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ORIGINAL PAPER

Development of a wheat genotype combining the recessive crossability alleles *kr1kr1kr2kr2* and the 1BL.1RS translocation, for the rapid enrichment of 1RS with new allelic variation

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Abstract The main objective of the present work was to develop a wheat genotype containing both the recessive crossability alleles (*kr1kr1kr2kr2*), allowing high crossability between 6x wheat and diploid rye, and the 1BL.1RS wheat/rye translocation chromosome. This wheat genotype could be used as a recipient partner in wheat–rye crosses for the efficient introduction of new allelic variation into 1RS in translocation wheats. After crossing the wheat cultivars 'Mv Magdaléna' and 'Mv Béres', which carry the 1BL.1RS translocation involving the 1RS chromosome arm from 'Petkus', with the line 'Mv9 kr1', 117 F_2 plants were analysed for crossability, ten of which had higher than 50% seed set with rye and thus presumably carried the *kr1kr1kr2kr2* alleles. Four of the ten plants contained the 1BL.1RS translocation in the disomic condition as detected by genomic in situ hybridization (GISH). The wheat × rye F_1 hybrids produced between these lines and the rye cultivar 'Kriszta' were analysed in meiosis using GISH. 1BL.1RS/1R chromosome pairing was detected in 62.4% of the pollen mother cells. The use of fluorescent in situ hybridization (FISH) with the repetitive DNA probes pSc119.2, Afa family and pTa71 allowed the 1R and 1BL.1RS chromosomes to be identified. The presence of the 1RS arm from 'Kriszta' besides that of 'Petkus' was demonstrated in the F_1 hybrids using the rye SSR markers RMS13 and SCM9. In four of the 22 BC_1 progenies analysed, only 'Kriszta'-specific bands were observed with these markers, though the presence of the 1BL.1RS translocation was detected using GISH. It can be concluded that recombination occurred between the 'Petkus' and 'Kriszta' 1RS chromosome arms in the translocated chromosome in these plants.

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Introduction

The 1BL.1RS wheat–rye translocation is the most widespread alien translocation, detected in hundreds of wheat cultivars worldwide (Bedő et al. 1993; Friebe et al. 1996; Rabinovich 1998; Lukaszewski 2000; Braun et al. 1998; Schlegel 2008). Most varieties with a 1BL.1RS translocation contain the short arm of the 1R chromosome from 'Petkus' rye (Zeller 1973; Schlegel and Korzun 1997). Unfortunately, most of the resistance genes (*Lr26*, *Yr9*, *Pm8*, *Sr31*) located on this chromosome arm are no longer effective against new biotypes of the diseases. However, the translocation was also postulated to have a yield-enhancing effect and to lead to the better adaptation of the carrier wheat varieties (Rajaram et al. 1990; Schlegel and Meinel 1994; Villareal et al. 1998). As it is probably of single origin (Schlegel and Korzun 1997) this 1RS arm lacks any genetic variation, so there is a need to introduce new allelic variation into 1RS from other 1RS chromosomes in order to exploit the rich gene reservoir of diploid rye. Other rye genotypes may have new resistance genes or alleles against various diseases and may have a less deleterious effect on bread making quality, probably the only negative consequence of the presence of the original 'Petkus' rye chromosome arm in wheat.

Several authors have reported the production of wheat cultivars carrying 1RS chromosome arms from various rye genotypes. The 1RS.1AL translocation in wheat cultivar

'Amigo' carries the 1RS arm of 'Insave' rye (Zeller and Fuchs 1983). 'Salmon', another 1BL.1RS wheat–rye translocation line, was derived from an F_3 seed from a hybrid between two octoploid triticale strains. When its cytoplasm was replaced by that of *Aegilops caudata*, *Ae. kotschyi* or certain other *Aegilops* species, Salmon produced haploid seedlings at high frequency (Tsunewaki 1964). A 1DL.1RS translocation was derived from the rye cultivar 'Imperial' (Shepherd 1973). Marais et al. (1994) used homologous recombination to transfer a gene from the short arm of chromosome 1R from 'Turkey 77' rye into the 1RS arm of the translocated chromosome in the wheat cultivar 'Veery'. A new 1BL.1RS wheat–rye translocation line was developed by Ko et al. (2002) from the backcross of the F_1 hybrid of wheat cv. 'Olmil' and rye cv. 'Paldanghomil'. Nevertheless, the 1BL.1RS combination involving the 1RS arm from 'Petkus' rye is now so widely distributed that it can be considered as part of the wheat gene pool. A fast, efficient method to introduce a substantial amount of allelic variation into this chromosome arm directly from diploid rye is urgently needed (Nagy et al. 2003; Lelley et al. 2004).

A method was elaborated by Lelley et al. (1999) to introduce new genetic variation into the 'Petkus' chromosome arm from other rye genotypes through homologous recombination. In the first step, new octoploid triticales were produced from a wheat genotype with high crossability with rye, developed by Molnár-Láng et al. (1996). These 8x triticales were then crossed with wheats containing the 1BL.1RS translocation. Homologous pairing and recombination were expected between the 1RS arms in the 1B.1R translocated wheat and in the octoploid triticale and was indeed observed using GISH by Nagy and Molnár-Láng (2000). Using the above method wheat–rye recombinants were produced and mapped with the help of various PCR-based markers (Nagy et al. 2003).

