflict of interest.

Author Contribution Statement

AS and ÉSZ selected the 1R, 4R and 6R wheat-*Secale cereanum* (perennial rye) addition lines from the backcross progenies of wheat-*Secale cereanum*, performed the FISH, GISH and molecular marker analyses and measured agronomic traits in the field and wrote the manuscript.

MR evaluated quality properties of the wheat-*Secale cereanum* addition lines and wrote these parts of the article.

MML started the wheat× *Secale cereanum* crossing programme, developed the BC_1 and BC_2 progenies, contributed to the manuscript writing and is the principal investigator in the OTKA K104382 project.

Key message

Wheat-*Secale cereanum* addition lines with yellow rust resistance (6R) and increased arabinoxylan content (1R, 4R, 6R) have been selected and identified in order to increase biodiversity of wheat.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Introduction

Cultivated and wild rye species containing R genomes that are non-homologous to those of wheat are important tertiary gene pools for wheat improvement. Rye (*Secale cereale* L. 2n=2x=14, RR) has some significant advantages over wheat and other crops. The rye plant is more winter hardy than wheat and produces economical yields in regions where soil and weather conditions are disadvantageous for wheat production. Compared to cultivated rye, the diploid wild mountain rye (*Secale montanum* Guss.) that includes numerous subspecies is perennial, has a large stature, strong tillering ability, frost tolerance and resistance to diseases. However, this wild perennial rye has poor agronomic value as a forage crop because of its small and scant leaf and breaking peduncle which are problems for seed production (Akgün and Tosun, 2004). The first *Secale cereale*×*S. montanum* hybrid plants (*S. cereanum*) in Hungary were developed in the 1950's (Kotvics 1970). The exact pedigree of the hybrids has not been published but the probable parents are *S. cereale* cv. Kisvárdai and *S. montanum* Guss. subsp. *anatolicum* (Boiss.) Tzvelev (on the basis of personal communication with G. Kotvics). The objective of developing these hybrids was, besides its botanical importance, to combine the best qualities of winter rye and perennial rye. The *S. cereanum* cultivar Kriszta (Kruppa 2001) has high green mass productivity, good nutritional parameters, is tolerant to frost and drought, and resistant to leaf rust, stem rust, yellow rust and powdery mildew. Owing to these attributes 'Kriszta' is an important gene source for widening genetic variability of bread wheat. 'Kriszta' has been used to develop new recombinant 1B.1R translocations in order to widen the genetic diversity of this rye chromosome arm (Molnár-Láng et al. 2010).

The dietary fibre (DF) content of rye is the highest among cultivated cereals. The typical rye grain contains 18 to 22% DF (Andersson et al. 2009). The major components of DF in wheat grain are the cell wall polysaccharides, arabinoxylan (AX) and (1-3)(1-4)-β-D-glucan (β-glucan). DF can be fractionated on the basis of solubility. Soluble DF, which is more fermentable in the colon than insoluble DF, results in both lower cholesterol and glucose absorption in the small intestine and lower postprandial blood insulin levels (Moore et al. 1998; Lewis and Heaton 1999). Reduced blood cholesterol is related to reduced risk of coronary heart disease, whereas reduced blood glucose is related to reduced risk of type II diabetes. Apart from its nutritional relevance, DF is also important from a technological point of view as it strongly affects wheat functionality during cereal processing, for example, in bread-making (Courtin and Delcour, 2002). Nowadays human healthcare and a healthy

lifestyle are in focus, and the demand for healthier food is expanding. Breeding of wheat cultivars rich in DF can satisfy this new social requirement.

Rye is an important reservoir for disease resistance genes. The short arm of rye chromosome 1R carries resistance genes against leaf rust (*Lr26*), stem rust (*Sr31*), stripe rust (*Yr9*) and powdery mildew (*Pm8*) (Friebe et al. 1996; McIntosh et al. 2003), therefore it was incorporated in tetraploid and hexaploid wheats in the form of either 1RS.1BL or 1RS.1AL chromosomal translocations (Zeller and Fuchs 1983; Friebe et al. 1989) and has been intensively used in breeding programs. The 1RS.1BL translocation, which is present in about 1050 wheat cultivars (Schlegel 2015), bears the 1R short arm of the rye cultivar Petkus. Due to the lack of allelic variation on this chromosome arm, resistance genes *Lr26, Pm8* and *Yr9* are no longer effective against new biotypes of diseases (Lutz et al. 1992; Bartoš 1993). More recently, virulence for *Sr31* has been also reported (Pretorius et al. 2000, 2010). For these reasons, the importance of searching for and introducing new resistance genes into wheat cultivars has grown (Lelley et al. 2004; Molnár-Láng et al. 2010; Tang et al. 2011a).

Rye can be crossed with wheat and its agronomic traits can be transferred via classic pre-breeding and breeding procedures from the hybrids into the wheat genome. In 1940 O'Mara demonstrated that the production of chromosome additions from amphidiploids backcrossed with wheat is a possible way to study the effects of individual alien chromosomes on wheat (O'Mara 1940). The first complete wheat-rye addition series created in this way was the ’Chinese Spring’-’Imperial’ (Driscoll and Sears 1971) which was later chosen as the standard series of rye additions (Sybenga 1983). Currently, at least two more complete wheat-*S. cereale* disomic addition sets are available; the ‘Chinese Spring’-‘King II’ (Miller 1973) and the ’Grana’-Dan´kowskie Złote (Miazga and Chrząstek 1987) addition sets. Addition lines deriving from the highly Al-tolerant Brazilian wheat line BH1146 and also tolerant rye cultivar Blanco (BH1146-Blanco and CS-Blanco sets) were produced by Lukaszewski (1988). The ‘Holdfast’-‘King II’ series (Riley and Chapman 1958) lacks chromosome 3R, while the ‘Kharkov’-‘Dakold’ series (Evans and Jenkins 1960) contains a 1R/3R translocation instead of chromosome 1R (Zeller 1977). Five of the additions of *S. montanum* to 'Chinese Spring' have also been established (Miller 1973) but it has not been possible to isolate the remaining two chromosomes (Miller 1984). Though addition lines cannot be considered as introgressions, they are stable enough to be maintained and used as starting point for the production of substitution and translocation lines.

Sophisticated molecular cytogenetic and molecular genetic methods help to analyse hybrids and their progenies in plants. Fluorescence in situ hybridization (FISH) is a powerful

technique for detecting specific nucleic acid sequences and localizing highly repetitive DNA sequences in specific regions of individual chromosomes, thus allowing their identification (Rayburn and Gill 1985; Leitch and Heslop-Harrison 1992; Mukai et al. 1993). Rye, as an allogamous (open-pollinated) species has a genome with high heterozygosity which manifests even in polymorphic FISH signals on chromosome pairs (Szakács and Molnár-Láng 2008), but the combination of various repetitive DNA probes and analyses using rye chromosome-specific SSR (single sequence repeat) markers enable more proper identification of rye chromosomes and chromosome segments in a wheat background.

The aim of the present work was to select and identify wheat-*S. cereanum* addition lines from the Mv9kr1-'Kriszta' F_1 hybrid progenies using molecular cytogenetic and molecular genetic techniques to study their morphological and quality properties and to test their resistance in the field.

Materials and methods

Plant materials

Production of wheat-S. cereanum addition lines

The Martonvásári 9 kr1 (Mv9kr1) winter wheat line which carries the recessive crossability allele kr1 transferred from 'Chinese Spring' (Molnár-Láng et al. 1996) was crossed with *S. cereanum* cv. Kriszta in 2002. The F_1 hybrids were sterile, thus young inflorescences were propagated in tissue culture (Molnár-Láng et al. 2002). The regenerated plants were backcrossed consecutively three times with the parental wheat genotype Mv9kr1 under controlled environmental conditions in 2003. The first selfing was carried out in 2005. Progeny plants were propagated and further selfed in the Martonvásár nursery. Selection of disomic addition lines from this genetic material started in 2012.

Control genotypes

In the marker analyses, the following plant materials were used as controls: wheat line Mv9kr1 and 'Mv Magdaléna' (Hungarian variety carrying 1BL.1RS translocation of 'Petkus' rye origin), wild rye *Secale montanum* ssp. *anatolicum*, rye cultivar Kriszta, and the 'Chinese Spring'-'Imperial' (CS-'Imperial') wheat-rye disomic addition series (Driscoll and Sears 1971).

Comparative FISH analysis

FISH patterns of chromosomes in the disomic additions were compared with those of *S. montanum* and rye cultivars Kriszta and Kisvárdai alacsony.

Fluorescence in situ hybridisation (FISH) and genomic in situ hybridisation (GISH)

The seeds of the Mv9kr1×'Kriszta' hybrid derivatives were germinated at room temperature for 24 h, incubated at 4°C for 48 h and then at 25°C for 26 h. Root tips were collected and treated in ice-cold sterile water for 24 h and fixed in a 3:1 (v/v) mixture of 100% ethanol and acetic acid. Root-tip squash preparations were made in 45% acetic acid. The coverslips were removed in liquid nitrogen, the preparations were air dried overnight and stored at –20°C until use. Total genomic DNA of Kriszta rye was labelled with digoxigenin-11-dUTP using nick translation mix (Roche Diagnostics, Mannheim, Germany) and used as a probe for GISH. The FISH probe pSc119.2, a 120 bp highly repeated sequence was amplified from rye genomic DNA and labelled with biotin-11-dUTP using PCR according to Contento et al. (2005). To shorten the time-consuming screening of the large amount of wheat-perennial rye progeny plants, FISH (using pSc119.2 probe) and GISH were carried out simultaneously following the protocol described by Molnár-Láng et al. (2010), with minor modifications. The synthetic oligonucleotide $(AAC)_5$ (Cuadrado et al. 2000) was amplified from rye genomic DNA using PCR according to Vrána et al. (2000) and labelled with digoxigenin-11-dUTP using nick translation mix (Roche). This DNA probe was hybridised to the slides after FISH-GISH analyses of the 'Mv9kr1'-'Kriszta' disomic addition lines. Digoxigenin and biotin signals were detected with anti-digoxigenin-rhodamine (Roche, Mannheim, Germany) and streptavidin-FITC (Roche), respectively.

Images were taken using Image Pro plus 5.1 software (Media Cybernetics, Silver Spring, USA) and a Spot CCD camera (Diagnostic Instruments, Inc., Sterling Heights, USA) attached to a Zeiss Axioscope 2 epifluorescence microscope.

SSR marker analysis

Genomic DNA was extracted from fresh young leaves with QuickGene DNA tissue kit (FujiFilm, Japan) according to the manufacturer's instructions. The list of the markers used in this study is presented in Table 1. The PCR amplification was performed under the conditions described in the original publications, with minor modifications (Cseh et al. 2011; Molnár et al. 2014). Touchdown PCR was carried out in an Eppendorf Mastercycler (Eppendorf, Hamburg, Germany) using the following reaction profiles: 94°C (3 min), 6 cycles of [94°C (20 sec), 65°C (20sec), 72°C (35 sec)], 35 cycles of [94°C (15 sec), primer annealing temperature (20 sec), 72°C (35 sec)], 72°C (2 min), hold at 15 °C. PCR products were separated using a Fragment Analyzer™ Automated CE System equipped with a 12-Capillary

Array Cartridge (effective length 33 cm) (Advanced Analytical Technologies, Ames, IA, USA). The results were analysed using PROsize v2.0 software.

Quality measurements

4 g of seeds were milled with a ball mill (Retsch Mixer Mill MM 200) and used for compositional quality analysis.

Crude protein content was analysed by the Kjeldahl method consistent with ICC method 105/2 using the Kjeltec 1035 Analyzer. Size Exclusion - High Performance Liquid Chromatography (SE-HPLC) was used to determine the glutenin, gliadin and albumin+globulin contents and the unextractable polymeric protein (UPP%=insoluble glutenin/soluble+insoluble glutenin) content using a modified method of Batey et al. (1991). 10 mg flour was suspended in 1 ml 0.5 % (w/v) SDS in phosphate buffer (pH 6.9) and sonicated for 15 s. After centrifugation, the supernatant was filtered on a 0.45 μm PVDF filter. Analyses were performed on a Phenomenex BIOSEP-SEC 4000 column in an acetonitrile buffer (0.05 % (v/v) triflouroacetic acid and 0.05 % (v/v) acetonitrile) with a running time of 10 m (2 ml/m flow rate). Proteins were detected by absorption at 214 nm.

A modification of the RP-HPLC method of Marchylo et al. (1989) was used to determine the relative amounts of the HMW glutenin subunits. The gliadins were extracted with 70% (v/v) ethanol followed by 50% (v/v) propan-1-ol. The glutenin polymers were then reduced with buffer (50% (v/v) propan-1-ol, 2M urea and 0.2M Tris-HCl, pH 6.6) containing 1% (w/v) DTT, and then alkylated with 4-vinylpyridine. HMW and LMW glutenin subunits were separated on a Supercosil LC-308 (300A, 3.5% carbon, 5μm, 5×4.6) column.

Total and water extractable pentosans (called arabinoxylans further on) were determined using a colorimetric method as described by Douglas (1981).

Field observations

The addition lines and the parental genotypes (Mv9kr1 and ’Kriszta’) were grown in the pesticide-free (organic) Tükrös nursery in Martonvásár in two consecutive seasons (2013-2014 and 2014-2015) with 10 seeds in each 1 m row and a row spacing of 15 cm. The same genotypes were sown in the Breeders nursery in Lászlópuszta in the 2014-2015 season in plots of 2 m^2. Ten plants were randomly selected from each genotype for analysis. Plant height and tillering (spikes per plant) were measured in the field immediately before harvest, the length of the main spike, number of spikelets per main spike, number of seeds per main spike, and thousand kernel weights were recorded after harvest.

Resistance to diseases of the Mv9kr1-‘Kriszta’ disomic addition lines was tested both in the pesticide-free and in the Breeders nursery.

Statistical analysis

Differences in morphological characteristics between the addition lines and the control Mv9kr1 genotype were determined by means of single factor ANOVA (analysis of variance) of the Microsoft Excel program. Values were calculated from 10 measurements and compared to the parental wheat line Mv9kr1. Because of the seeding density in the Breeders nursery, *S. cereanum* cv. Kriszta had such a high number of tillers that it was impossible to separate each plant, therefore statistical analysis was carried out only from measurements of 10 spikes.

Average arm ratio of the 1R chromosomes (1RL/1RS) and relative length of the 1RS and 1RL arms (1RS/1R and 1RL/1R, respectively) were calculated by means of ImageJ v1.49, a public domain Java-based image processing program. Statistical analysis of values measured in twenty cells of each 1R addition line was carried out using single factor ANOVA.

Results

In situ hybridisation

Identification of Mv9kr1-‘Kriszta’ addition lines using simultaneous FISH (with pSc119.2 probe, yellow-green colour) and GISH was carried out in the progenies of the wheat-*S. cereanum* hybrids to detect and identify perennial rye chromosomes in the wheat background, while the repetitive DNA probe $(AAC)_5$ (red colour) was used to identify rye chromosomes more accurately. Based on their FISH patterns, three disomic additions (1R, 4R and 6R) have been identified (Fig. 1a-f).