The aim of the present research was to develop a wheat genotype containing both the recessive crossability alleles (*kr1kr1kr2kr2)* in the homozygous state and the 1BL.1RS translocation. Hungarian wheat cultivars with the 1BL.1RS translocation have the *Kr1Kr1Kr2Kr2* allele composition at the crossability locus, like most European wheat cultivars (Zeven 1987). The transfer of the recessive crossability alleles into a wheat cultivar carrying the 1BL.1RS translocation would give the 1RS arm of the 1BL.1RS chromosome the chance to recombine with the 1RS arm of diploid rye. This genotype could then be used in wheat–rye crosses as a recipient to create new 1RS recombinants in a wheat background. The advantage of this method, compared with the technique used by Lelley et al. (1999), is that there is no need to develop octoploid triticale lines and cross them with wheat cultivars carrying the 1BL.1RS translocation, as recombination can occur directly in the wheat × rye F_1 hybrids between the 1R rye chromosome arm and the 1BL.1RS translocation.

The high crossability alleles were introduced into translocated wheats and the genome composition of the wheat × rye hybrids was analysed using genomic in situ hybridization (GISH). Fluorescent in situ hybridization (FISH) with repetitive DNA probes was used to confirm pairing between the 1BL.1RS chromosome and the 1R rye chromosome in meiosis. The presence of the 1RS arm of the pollinator rye genotype cv. 'Kriszta' in the wheat × rye hybrids was detected with the help of SSR markers RMS13 and SCM9. Recombinations in the 1RS arm of the 1BL.1RS translocated chromosome were detected with the same markers in the backcross progenies.

Materials and methods

Plant material

Wheat (*Triticum aestivum* L.) line 'Martonvásári 9 kr1' (Mv9 kr1) (Molnár-Láng et al. 1996), which carries the *kr1kr1kr2kr2* genes, and Martonvásár wheat cultivars 'Mv Magdaléna' and 'Mv Béres', which contain the 1BL.1RS translocation, were crossed to develop a wheat genotype containing both the recessive crossability alleles and the 1BL.1RS translocation.

The pedigree of 'Mv Magdaléna' is 'Yubileinaya 50'/'Fundulea 29'//'Mv Ma'. The 1BL.1RS translocation in this cultivar can be traced back to 'Fundulea 29', which received the 1B.1R translocation from the Russian wheat cultivar 'Avrora'. The cultivar 'Avrora' is a derivative of the 'Neuzucht'/'Bezostaya 4'//'Bezostaya 1' combination, so the 1RS chromatin in this cultivar originates from the German cultivar 'Neuzucht', which was developed from the 'Criewener 104'/'Petkus' rye cross by Riebesel in Germany in the first half of the twentieth century (Schlegel and Korzun 1997; Rabinovich 1998).

'Mv Béres' is a derivative of 'Erythrospermum 352'/'Mv Magdaléna', so the 1BL.1RS translocation can be traced back to 'Petkus' rye through the wheat cultivar 'Neuzucht', as in 'Mv Magdaléna'.

Rye (*Secale cereale* L.) cultivars 'Kisvárdai alacsony', 'Lovászpatonai' (Hungarian), 'Haru 4' (Japanese), 'Blanco' (Brazilian), 'Petrus', 'Mercator' (German) and 'Porto' (Portugal) were used in wheat–rye hybridization experiments to test the crossability of various wheat genotypes.

Perennial rye cultivar 'Kriszta', originating from a hybrid between *S. cereale* and the wild species *S. montanum* (Kotvics et al. 1999; Kruppa 2001), was used to develop new wheat–rye hybrids.

Seeds of the 'Petrus' rye cultivar were kindly provided by the Gatersleben Genebank (Leibnitz Institute of Plant Genetics and Crop Plant Research, Gaterleben, Germany), seeds of the 'Haru 4', 'Blanco' and 'Porto' rye cultivars by the USDA ARS (National Small Grain Collection, Aberdeen, ID, USA) and seeds of the 'Mercator' rye cultivar by the Research Institute of Cereal Crops, Kromeriz, Czech Republic.

Methods

Pollination and statistical analysis

The wheat plants were pollinated 3–5 days after emasculation in the field nursery or in the phytotron under controlled environmental conditions (Conviron, PGR-15 cabinet, 21°C, 80% humidity, 600 µmol/m^{2}s; Tischner et al. 1997). Seed set was assessed by calculating the ratio of developing seeds to the total number of florets pollinated.

The relationship between genotype (*kr* allele composition) and phenotype (percentage of crossability with rye) was determined according to Lein's classification (1943):

Genotype	Crossability percentage with rye
Kr1Kr1Kr2Kr2	Lower than 5
Kr1Kr1kr2kr2	10–30
kr1kr1Kr2Kr2	30–50
kr1kr1kr2kr2	Higher than 50

Chi-square was calculated for the comparison of the expected and observed ratios of plants with *kr1kr1kr2kr2* allele composition among plants heterozygous for both genes.

In situ hybridization

Chromosome preparations

Root tip chromosome preparations were made as described by Jiang et al. (1994). Anthers containing pollen mother cells (PMCs) at metaphase I of meiosis were fixed in 1:3 (v/v %) acetic acid/ethanol and squashed in 45% acetic acid.

DNA probes and labelling

Total genomic DNA was isolated from rye (*S. cereale*) and wheat (*T. aestivum*) according to Sharp et al. (1988). The rye DNA was sonicated and labelled with biotin (biotin-16-dUTP, Roche, Mannheim, Germany) by nick translation and used as hybridization probe to detect rye chromatin using genomic in situ hybridization (GISH).

The repetitive DNA sequences of pSc119.2 (Bedbrook et al. 1980) and Afa-family (Nagaki et al. 1995) were amplified and labelled with biotin-16-dUTP (Roche) and digoxigenin-11-dUTP (Roche), respectively, using PCR (Nagaki et al. 1995; Contento et al. 2005). The 18S-5.8S-26S rDNA clone pTa71 (Gerlach and Bedbrook 1979) was labelled with 50% biotin-16-dUTP and 50% digoxigenin-11-dUTP. Digoxigenin and biotin were detected using anti-digoxigenin-rhodamine Fab fragments (Roche) and streptavidin-FITC (Roche), respectively.