Mv9kr1-‘Kriszta’ progeny plants with identification numbers 132941, 132948 and 132949 were selected as 1R disomic additions. Two types of 1R chromosomes, each differing in pSc119.2 FISH patterns from those of *S. cereale*, were detected in these lines (Fig. 1a). Chromosome 1R of the line ‘132941’ showed hybridisation sites terminally on the long arm, in an interstitial position on the short arm, and subterminally on the satellite (Fig. 1b). The average arm ratio, and the relative length of the 1RS and 1RL arms of this metacentric chromosome were 1.037, 0.488 and 0.512, respectively (Table 2). Location of the pSc119.2 signals on chromosome arm 1RS of the line ‘132949’ was similar to that observed in the line ‘132941’, but a double interstitial signal was visible on the long arm (Fig. 1c). The average arm ratio, and the relative length of the 1RS and 1RL arms of this submetacentric chromosome were 1.443, 0.409 and 0.590, respectively, that differed significantly from those

found in the line ‘132941’ (Table 2). Line ‘132948’ carried both types of chromosome 1R (Fig. 1d, Table 2). Using the microsatellite repetitive sequence $(AAC)_5$, no relevant differences were detected in FISH patterns on the 1R chromosomes of *S. cereale*, *S. montanum*, *S. cereanum* and the Mv9kr1-‘Kriszta’ 1R addition lines, except for an interstitial signal on the long arm of one member of the chromosome pair in *S. cereanum*.

The addition line with identification number ‘12641’ was disomic for chromosome 4R, which could easily be identified by means of the two repetitive DNA probes. The submetacentric rye chromosome pair in this line showed a terminal pSc119.2 hybridisation site on the short arm, and an interstitial pSc119.2 and a terminal $(AAC)_5$ signal on the long arm (red colour). The FISH pattern and the arm ratio of the 4R chromosome found in the line ‘12641’ showed the greatest resemblance to those observed in *S. cereale* (Fig. 1a and e).

The rye chromosome pair of the Mv9kr1-‘Kriszta’ progeny line ‘13321’ carried a double interstitial pSc119.2 signal on the long arm and a single terminal signal on the short arm. It was identified as 6R, despite the lack of a terminal pSc119.2 hybridisation site specific for both the *S. montanum* and the ‘Kriszta’ 6RL chromosome arm, and an $(AAC)_5$ signal in the same position specific for *S. cereal*e 6RL. Chromosome 6R of this addition line seemed to be more metacentric than that of the studied rye species (Fig. 1a and f).

Molecular marker analysis

Mv9kr1-‘Kriszta’ disomic addition lines 1R, 4R and 6R were also analysed with rye chromosome-specific PCR-based markers (Table 1) to confirm molecular cytogenetic results. PCR products of the 1RS-specific markers SCM9, TSM120, TSM81 and TSM103 were compared to the Martonvásár wheat cultivar Mv Magdaléna carrying 1RS.1BL translocation. All the four 1RS-specific markers amplified bands in the three (‘132941’, ‘132948’ and ‘132949’) 1R addition lines (Table 3), but none of them showed fragment length polymorphism compared to Mv Magdaléna (Fig. 3). 1RL-specific markers GRM0077, GRM0325 and GRM0350 produced PCR amplicons in addition lines ‘132948’ and ‘132949’, but no 1RL-specific bands were present in the lane containing DNA from the line ‘132941’ (Fig. 3, Table 3). All of the 4RS- (GRM0146, GRM0324 and GRM1008) and 4RL-specific (GRM0277, MWG652, GRM1019) markers amplified PCR products both in the Chinese Spring-‘Imperial’ and in the Mv9kr1-‘Kriszta’ 4R addition lines, except for GRM1019 which produced no PCR amplicons in the Chinese Spring- ‘Imperial’ 4R addition line (Fig. 3, Table 3). From the six 6R-specific markers (GRM0059, GRM0609, SCM304, SCM2, SCM28, and SCM180, Table 1). SCM2 specific for the long arm of the 6R chromosome produced no rye-

specific bands in the CS-'Imperial' 6R addition, while in the Mv9kr1-'Kriszta' 6R addition line multi-banded PCR amplicons were observed (Table 3). SSR markers SCM304 and GRM0609 (specific for 6RS), and SCM28 (specific for 6RL) showed rye-specific banding patterns in both the CS-'Imperial' and the Mv9kr1-'Kriszta' 6R addition lines, while markers GRM0059 (specific for 6RS) and SCM180 (specific for 6RL) amplified the expected PCR products only in the CS-'Imperial' 6R addition line (Fig. 3, Table 3).

Morphology and yellow rust resistance of the wheat-*S. cereanum* addition lines

The morphological measurements from the Mv9kr1-'Kriszta' 1R, 4R and 6R disomic addition lines and the parental species were taken in both the Breeders and the pesticide-free nurseries, and data were compared to those of the parental wheat line Mv9kr1 (Tables 4 and 5). Spike morphology of these lines bore a strong resemblance to that of the parental wheat line Mv9kr1, but having awnlets towards the tip, the 1R addition showed the greatest similarity. Diversity of 1R addition lines '132941', '132948' and '132949' found in their pSc119.2 FISH patterns was not expressed in the spike morphology. The 4R addition line carried well-developed supernumerary spikelets. Ears of the 6R addition line were compact with a denser part at the top and were shorter than those of the 1R and 4R addition lines (Fig. 2a and c). Plants of the 1R addition lines in both nurseries were significantly shorter than those of the other addition lines and the Mv9kr1 wheat line. The addition of chromosomes 1R and 6R to the Mv9kr1 genome significantly increased the tillering capacity of these introgression lines in the Breeders nursery, but no significant differences were observed in the pesticide-free nursery (Tables 4 and 5). The 4R addition line had significantly longer while the 6R addition line had significantly shorter spikes both in the pesticide-free and in the Breeders nursery (Tables 4 and 5). The presence of the chromosome 4R in the wheat genetic background resulted in significantly higher number of spikelets per spike in the Breeders nursery, but this effect was not recognized in the pesticide-free nursery. No significant differences in the number of seeds per main spikes were found between the wheat line Mv9kr1 and the additions in the Breeders nursery, but the added rye chromosomes in the 1R and 6R addition lines caused a significant decrease in the number of seed per spike in the pesticide-free nursery. Thousand kernel weight (TKW) data were measured in the pesticide-free nursery. It was the lowest in the 1R, and the highest in the 6R addition line.

In 2014 and 2015 there were serious yellow rust epidemics in Hungary. Disease resistance of the addition lines was examined in both years and in both nurseries. Among the Mv9kr1-'Kriszta' disomic addition lines only the 6R addition displayed yellow rust resistance from

tillering to maturity. It had darkish green leaves without any symptoms. The infection on the plants of the 1R and 4R addition lines was just as severe as on the highly susceptible wheat parent Mv9kr1 (Fig. 2b and c).

Quality properties of the wheat-*S. cereanum* addition lines

Quality properties of the wheat-*S.cereanum* disomic addition lines were studied, especially focusing on the protein and dietary fibre components (Table 6). The total protein content of the 4R and 6R addition lines significantly increased, but the compositional properties of their proteins were very similar to the wheat control line, Mv9kr1 (Glu/Gli ratio, UPP%). Only the quantitative ratio of the HMW glutenin subunits increased significantly in these lines, related to the LMW glutenins and there is also a small change in the Glu/Gli ratio of the 6R addition line. The total protein content and the Glu/Gli ratio of the Mv9kr1 did not change due to the addition of the 1R chromosome from Kriszta, but significant decrease were found in the UPP% and in the ratio of the HMW and LMW glutenin subunits. It means, that the compositional properties of the glutenins changed in a way that the ratio of the large unextractable polymeric glutenins and the high molecular weight glutenins decreased, and shorter glutenin chains appeared. From qualitative point of view, it is resulting in a softer dough with smaller dough stability and poor bread-making quality.