Sequential FISH and GISH

FISH was carried out on meiotic chromosome spreads of wheat × rye F_1 hybrids using repetitive DNA probes (pSc119.2, Afa family, pTa71) according to Molnár et al. (2009) after the pretreatment and stringency washing of the slides. After documentation of the FISH sites, the slides were washed (4× SSC Tween at 25°C overnight) and rehybridized using rye genomic probe.

GISH was carried out on mitotic preparations made from the recipient wheat genotype and from the wheat × rye hybrids. Unlabelled wheat genomic DNA was sheared by autoclaving and used as a competitor at 30 times the quantity of the probe. GISH analysis was performed according to Reader et al. (1994) with minor modifications (Molnár-Láng et al. 2000a). The chromosomes were examined with a Zeiss Axioskop 2 epifluorescence microscope equipped with Filter 10 for FITC, Filter 15 for Rhodamine, Filter 01 set for DAPI and double band Filter 24 set for FITC and Rhodamine (Carl Zeiss Mikroskopie, Jena, Germany). The images were captured with a Spot CCD camera (Diagnostic Instruments, Sterling Heights, MI, USA), and compiled with Image Pro Plus software (Media Cybernetics, Silver Spring, MA, USA).

SSR marker analysis

Genomic DNA was isolated according to Anderson et al. (1992) from the following genotypes: wheat line 'Mv9 kr1' (six plants), wheat cultivars 'Mv Béres' and 'Mv Magdaléna' (six plants each), rye cultivar 'Kriszta' (ten plants), wheat × rye hybrids [combinations: ('Mv Béres' × 'Mv9 kr1') × 'Kriszta', ('Mv Magdaléna' × 'Mv9 kr1') × 'Kriszta', 12 plants each], and progenies obtained by backcrossing these wheat × rye hybrids with wheat line 'Mv9 kr1' (22 plants). The PCR reaction mixture for the 1RS-specific SSR markers RMS13 (Korzun, unpublished; Nagy et al. 2003) and SCM9 (Saal and Wricke 1999) contained 20 ng of template DNA, 2 × GoTaq Green Master Mix (Promega, USA) and 0.2 µM of each primer in a total volume of 25 µl. Amplification was carried out in an Eppendorf Mastercycler (Eppendorf, Germany) using the following profile: 90 s at 94°C; 36 cycles of 30 s at 94°C, 40 s at 60°C, 40 s at 72°C and a final extension at 72°C for

5 min. The PCR products were separated using 2.6% SeaKem agarose gels (Cambrex, USA) and the fragments were stained with ethidium bromide (Schneider and Molnár-Láng 2009). A 50-bp DNA ladder (Fermentas, Lithuania) was used to estimate molecular weights. The patterns were documented and analysed using a GeneGenius gel documentation system (Syngene, UK).

Results

Transfer of *kr1kr2* genes into Hungarian wheat cultivars with the 1BL.1RS translocation

The crossability of 'Mv Béres', 'Mv Magdaléna' and 12 other Hungarian wheat cultivars carrying 1BL.1RS or 1AL.1RS translocations with rye cultivar 'Mercator' was tested in the field nursery in 2005. The seed set was very low (0–2.6%), confirming that these cultivars had *Kr1Kr1Kr2Kr2* allele composition. In order to transfer the *kr1kr2* recessive crossability alleles into 'Mv Béres' and 'Mv Magdaléna', they were crossed with the wheat line 'Mv9 kr1' in the field nursery in the same year.

The presence of a single 1BL.1RS wheat/rye translocation in the F_1 progenies was proved using GISH, thus confirming hybridization between the genotypes. The segregation of the 1BL.1RS translocation in the F_2 progenies was analysed using GISH. Eight of 75 F_2 progenies from the 'Mv Béres' × 'Mv9 kr1' cross contained a pair of 1BL.1RS translocation chromosomes (Fig. 1a). Twenty-two F_2 plants had no translocation, and 45 plants had one 1BL.1RS chromosome.

The crossability of 76 'Mv Béres' × 'Mv9 kr1' and 41 'Mv Magdaléna' × 'Mv 9 kr1' F_2 progenies was analysed by pollinating the plants with rye. Classes of expected allele composition were created according to Lein's classification (1943) based on seed set (Table 1). A total of 183 spikes on 117 F_2 plants were pollinated with rye. The seed set was less than 5% on 84 plants, but ten plants (8.54%) gave more than 50% seed set with rye, suggesting that they had *kr1kr1kr2kr2* allele composition. According to the Chi square test, this ratio does not differ significantly from the 6.25%, ($\chi^2 = 0.0423$, $P = 0.95$) expected for two independent genes in the progeny of a heterozygous F_1 plant. The rye cultivar used as pollinator did not influence the seed set. With 'Kisvárdai alacsony' the average seed set was 8.67% on 145 pollinated spikes, while with 'Kriszta' it was 9.89% on 33 pollinated spikes. The seed set of the ten selected F_2 plants assumed to carry the recessive *kr1kr2* alleles is shown in Table 2.

Four of the ten F_2 progenies, two each from the crosses 'Mv Béres' × 'Mv9 kr1' and 'Mv Magdaléna' × 'Mv 9 kr1', contained the 1BL.1RS translocation in the disomic condition together with the *kr1kr1kr2kr2* alleles. The crossability of the progenies of the four selected plants was analysed in the F_3 generation (Table 2). A total of 17 spikes on 14 plants were pollinated with rye cultivar 'Kriszta'. The average seed set was 54.8% (65.5, 48.4, 44.4 and 64.7% of the progenies of the four different lines) confirming that these lines had recessive *kr1 kr2* alleles in the homozygous state.