Significant difference was found in the dietary fibre content of wheat and rye parents, especially in the total (TOTAX) and water-extractable arabinoxylan (WEAX) content. Therefore, it was expected that the addition of certain rye chromosomes would increase the fibre content of wheat. In our experiment, all the three chromosome additions significantly increased both the TOTAX and the WEAX. Addition of the *S. cereanum* chromosome 1R to the wheat genome almost doubled the level of the water-extractable fraction, thus exceeded the effect of chromosomes 4R and 6R.

Discussion

Rye, as an open-pollinated crop, has significant genetic diversity which can be expressed in the patterns of repetitive DNA sequences within and among species (Cuadrado and Jouve 1995, 2002; Alkhimova et al. 1999), and even among cultivars (Szakács and Molnár-Láng 2008). Recent characterization of 360 repetitive elements in six species of the genus *Secale* implies that repetitive DNA sequences have played important roles in the course of chromosome evolution of rye (Tang et al. 2011b).

In the present study, comparison of the 1R, 4R and 6R chromosomes of the Mv9kr1-'Kriszta' addition lines with those of *S. cereale*, *S. montanum* and *S. cereanum* has also revealed high level of rye chromosome polymorphism (Fig. 1a). The genetic variability manifested in FISH polymorphism caused difficulties during the cytological identification of the rye chromosomes in the Mv9kr1-'Kriszta' addition lines. Using the repetitive DNA probe pSc119.2, the 1R chromosome showed polymorphic FISH signals both on the short and the long arm. Identification of 1RS was easy owing to its satellite, but the two strong FISH signals on the 1RL arm were similar to those on chromosome 6R. Using this probe, Cuadrado and Jouve (1995) described similar double hybridisation sites on the 1RL of *S. montanum*. It is not probable that the Mv9kr1-'Kriszta' addition lines '132948' and '132949' carry entire *S. montanum* 1R chromosomes, because they were susceptible to diseases, but it cannot be excluded that these chromosomes are translocations between *S. cereale* (1RS) and *S. montanum* (1RL). SSR marker analysis of these lines with the 1RS-specific SCM9 confirms the *S. cereale* origin of the chromosome 1R short arm. The arm ratio of the metacentric and submetacentric 1R chromosomes differed significantly in the line '132948'. The presence of the double interstitial pSc119.2 hybridisation sites on the long arm of the submetacentric 1R chromosome and the absence of any signal in the same position on the metacentric one, as well as the variance in the length of 1RL arms rather suggest that this chromosome polymorphism is the consequence of a translocation between 1R long arms carrying a single interstitial signal. The lack of any PCR amplifications in line '132941' using 1RL-specific markers confirms that some kind(s) of rearrangement(s) occurred in the long arm of the metacentric chromosome. Short arms of the metacentric and submetacentric chromosomes also differed in length which may be caused by an additional translocation. Presumably, lines with only one type of 1R chromosome ('132941' and '132949') derive, through chromosome segregation, from lines in which the above-mentioned translocation took place.

Regarding in situ hybridisation patterns with repetitive sequences, 4R is considered to be the most conservative among the rye chromosomes (Cuadrado and Jouve 1995). On the basis of the arm ratio and the distribution of FISH signals, particularly the terminal $(AAC)_5$ pattern on the long arm, it was clear that the Mv9kr1-'Kriszta' disomic addition line '12641' carried a pair of chromosome 4R, and supposedly derived from *S. cereale*. The expression in this addition of the supernumerary spikelet trait confirmed the presence of the rye chromosome 4R. It seems that homologous group 4 of cereals plays an important role in the development of this spike deformity. In introgression lines bearing the chromosomes $4M^g$ of *Aegilops geniculata* (Friebe et al. 1999) and 4H of *Hordeum vulgare* (Kruppa et al. 2013; Aranyi et al.

2014) this morphological feature was also observed. Monosomic analyses in bread wheat revealed that, beside 2D and 5A, chromosomes 4A and 4B carried genes for supernumerary spikelet character (Sun et al. 2009).

Differences in FISH patterns and chromosome morphology between the 6R chromosome of the 'Mv9kr1'-'Kriszta' disomic addition line and the studied rye species suggests that significant changes occurred in this chromosome during the development of the line '13321'. From the observation that the repetitive DNA sequences pSc119.2 and $(AAC)_5$ did not show any terminal hybridisation signal on the 6R long arm, it can be concluded that a deletion took place in this chromosome segment. The change in the length of the 6RL chromosome arm may be the consequence of this deletion. The variability in PCR amplicon length of the 6R-specific SSR markers suggests that the Mv9kr1-'Kriszta' 6R addition line has remarkable DNA sequence difference in primer binding sites compared to the CS-'Imperial' 6R addition line (Fig. 3 and Table 2). As the excellent yellow rust resistance of this line can exclusively originate from the *S. montanum* parental species of 'Kriszta' rye, its 6R is either a *S. cereale* chromosome containing chromosome segment(s) from *S. montanum* or a *S. montanum* 6R. There are evidences that chromosome 6R of *S. montanum* carries a pericentromeric inversion (Devos et al. 1993; Cuadrado and Jouve 1995) which can also cause the variation in the length of the PCR product. One of the possible explanations of the discrepancies found in the chromosome FISH signals is the mutagenic effect of the tissue culture used for the propagation of the wheat-*S. cereanum* hybrid. The chromosome-breaking nature of this in vitro technique has been described and well-studied in many plant species (Larkin and Scowcroft 1981; Phillips et al. 1994).

SSR markers are powerful tools to identify and follow alien chromosome segments in wheat introgression lines. However, sequence data are not available for *S. montanum*, one of the parental species of *S. cereanum*. Consequently, markers specific for this rye species have not been designed yet. In the present study, *S. cereale*-specific SSR markers were applied to verify the FISH identification of 'Kriszta' chromosome in the wheat-*S. cereanum* addition lines. Chromosomal location of the rye chromosome-specific SSR markers were originally assigned in CS-'Imperial' disomic addition lines (Saal and Wricke 1999), but the majority of these SSR markers produced chromosome-specific amplicons in the parental 'Kriszta' rye and the 1R, 4R and 6R wheat-*S. cereanum* addition lines as well (Fig. 3 and Table 2), which proves the relatively high transferability of SSR markers from *S. cereale* into *S. cereanum*.

The introgression of chromosomes 1R, 4R and 6R to the wheat line Mv9kr1 resulted in changes in plant and spike morphology, but their effects on these traits were not the same in

the organic and the Breeders nurseries. From the breeders' point of view, tillering and fertility are the two most important yield components. The number of tillers per plant determines the number of spikes per plant. It is influenced by environmental factors (water and nutrient supplies, temperature, etc.) and is under genetic control. Addition lines 1R and 6R showed significantly higher tiller number compared to Mv9kr1 in the Breeders nursery (Lászlópuszta), but no such a difference was manifested in the organic nursery (Tükrös). The two additions significantly decreased the number of seeds per main spike in Tükrös, but this effect was not noticeable in Lászlópuszta where fertility of the 1R, 4R and 6R additions was practically equal to that of the parental wheat line. These data reflect well the differences in the environmental conditions of the two nurseries. The supernumerary spikelet trait of the Mv9kr1-'Kriszta' 4R addition line was also observable only in Lászlópuszta, though, because of the sterility of the extra spikelets, it did not increase the number of seeds per main spike.