The crossability of the selected lines was checked in the field nursery in 2008. When 53 F_3 plants from the two combinations were pollinated with five different rye cultivars the average seed set was 46.9%, giving a total of 799 wheat–rye F_1 hybrid seeds (Table 3). The crossability of these lines was relatively high with all five rye cultivars.

GISH and FISH analysis in mitosis and meiosis of wheat–rye hybrids produced with the new recipient wheat genotypes: demonstration of 1BL.1RS-1R chromosome pairing

The genome composition of the F_1 wheat–rye hybrid seeds produced with the rye cultivar 'Kriszta' on F_3 progenies of selected wheat lines (061229, 061187, 061234, 061254) from the 'Mv Béres' × 'Mv9 kr1' and 'Mv Magdaléna' × 'Mv9 kr1' crosses (Table 2) was analysed by GISH using total genomic rye DNA. Strong fluorescent signals were obtained for seven rye chromosomes and a rye chromosome arm in the 1Bl.1RS translocated chromosome (Fig. 1b).

The meiotic behaviour of wheat–rye hybrids produced between the recipient wheat genotype 'Mv Béres' × 'Mv9 kr1' F_3 and 'Kriszta' rye was analysed with GISH. The rye chromatin was clearly discriminated in the wheat genetic background on the basis of the green fluorescent signal. Pairing between the 1BL.1RS translocated chromosome and a rye chromosome was detected in metaphase I of meiosis in the wheat–rye F_1 hybrid plants (Fig. 1d, f). Rod bivalents (1BL.1RS-1R) were observed in 113 of the 181 cells analysed (62.4%). Data on the chromosome pairing observed in the F_1 hybrid are presented in Table 4.

The chromosomes forming bivalents in metaphase I of wheat–rye hybrids were identified with the help of repetitive DNA probes (Fig. 1c, e). pTa71 gives a strong fluorescent signal in the NOR region of the satellited rye chromosome 1R. FISH confirmed that the pairing revealed using GISH in 62% of the cells occurred between the 1BL.1RS translocated chromosome and the 1R rye chromosome. The probe pSc119.2 gives strong fluorescent signals on the telomeres of rye chromosomes and on the telomere of the long arm of 1B, while 1RL is differentiated by an intense interstitial pSc119.2 band.

Most of the wheat and rye chromosomes can be identified from the FISH signals given by the three DNA probes (pSc119.2, Afa family, pTa71) on the meiotic chromo-