Boros et al. (2002) reported that *S. cereale* chromosomes 2R, 5R and 6R, in the order from the smallest to the largest effect, added to 'Chinese Spring' significantly increased arabinoxylan content in these addition lines. Based on the evaluation of DF content in disomic wheat-rye addition lines and octoploid triticale, Cyran et al. (1996) found that chromosomes 4R and 6R had an impact on the high expression of total arabinoxylan in wheat grain. The present study confirmed the positive effect of *S. cereanum* chromosome 4R and 6R on total fibre content, and also revealed their role in increasing the level of WEAX in wheat. Contrary to the above-mentioned publications, our results indicate that the expression of both the TOTAX and the WEAX are also affected by chromosome 1R. Changes in the WEAX content are really outstanding especially in the case of chromosome additions 1R and 6R, but a question arises relating to this finding. Chromosome addition could also result in a decrease in the thousand kernel weight (TKW) affecting the compositional properties of the seeds in this way. However, as 1R chromosome resulted in a small decrease in the TKW while 6R chromosome resulted in a small increase in it beside similar changes in their fibre content, the effect of the TKW on the fibre content could be excluded. 'Kriszta' contains both *S. cereale* and *S. montanum* genomes, but its exact chromosome composition is unknown. *S. montanum* can be present as entire chromosomes or translocated chromosome segments. As the R genome of the two species is very close (Riley 1955; Stutz 1957; Shewry et al. 1985), and there are no molecular markers specific for *S. montanum*, neither multi-colour GISH nor marker techniques are available to distinguish them. The unique behaviour of 'Kriszta' chromosome 1R raises the question whether this chromosome contains, as a result of

recombination, segment(s) from *S. montanum* 1R carrying, by any chance, gene influencing DF content, or this gene came into 1R from chromosomes 4R or 6R through translocation.

Secalins are the major storage proteins of rye, which could be classified into four major groups that differ in their amino acid composition and relative molecular mass (Mr). These are the high molecular weight (HMW) secalins, ω-secalins, small γ-secalins (Mr=40000, 40 kDa), and large γ-secalins (Mr=75000, 75 kDa) (Shewry et al. 1983a; Kasarda et al. 1983). Genes for all the HMW secalins are present on chromosome 1RL, while all the monomeric ω-secalins and the small γ-secalin genes are present on chromosome 1RS both in *Secale cereale* and *Secale montanum*. In contrast, the genes for the large γ-secalins are located on 2RS in *Secale cereale* but on 6R in *Secale montanum* (Shewry et al. 1986). It means that rye secalins in our wheat/rye 1R addition lines could originate from both *Secale cereale* and *Secale montanum*, while the effect of the *Secale montanum* 6R chromosome could be seen on the protein composition of the 6R addition line. The small UPP% and higher LMW of the 1R addition line refers to the presence of the HMW secalins, ω-secalins and/or the small γ-secalins of rye in wheat. In the 6R addition line, the presence of the large γ-secalins might be responsible for the significant increase in the quantity of the proteins, gliadins and HMW glutenins. Despite partial homology, the rye HMW-secalin differ significantly from wheat HMW glutenin subunits with respect to their structural and quantitative parameters, which are especially important for the formation and properties of wheat gluten (De Bustos and Jouve 2003; Köhler and Wieser 2000). According to previous studies, the incorporation of the locus Glu-R1 into wheat had negative impact on the wheat processing quality (Shewry and Bechtel 2001; Lukaszewski 2006). The presence of this protein might be a reason for the poor gluten quality in our 1R addition line. Omega-secalins (ω-secalins) are regarded as the major cause of poor grain quality in rye, triticale or wheat 1BL/1RS translocation lines (Dhaliwal et al. 1990). They may have unique functions, different from those of other seed proteins (Jiang et al. 2010) and surely contribute to the quality changes in our 1R wheat-rye addition line. The 75 kDa γ-secalins are the most abundant group of secalins, accounting for greater than 50% of the total proteins in rye (Shewry et al. 1983 a,b; Gellrich et al. 2003). The 75 kDa γ-secalin is much larger in molecular weight than the 40 kDa γ-secalin mainly due to the insertion of sequence in the repetitive domain (Shewry et al. 1982). This suggested that the variation in the length of the repetitive domain was related to the protein structure and the quality of rye dough. Moreover, it was predicted that the presence of the large proline/glutamine repetitive domain in the 75 kDa γ-secalin protein may also affect dough properties (Murray et al, 2001). In our experiment, this important type of rye protein had positive effect on wheat quality in

the 6R addition line by increasing the total protein content and the ratio of HMW proteins so the chromosome addition had dough strengthening effect.

Stripe rust (yellow rust) caused by *Puccinia striiformis f. sp. tritici* (Pst) is a serious disease infecting in wheat producing areas with cool and moist weather conditions during the growing season. Currently, it is considered to be the most damaging rust disease all over the world including Hungary. So far, *Yr9* gene located on the 1RS arm of the 1RS.1BL translocation has been the only known resistance gene against Pst in wheat, but it no longer provides protection. The Mv9kr1-'Kriszta' 6R disomic addition line is highly resistant to stripe rust as no symptoms were visible on the plants grown in the pesticide-free and the Breeders nursery in the consecutive seasons of 2013-2014 and 2014-2015, and can be considered as an excellent candidate to transfer new, effective yellow rust resistance gene(s) from rye into cultivated bread wheat. The stripe rust resistance of 'Kriszta' is obviously originates from the wild parental species *S. montanum*. It is remarkable that according to our results the resistance gene is located on chromosome 6R, while it is present on the 2RL chromosome arm of *S. montanum* ssp. *africanum* (Lei et al. 2013). It indicates considerable genetic diversity among the subspecies of *S. montanum*, and makes it also probable that further resistance genes are located on the individual chromosomes of *Secale cereanum*.

The Mv9kr1-'Kriszta' 1R, 4R and 6R disomic additions represent a large reservoir of agronomically useful genes. Among the tribe Triticeae, rye is the most tolerant species to Al toxicity (Aniol and Gustafson 1984; Gallego et al. 1998). According to previous papers, major genes influencing Al tolerance are located on chromosomes 3R, 4R, and the short arm of 6R. It appeared that Al tolerance was mainly due to chromosome arm 6RS and was significantly affected by the wheat background (Aniol and Gustafson 1984). Gallego and Benito (1997) found that Al tolerance is controlled by at least two major dominant and independent loci (*Alt1* and *Alt3*) located on chromosomes 6RS and 4R. Powdery mildew resistance genes were also found on chromosomes 4R and 6R (Heun et al. 1990; An et al. 2013, 2015). Chromosome 6R carries a gene for resistance to cereal cyst nematode (*Heterodea avenae* Woll.,) as well (Asiedu et al. 1990), and a Russian wheat aphid (*Diuraphis noxia* Mordvilko) single dominant gene was located on chromosome 4R (Nkongolo et al. 1996).

On the basis of the results presented here and the potentials inherent in the Mv9kr1-'Kriszta' 1R, 4R and 6R disomic additions, these lines can be regarded as important gene resources for wheat breading. Translocation lines deriving from these additions make possible the direct and stable transfer of valuable features such as high dietary fibre and protein

content, as well as yellow rust resistance from *S. cereanum* to modern wheat cultivars that can be utilised in further pre-breeding and breeding projects.

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Figure captions

Figure 1a: Fluorescence in situ hybridisation (FISH) patterns of the 1R, 4R and 6R chromosomes of the Hungarian rye cultivar Kisvárdai alacsony (1st row), *Secale montanum* (2nd row), *Secale cereanum* cv. Kriszta (3rd row) and the 1R, 4R and 6R chromosomes of the Mv9kr1-'Kriszta' addition lines (4^{th} row) with pSc119.2 and $(AAC)_5$ repetitive DNA probes. Fig. 1b, c, d: FISH and GISH patterns on the partial cells of the three different Mv9kr1-'Kriszta' 1R disomic addition lines with diverse pSc119.2 patterns. Fig. 1e, f: Simultaneously applied GISH and FISH on the partial cells of the 4R and 6R disomic Mv9kr1-'Kriszta' addition lines. The 1R, 4R and 6R chromosomes found in the Mv9kr1-'Kriszta' disomic addition lines are enlarged in the bottom right corner.