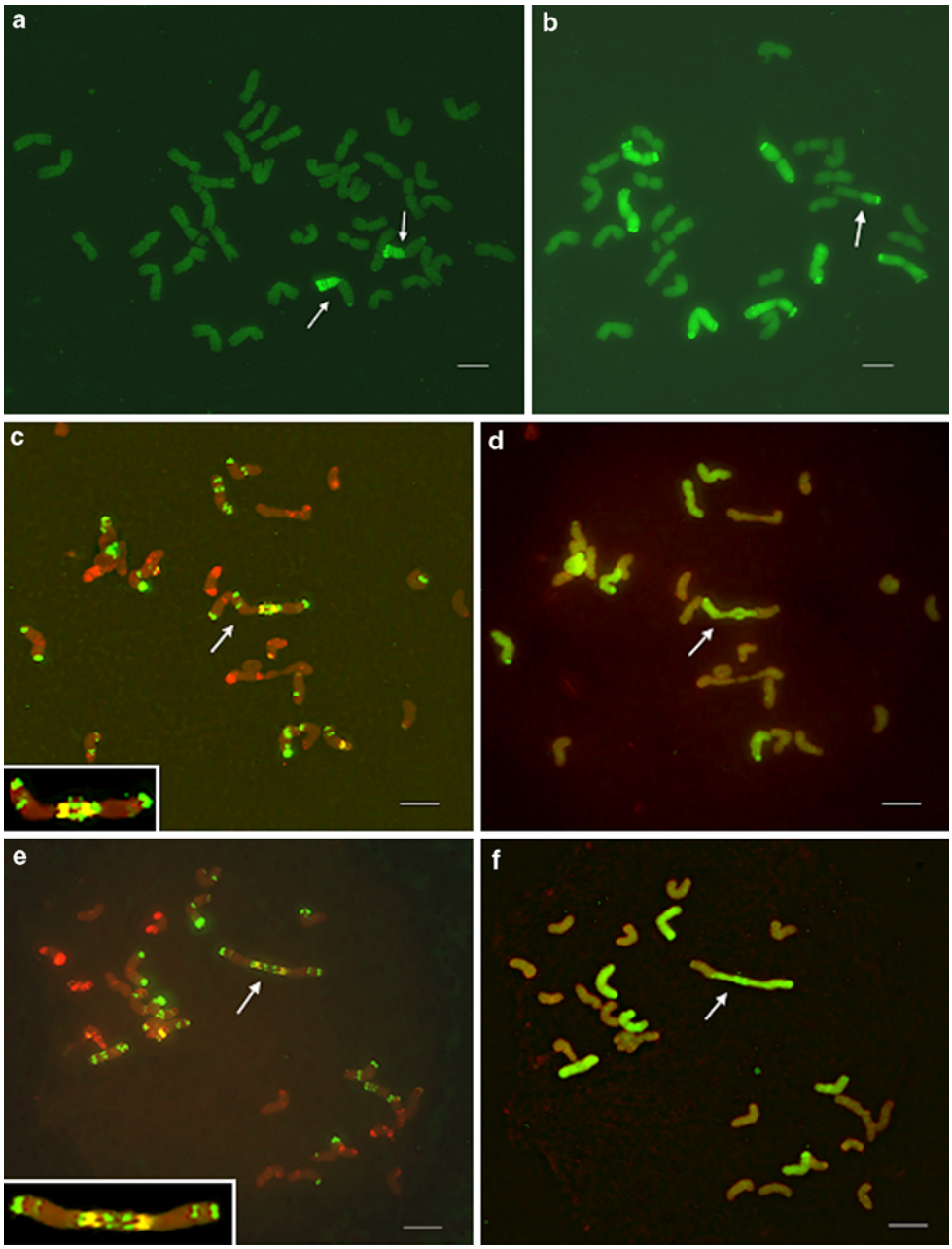

Fig. 1 In situ hybridization on mitotic and meiotic chromosomes: **a** Genomic in situ hybridization (GISH) on somatic chromosomes of the 'Mv Béres' × 'Mv 9 kr1' F_3 line with the 1BL.1RS translocation in the disomic condition. Total rye genomic DNA was labelled with biotin and detected by streptavidin-FITC. The rye chromosome arms indicated by *arrows* in the 1BL.1RS translocated chromosomes are *yellowish-green* (Filter 10 for FITC). **b** GISH on somatic chromosomes of the wheat × rye F_1 hybrid produced between the 'Mv Béres' × 'Mv9 kr1' F_3 line and the rye cv. 'Kriszta'. Seven rye chromosomes and the 1RS arm in the 1BL.1RS translocated chromosome (*arrow*) are *yellowish-green*. Twenty wheat chromosomes and the 1BL arm of the 1BL.1RS chromosomes are unlabelled (Filter 10 for FITC). **c** FISH with repetitive DNA probes pSc119.2 (*green*), Afa family (*red*) and pTa71 (*yellow*) on meiotic metaphase I chromosomes of wheat × rye hybrids. Pairing was detected by FISH between the 1BL.1RS and 1R chromosomes (*arrow*). The 1BL.1RS-1R bivalent is enlarged in the corner. **d** GISH on the same cell. One rod bivalent detected between a rye chromosome and the 1BL.1RS chromosome and 6 rye univalents are labelled, 16 wheat univalents and 2 wheat rod bivalents are unlabelled (double band Filter set 24) **e** FISH with repetitive DNA probes on meiotic metaphase I chromosomes. 1BL.1RS-1R pairing was identified using FISH (*arrow*). The 1BL.1RS-1R bivalent is enlarged in the corner. **f** GISH on the same cell. One rod bivalent detected between a rye chromosome and the 1BL.1RS chromosome (*arrow*) and 6 rye univalents are *yellowish-green*, 20 wheat univalents are unlabelled (double band Filter set 24)

somes of wheat × rye hybrids. Afa family signals were mainly seen on the D genome and pSc119.2 signals on the rye and B genome chromosomes, while the FISH pattern of the univalents was very similar to that described for the mitotic chromosomes in wheat and rye (Sepsi et al. 2008; Szakács and Molnár-Láng 2008).

Table 1 Gene composition at the *kr* loci in F_2 progenies of 'Mv Béres' × 'Mv 9 kr1' and 'Mv Magdaléna' × 'Mv9 kr1' hybrids based on seed set % when pollinated with rye in the Martonvásár phytotron in 2007

Genotype	No. of plants					
	0–5%	5–10%	10–30%	30–50%	≥ 50%	Total no. of plants pollinated with rye
	Kr1Kr1Kr2Kr2	*Kr1Kr1Kr2kr2*	*Kr1Kr1kr2kr2*	*kr1kr1Kr2Kr2*	*kr1kr1kr2kr2*	
			Kr1kr1Kr2Kr2	*kr1kr1Kr2kr2*		
			Kr1kr1kr2kr2			
			Kr1kr1Kr2kr2			
'Mv Béres' × 'Mv9 kr1' F_2	51	3	8	6	8	76
'Mv Magdaléna' × 'Mv9 kr1' F_2	33	3	1	2	2	41
Total	84	6	9	8	10	117

Classes of expected allele composition were created according to Lein's classification (1943)

145 spikes were pollinated with rye cultivar Kisvárdai alacsony, 33 with Kriszta, 4 with Mercator and 1 with Lovászpatonai

Table 2 Seed set of selected 'Mv Béres' × 'Mv9 kr1' and 'Mv Magdaléna' × 'Mv9 kr1' F_2 plants and F_3 progenies assumed to carry the *kr1kr1kr2kr2* alleles and grown under controlled conditions in a phytotron in 2007 and 2008

Generation	Plant code	Genotype (parental plant code in brackets)	No. of 1BL.1RS	Seed set %			Pollinator rye genotype
				1st spike	2nd spike	Average of several pollinated spikes (No. in brackets)	
F_2	061215	'Mv Béres' × 'Mv9 kr1' F_2	1	63.3	25.0		'Kisv'[a]
	061229		2	87.5	25.0		'Kisv'
	061231		1	70.0	–		'Kisv'
	061203		1	53.1	61.5		'Kisv'
	061187		2	47.0	50.0		'Kisv'
	061579		1	76.5	71.4		'Kisv', 'Mercator'
	061567		1	20.0	61.5		'Kriszta',
	061584		1	70.5	44.1		'Lovász'[b], 'Kriszta'
	061234	'Mv Magdaléna' × 'Mv9 kr1' F_2	2	46.2	50.0		'Kisv'
	061254		2	50.0	83.3		'Kisv', 'Kriszta'
F_3	07807-09	'Mv Béres' × 'Mv9 kr1' F_3 (061187)	2			65.5 (4)	'Kriszta'
	07810-14	'Mv Béres' × 'Mv9 kr1' F_3 (061229)	2			48.4 (6)	'Kriszta'
	07816-19	'Mv Magdaléna' × 'Mv9 kr1' F_3 (061234)	2			44.4 (4)	'Kriszta'
	07821-23	'Mv Magdaléna' × 'Mv9 kr1' F_3 (061254)	2			64.7 (3)	'Kriszta'
P_1	–	'Mv Magdaléna'	2			2.3 (8)	'Mercator'
	–	'Mv Béres'	2			2.5 (7)	'Mercator'

Data on the pollination of cultivars 'Mv Magdaléna' and 'Mv Béres' with rye were collected in the field in 2005

[a] 'Kisvárdai alacsony'

[b] 'Lovászpatonai'

SSR analysis on wheat–rye hybrids and backcross derivatives; detection of recombinants

The RMS13 rye microsatellite marker specific to the 1RS rye chromosome arm did not amplify any products on 'Mv9 kr1' wheat DNA. However, wheat cultivars 'Mv Béres' and 'Mv Magdaléna', containing the 1BL.1RS translocation, gave three distinct amplification products measuring approx. 134, 150 and 168 bp (Fig. 2). These amplification products are 'Petkus'-specific bands, as wheat cultivars

Table 3 Number of wheat–rye hybrid seeds produced with different rye genotypes in the field in 2008

Wheat genotype	Pollinator rye	Pollinated florets/spike	No. of hybrid seeds	Seed set %	Total number of hybrid seeds	Average seed set %
'Mv Béres' × 'Mv9 kr1' F_3	'Haru-4'	194/6	141	72.7	428	52.7
	'Blanco'	118/4	57	48.3		
	'Petrus'	178/6	87	48.9		
	'Kisvárdai alacsony'	190/6	71	37.4		
	'Porto'	132/4	72	54.5		
'Mv Magdaléna' × 'Mv9 kr1' F_3	'Haru-4'	136/4	51	37.5	371	41.6
	'Blanco'	158/5	76	48.1		
	'Petrus'	264/8	112	42.4		
	'Kisvárdai alacsony'	334/10	132	39.5		
Total		1,704/53	799		799	46.9

Table 4 Mean chromosome pairing at metaphase I of meiosis in F_1 hybrids between wheat ('Mv Béres' × 'Mv9 kr1' F_3 progenies carrying the 1BL.1RS translocation) and rye (cv. 'Kriszta')

Genotype	No. of cells scored	Rye			Wheat			1BL.1RS-Rye rod bivalents
		Univalents	Bivalents		Univalents	Bivalents		
			Rod	Ring		Rod	Ring	
081302	120	6.308	0.016	–	19.016	0.641	0.025	0.658
081274	61	6.442	–	–	20.049	0.196	–	0.557
Total	181	6.353	0.011	–	19.364	0.491	0.016	0.624

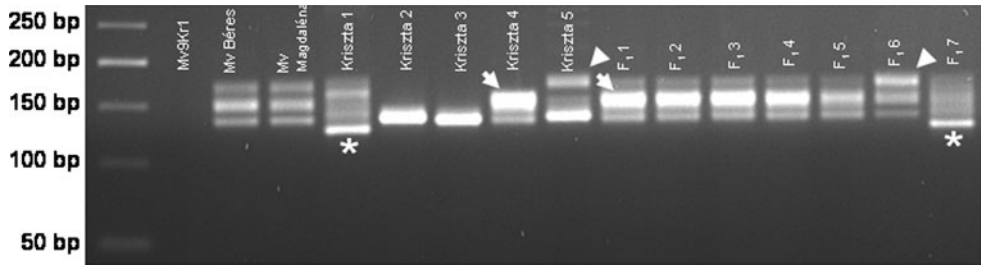

Fig. 2 Electrophoretic patterns of the 1RS-specific marker RMS13 in the wheat line 'Mv9 kr1', the wheat cultivars 'Mv Béres' and 'Mv Magdaléna', carrying the 1BL.1RS translocation involving the 1RS arm of rye cultivar 'Petkus', five plants of rye cultivar 'Kriszta' and the wheat–rye F_1 hybrid plants (F_1 1–7). F_1 hybrids No. 1–3 are from the ('Mv Béres' × 'Mv9 kr1') × 'Kriszta' combination, and F_1 hybrids No. 4–7 from the ('Mv Magdaléna' × 'Mv9 kr1') × 'Kriszta' cross. The *asterisk*, *arrow* and *arrowhead* indicate bands specific to 'Kriszta' in F_1 plants

'Mv Magdaléna' and 'Mv Béres' carry 1RS chromatin originating from 'Petkus' rye (for pedigrees, see "Materials and methods"). Ten seeds originating from the 'Kriszta' plants used as pollinator for producing wheat–rye hybrids were analysed with the SSR markers. Five allele combinations were found, composed of at least seven different alleles (bands).

F_1 hybrids Nos. 1–5 gave one strong (approx. 154 bp) band, which originated from 'Kriszta' plant No. 4 (Fig. 2, arrows), F_1 hybrid No. 6 amplified an approx. 172 bp polymorphic band originating from 'Kriszta' plant No. 5 (arrowhead) and F_1 hybrid No. 7 amplified an approx. 126 bp product (Fig. 2, asterisk), which was present in 'Kriszta' rye plant No. 1. All the F_1 hybrids also amplified bands from the 'Petkus' 1RS chromosome arm present in wheat cultivars 'Mv Magdaléna' and 'Mv Béres'.