Figure 2a, b: From left to right spikes and leaves of the wheat line Mv9kr1, 'Kriszta' perennial rye (*S. cereanum*), the 1R, 4R and 6R wheat-*S. cereanum* disomic addition lines. Fig. 2c: Spikes of the 1R, 4R and 6R Mv9kr1-'Kriszta' addition lines in the pesticide-free nursery in 2014. 'Kriszta' perennial rye and the Mv9kr1-'Kriszta' 6R disomic addition line showed yellow rust resistance in the pesticide-free nursery in 2014.

Figure 3: PCR amplification patterns of the SSR markers SCM9, GRM0350, GRM0324, GRM1019, GRM0609 and SCM28 on the following DNA templates (from left to right): Mv9kr1 wheat line (Mv9), 'Kriszta' perennial rye (Kr), *Secale montanum* (Mon), CS-'Imperial' 1R, 2R, 3R, 4R, 5R, 6R and 7R disomic addition lines (I1R-I7R), and Mv9kr1-*S. cereanum* cv. Kriszta 1R or 4R or 6R disomic addition lines (K1R, K4R and K6R) according to the rye chromosome-specificity of the SSR markers used. Banding patterns of the wheat-*S. cereanum* cv. Kriszta 1R disomic addition line produced with the 1RS chromosome-specific SSR marker SCM9 were compared to the Martonvásár wheat cultivar Mv Magdaléna (Mag) carrying 1RS.1BL translocation of 'Petkus' rye origin. Sample order of the wheat-'Kriszta' 1R addition lines (Kr1R) is '132941', '132948' and '132949'. PCR products of the markers (SCM9 and GRM0350, GRM0324 and GRM1019, GRM0609 and SCM28) specific for the rye chromosomes 1R, 4R and 6R, respectively, are marked with arrows. L: DNA ladder

Tables

Table 1: Rye chromosome-specific SSR markers used in the present study

Marker	Annealing temperature (°C)	Chromosome location in *S. cereale*	Reference
SCM9	60	1RS	Saal and Wricke 1999
TSM81	60	1RS	Kofler et al. 2008
TSM103	60	1RS	Kofler et al. 2008
TSM120	60	1RS	Kofler et al. 2008
GRM0077	60	1RL	Martis et al. 2013
GRM0325	60	1RL	Martis et al. 2013
GRM0350	60	1RL	Martis et al. 2013
GRM0146	60	4RS	Martis et al. 2013
GRM0324	60	4RS	Martis et al. 2013
GRM1008	60	4RS	Martis et al. 2013
GRM0277	60	4RL	Martis et al. 2013
GRM1019	60	4RL	Martis et al. 2013
MWG652*	55-58	4RL	Graner et al. 1991
GRM0059	60	6RS	Martis et al. 2013
GRM0609	60	6RS	Martis et al. 2013
SCM304	60	6RS	Saal and Wricke 1999
SCM2	55	6RL	Saal and Wricke 1999
SCM28	60	6RL	Saal and Wricke 1999
SCM180	60	6RL	Saal and Wricke 1999

*RFLP marker converted into STS-PCR marker (GrainGenes Database, http://wheat.pw.usda.gov/cgi-bin/GG3/report.cgi?class=marker;name=cMWG652;show=locus)

Table 2: Arm ratio of rye chromosomes 1R, and relative length of the 1RS and 1RL arms in the Mv9kr1-'Kriszta' disomic addition lines '132941', '132948' and '132949'

Cytology number	1R morphology	Arm ratio (1RL/1RS)	Relative length 1RS (1RS/1R)	Relative length 1RL (1RL/1R)
132941	metacentric	$1.037^{a} \pm 0.018$	$0.488^{c} \pm 0.004$	$0.512^{e} \pm 0.041$
132949	submetacentric	$1.443^{b} \pm 0.045$*	$0.409^{d} \pm 0.007$*	$0.590^{f} \pm 0.008$*
132948	metacentric	$1.051^{a} \pm 0.025$	$0.488^{c} \pm 0.004$	$0.512^{e} \pm 0.004$
	submetacentric	$1.448^{b} \pm 0.029$*	$0.409^{d} \pm 0.009$*	$0.591^{f} \pm 0.009$*

*Significantly different from metacentric at the $P < 0.01$ level

a, b: Arm ratios are not significantly different

c, d, e, f: Relative lengths are not significantly different

Table 3: Production of PCR amplicons in wheat-*Secale cereale* (CS-'Imperial') and wheat-*Secale cereanum* (Mv9kr1-'Kriszta') 1R, 4R and 6R disomic addition lines using rye chromosome-specific SSR markers

Marker (location)	CS-'Imperial'			Mv9kr1-'Kriszta'		
	1R	4R	6R	1R	4R	6R
SCM9 (1RS)	+	-	-	+	-	-
TSM81 (1RS)	+	-	-	+	-	-
TSM103 (1RS)	+	-	-	+	-	-
TSM120 (1RS)	+	-	-	+	-	-
GRM0077 (1RL)	+	-	-	+	-	-
GRM0325 (1RL)	+	-	-	+	-	-
GRM0350 (1RL)	+	-	-	+	-	-
GRM0146 (4RS)	-	+	-	-	+	-
GRM0324 (4RS)	-	+	-	-	+	-
GRM1008 (4RS)	-	+	-	-	+	-
GRM0277 (4RL)	-	+	-	-	+	-
GRM1019 (4RL)	-	-	-	-	+	-
MWG652 (4RL)*	-	+	-	-	+	-
GRM0059 (6RS)	-	-	+	-	-	-
GRM0609 (6RS)	-	-	+	-	-	+
SCM304 (6RS)	-	-	+	-	-	+
SCM2 (6RL)	-	-	-	-	-	+
SCM28 (6RL)	-	-	+	-	-	+
SCM180 (6RL)	-	-	+	-	-	-

+: PCR product; - : no PCR product; *RFLP marker converted into STS-PCR marker

Table 4: Morphological features of the Mv9kr1-'Kriszta' (wheat-*S. cereanum*) disomic 1R, 4R and 6R addition lines (Breeders nursery, 2015). Values are the means of 10 measurements and were compared to the parental wheat line Mv9kr1.

Genotypes	Plant height (cm)	Tillering (spikes/plant)	Length of the main spike (cm)	Spikelets/ main spike	Seeds/main spike
Mv9kr1	68.90±2.33	5.60±2.17	8.31±0.48	19.90±1.52	50.10±4.03
'Kriszta'	139.20±12.90	no data	16.20±11.80	46.60±4.97	66.20±12.95
addition line 1R	62.40±4.59**	8.00±2.82*	8.85±0.89	20.40±1.26	54.30±8.36
addition line 4R	70.10±2.68	7.80±1.68	10.65±0.50**	22.30±0.94**	51.10±8.14
addition line 6R	85.20±6.71**	8.60±3.20*	7.65±0.74*	21.20±1.98	50.10±15.17

*Significantly different from Mv9kr1 at the $P < 0.05$ level

**Significantly different from Mv9kr1 at the $P < 0.01$ level

Table5: Morphological features of the Mv9kr1-'Kriszta' (wheat-*S. cereanum*) disomic 1R, 4R and 6R addition lines (pesticide-free nursery, 2015). Values are the means of 10 measurements and were compared to the parental wheat line Mv9kr1.

Genotypes	Plant height (cm)	Tillering (spikes/ plant)	Length of the main spike (cm)	Spikelets/ main spike	Seeds/main spike	TKW[1] (g)
Mv9kr1	75.30±4.57	5.20±0.78	9.10±0.73	19.60±2.50	53.80±8.43	36.20
'Kriszta'	178.70±8.42	11.50±4.83	13.85±1.82	38.80±6.28	63.40±27.60	19.30
addition line 1R	54.85±4.56**	5.50±0.84	8.78±1.18	18.42±1.71	44.20±7.67*	27.10
addition line 4R	75.70±2.71	5.80±1.68	9.95±0.83*	19.00±2.05	46.80±10.51	29.00
addition line 6R	69.70±6.25	5.80±1.31	6.95±0.43**	18.60±1.83	39.90±16.05*	39.90

[1] 1,000-kernel weight

*Significantly different from Mv9kr1 at the $P < 0.05$ level

**Significantly different from Mv9kr1 at the $P < 0.01$ level

Table 6: Quality properties of the parental Kriszta perennial rye (*S. cereanum*), Mv9kr1 wheat line and Mv9kr1-'Kriszta'1R, 4R and 6R disomic addition lines grown in the pesticide-free nursery in 2014. The results are presented as the means of 2 or 3 replicates ± standard deviation. The wheat-*S. cereanum* disomic addition lines were compared to the parental wheat genotype Mv9kr1.

Genotypes	total protein %[a]	Glu/Gli[b]	UPP%[b]	HMW/LMW[b]	TOTAX[a] (mg/g)	WEAX[a] (mg/g)
Kriszta	19.00±0.08	0.38±0.01	26.52±2.38	0.73±0.023	56.45±5.70	24.3±1.00
Mv9kr1	14.90±0.05	0.82±0.02	43.18±2.53	0.77±0.004	41.39±1.73	6.44±0.19
1R addition line	13.70±0.04	0.88±0.05	12.31±0.20**	0.68±0.003**	55.00±1.73**	12.14±0.78**
4R addition line	17.80±0.07**	0.73±0.08	41.30±0.47	0.89±0.006**	51.52±2.45**	7.84±0.12**
6R addition line	19.80±0.06**	0.70±0.03*	41.26±2.83	0.86±0.015**	54.67±2.12**	10.34±0.24**

a: 2 replicates; b: 3 replicates

*, **Significantly different from Mv9kr1 at the $P < 0.05$ level and at the $P < 0.01$ level, respectively

abbreviations: Glu - glutenin; Gli - gliadin; HMW - high molecular weight glutenins; LMW - low molecular weight glutenins; TOTAX - total arabinoxylan; UPP% - unextractable polymeric protein; WEAX - water extractable arabinoxylan.

Fig. 1

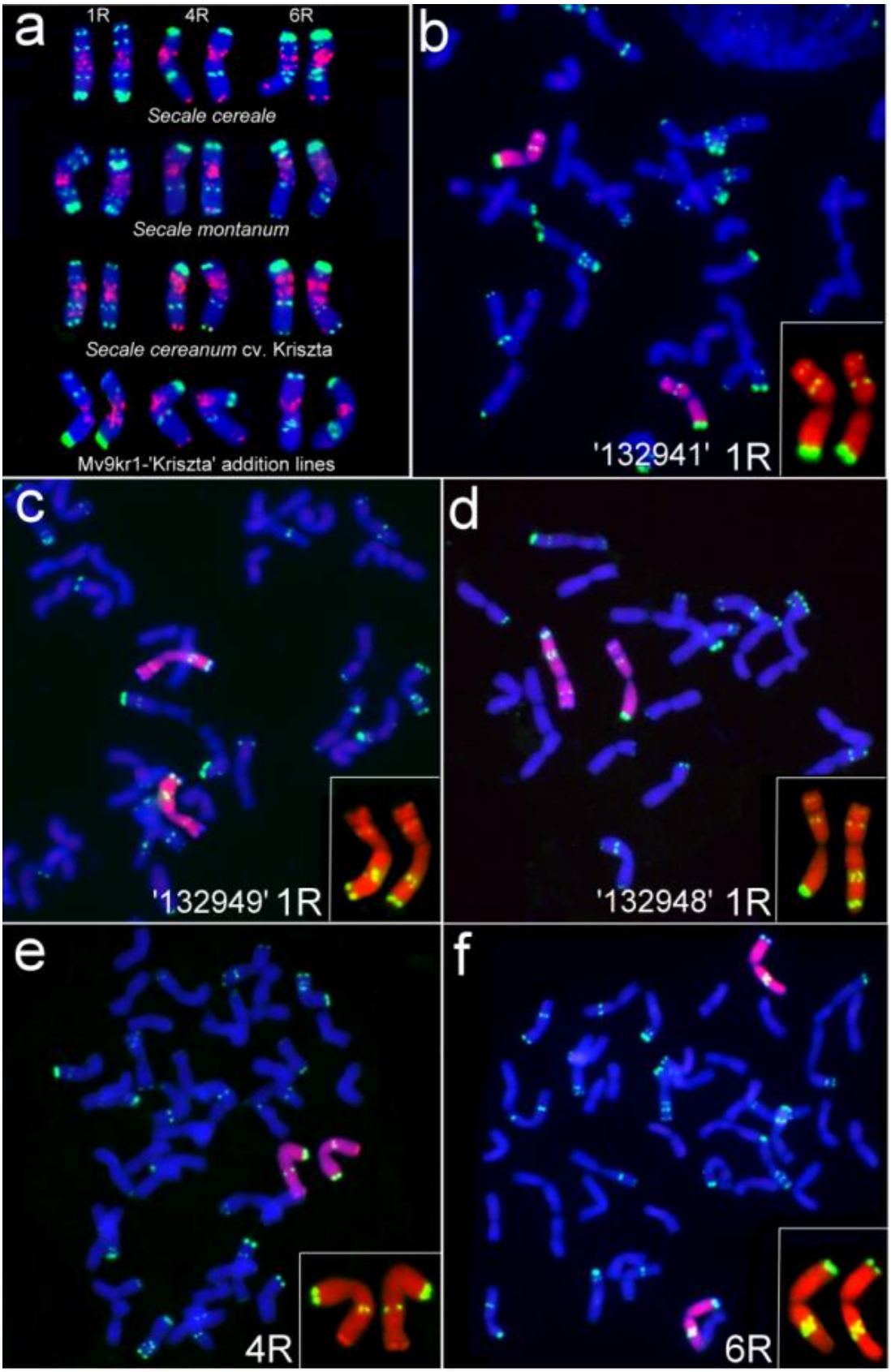

a
1R
4R
6R
Secale cereale
Secale montanum
Secale cereanum cv. Kriszta
Mv9kr1-'Kriszta' addition lines
b
'132941' 1R
c
'132949' 1R
d
'132948' 1R
e
4R
f
6R