The expected genome composition of the BC_1 progenies was 20 wheat chromosome pairs + one 1BL.1RS chromosome + one 1B chromosome + 0–7 rye chromosomes.

All the BC_1 progenies were expected to contain one 1BL.1RS chromosome, and the GISH analysis confirmed this assumption for all the BC_1 plants analysed. The original 1BL.1RS chromosome contained the chromosome arm

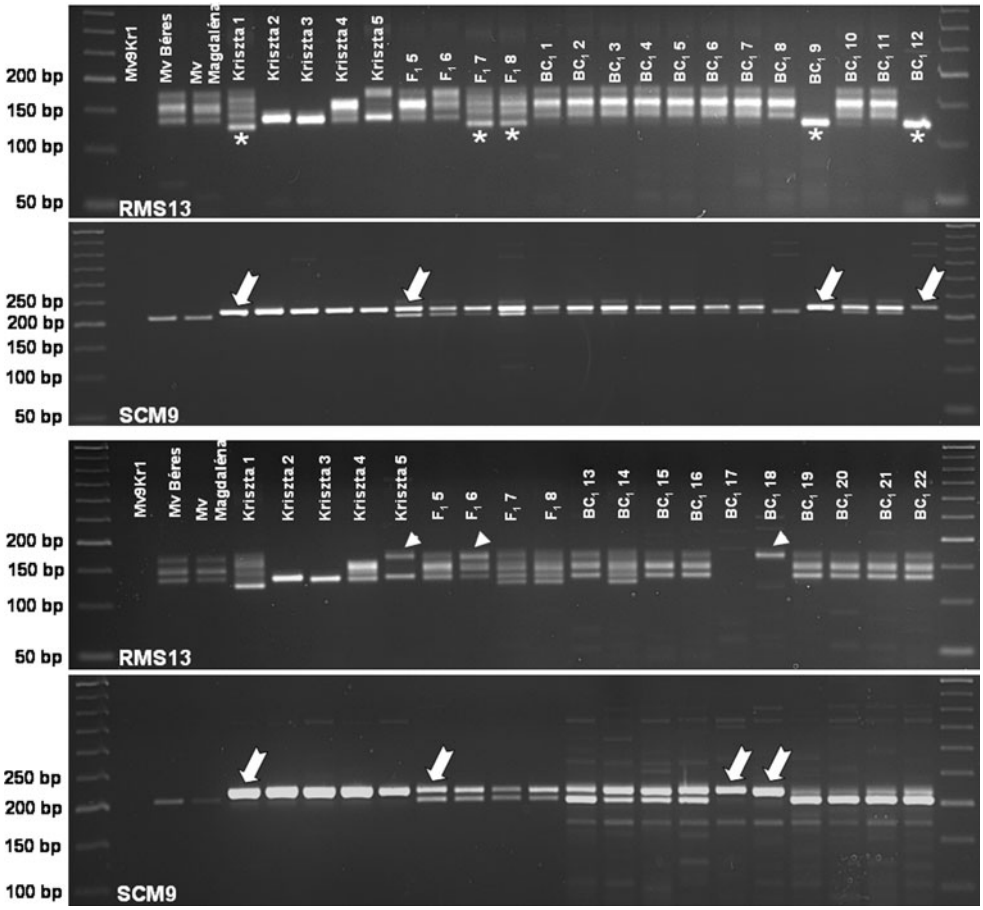

Fig. 3 Electrophoretic patterns of 1RS-specific markers (RMS13, SCM9) in the wheat line 'Mv9 kr1', the wheat cultivars 'Mv Béres' and 'Mv Magdaléna', carrying the 1BL.1RS translocation involving the 1RS arm of rye cultivar 'Petkus', five plants of rye cultivar 'Kriszta', four wheat–rye F_1 hybrid plants ($F_1$5–8) and the wheat–rye hybrids backcrossed to wheat line 'Mv9 kr1' ($BC_1$1-$BC_1$22). The *asterisks*, *arrows* and *arrowheads* indicate bands specific to 'Kriszta' in F_1 and BC_1 plants

from 'Petkus' rye, so the electrophoretic patterns of 'Mv Magdaléna', 'Mv Béres' and the F_1 hybrids had 'Petkus'-specific bands with the two markers.

The electrophoretic patterns of 22 BC_1 plants were analysed with two 1RS-specific SSR markers (RMS13 and SCM9). Both markers amplified 'Petkus'-specific bands in the majority of the BC_1 plants (1–8, 10–11, 13–16, 19–22), demonstrating that these plants contained the original 1BL.1RS translocation, carrying the 1RS from rye cultivar 'Petkus'. Four BC_1 plants (Nos. 9, 12, 17, 18), however, did not exhibit 'Petkus'-specific bands. Instead, 'Kriszta'- specific bands could be observed in BC_1 plants Nos 9, 12 and 18 corresponding to the alleles of 'Kriszta' rye plants No. 1 and No. 5. This can be explained by the introgression of 'Kriszta' rye chromatin into the 1RS.1BL chromosome in the place of the 'Petkus' rye chromatin.

BC_1 plant No. 17 showed no PCR product when marker RMS13 was used (even after three replicates), while the SCM9 marker only amplified the 'Kriszta'-specific band (Fig. 3). The absence of the 'Petkus'-specific bands previously detected in cultivars 'Mv Magdaléna' and 'Mv Béres' and the presence of 'Kriszta'-specific PCR products in BC_1 plants Nos. 9, 12, 17 and 18 indicated that a recombination event had occurred between the 'Petkus' and 'Kriszta' 1RS regions in the 1BL.1RS translocation.