Fig. 2

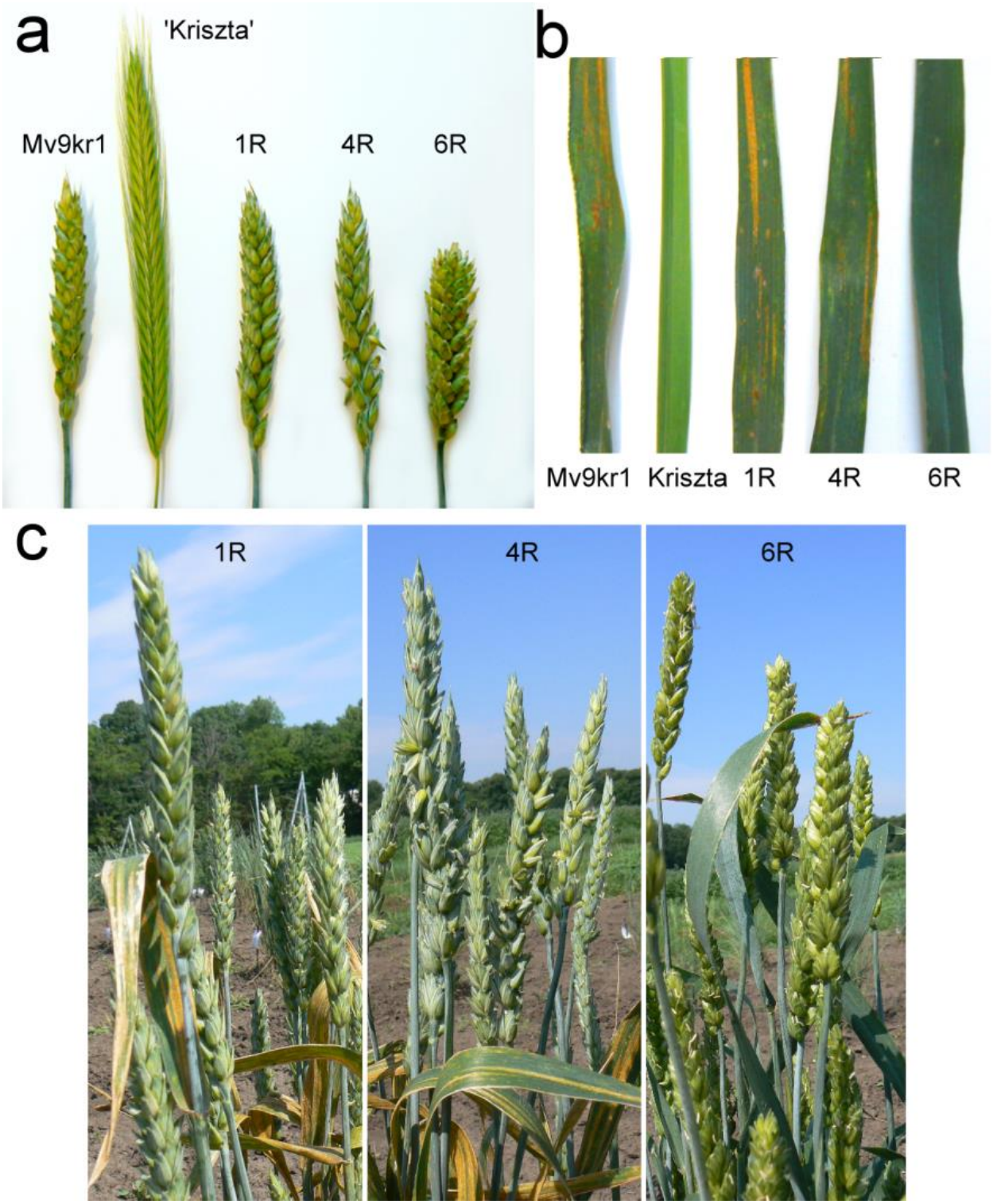
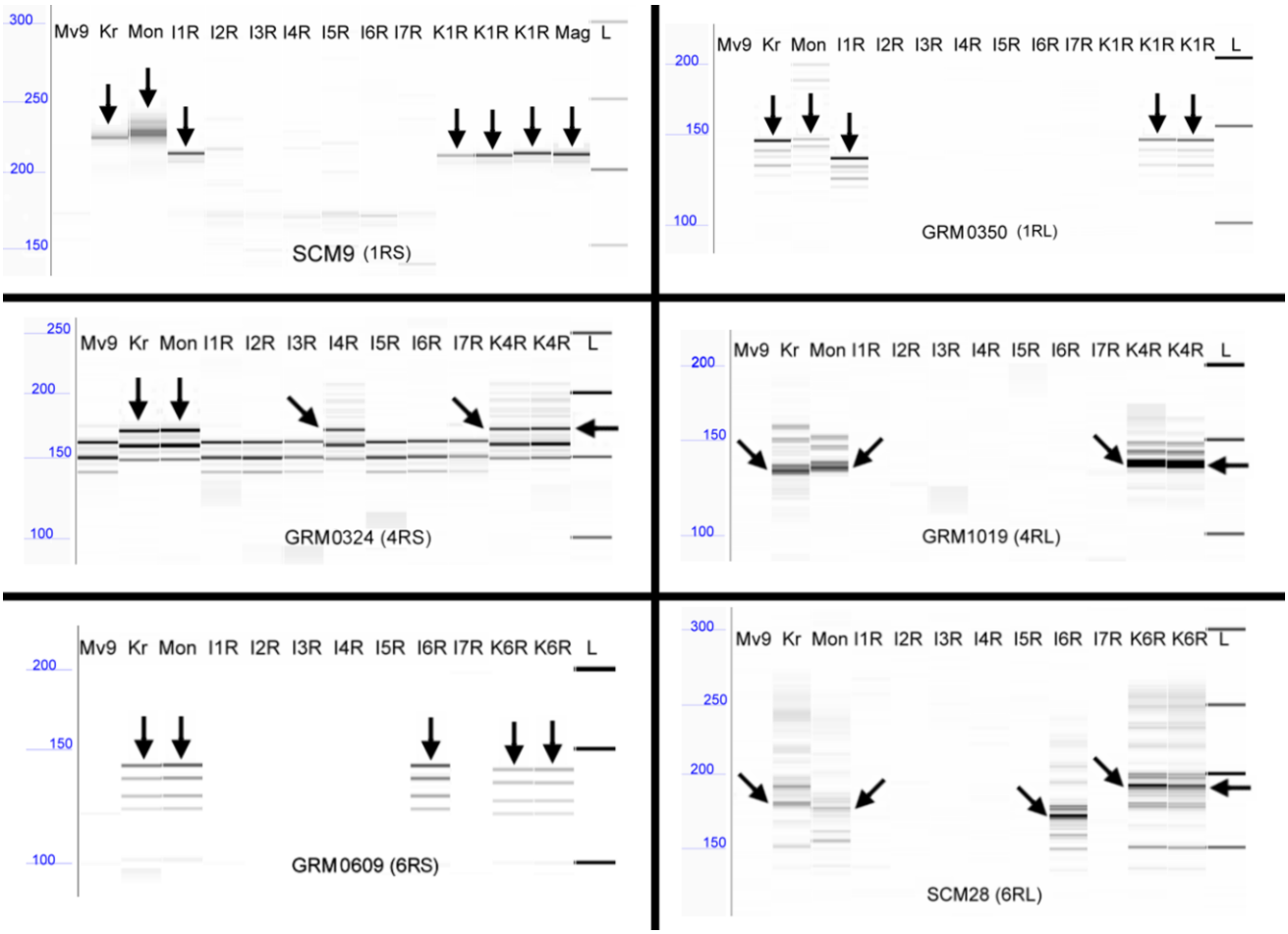


Fig. 3


300
250
200
150
Mv9 Kr Mon I1R I2R I3R I4R I5R I6R I7R K1R K1R K1R Mag L
SCM9 (1RS)
200
150
100
Mv9 Kr Mon I1R I2R I3R I4R I5R I6R I7R K1R K1R K1R L
GRM0350 (1RL)
250
200
150
100
Mv9 Kr Mon I1R I2R I3R I4R I5R I6R I7R K4R K4R L
GRM0324 (4RS)
200
150
100
Mv9 Kr Mon I1R I2R I3R I4R I5R I6R I7R K4R K4R L
GRM1019 (4RL)
200
150
100
Mv9 Kr Mon I1R I2R I3R I4R I5R I6R I7R K6R K6R L
GRM0609 (6RS)
300
250
200
150
Mv9 Kr Mon I1R I2R I3R I4R I5R I6R I7R K6R K6R L
SCM28 (6RL)