Discussion

As there is no genetic variation in the 1RS chromatin present in the hundreds of wheat cultivars around the world that carry the 1BL.1RS wheat/rye translocation, it is necessary to introduce new allelic variation into the 1RS arm of this chromosome by tapping the rich variation available in the rye genepool (Lelley et al. 1999, 2004). To develop a 'recipient' wheat genotype for the efficient production of wheat × rye hybrids the recessive *kr1* and *kr2* crossability alleles from the 'Mv9 kr1' line, developed by Molnár-Láng et al. (1996), were introduced into two state-registered modern Martonvásár wheat cultivars, 'Mv Magdaléna' and 'Mv Béres' which carry the 1BL.1RS translocation. The 1RS rye chromosome arm in these cultivars can be traced back to the Russian wheat cultivar 'Avrora', which received this translocation from the German wheat cultivar 'Neuzucht', the well-known progenitor of the 1BL.1RS translocations containing the 'Petkus' rye chromosome arm (Schlegel and Korzun 1997; Rabinovich 1998). The crossability of the F_2 plants was analysed by pollinating with rye, and the presence of the recessive *kr* alleles in the F_2 progeny was determined on the basis of seed set data. Seed set is greatly influenced by environmental factors, but the experiment was carried out under controlled conditions in a

phytotron, and the seed set data for two neighbouring spikes on the same plant were in most cases very similar. A seed set of over 50% can only be achieved on plants carrying the recessive *kr1kr2* alleles in the homozygous condition. The good crossability of the selected plants was inherited in the F_3 generation, as confirmed for several progenies under controlled environmental conditions and in the field. The new recipient wheat genotypes developed from the cultivars 'Mv Béres' and 'Mv Magdaléna' thus allow new rye chromatin to be introduced directly into the 1RS chromosome arm.

The method first described by Lelley et al. (1999) involved the production of octoploid triticale using the 'Mv9 kr1' wheat line and various rye cultivars having secalin banding patterns different from that of the 1BL.1RS translocation wheats. These triticales were then pollinated with wheat cultivars carrying the 1BL.1RS translocation. In the F_1 progenies the rye chromosome arms were detected by GISH, revealing the presence of trivalents (wheat–1BL.1RS–rye) and bivalents (wheat–1BL.1RS; 1BL.1RS–rye; Nagy and Molnár-Láng 2000).

In earlier studies using GISH (King et al. 1994; Miller et al. 1994) a very low frequency of pairing between wheat and rye chromosomes was found in wheat–rye hybrids. In the present experiments no wheat–rye bivalents were detected. The frequency of pairing between the chromosomes of related genera in F_1 hybrids is generally low, as demonstrated earlier using GISH in wheat × barley hybrids (Molnár-Láng et al. 2000b). A high level of homoeologous chromosome pairing was detected by Cuadrado et al. (1997) in wheat × rye hybrids produced with the 'Chinese Spring *ph1b*' mutant line, where chromosomes were identified using FISH in meiosis with the help of repetitive DNA probes. The average number of wheat–wheat homoeologous chromosome arm associations in the present experiments was 0.52, slightly higher than that reported by Miller et al. (1994) in wheat × rye ('Chinese Spring' × 'Petkus') hybrids (0.48), but lower than that found by Schlegel and Weryszko (1979) for 'Chinese Spring' × 'Danae' hybrids (0.71), and much lower than that observed in hybrids developed using the 'CS *ph1b'* mutant line by Cuadrado et al. (1997). The number of rye-rye chromosome arm associations per cell was very similar to that given in earlier reports (Miller et al. 1994; Mettin et al. 1976; Schlegel and Weryszko 1979).

The main purpose of the meiotic studies in the present experiment was, however, to demonstrate the high frequency of homologous chromosome pairing between the 1RS arm of the 1BL.1RS translocation chromosome and the 1RS arm from diploid rye, which could be detected in 62% of the pollen mother cells in F_1 wheat–rye hybrids. In the octoploid triticale × 1BL.1RS wheat hybrids the pairing between 1BL.1RS and 1RS was only 32.8%, though pairing between 1BL.1RS and 1BL was much higher (Nagy and Molnár-Láng 2000). The 1BL.1RS translocated chromosome and the 1R chromosome could be clearly identified in meiosis with the help of repetitive DNA probes (pSc119.2, pTa71), the FISH pattern on meiotic chromosomes being similar to that observed earlier on mitotic chromosomes (Cuadrado et al. 1997; Schneider et al. 2003; Sepsi et al. 2008; Szakács and Molnár-Láng 2008).

The high frequency of chromosome associations between the 1BL.1RS and 1R chromosomes suggests a high frequency of recombination, though several authors have reported that the number of recombinations in the progenies was much lower than the number of chromosome arm associations in meiosis (Marais et al. 1994; Singh et al. 1990). The frequency of associations between wheat and rye chromosomes greatly exceeded the level of wheat–rye recombination found in the hybrids examined by Orellana (1985) and Benavente et al. (1996). The very high level of homologous chromosome pairing between the 1Bl.1RS chromosome and the new 1R chromosome in the F_1 hybrid can be expected to result in several recombinants in the backcrossed progenies.

Recombinations between the rye chromosome arms were detected earlier with the help of molecular markers (Nagy et al. 2003; Nagy and Lelley 2003). Several molecular markers have been developed for the 1RS chromosome arm of diploid rye (Börner and Korzun 1998), but most of them are restriction fragment length polymorphism (RFLP) markers, along with a few AFLP, isozyme, protein and RAPD markers.

In the present study, polymorphic bands were observed with the 1RS-specific rye SSR markers RMS13 and SCM9 in rye cultivar 'Kriszta' compared with the 'Petkus' 1RS chromosome arm in wheat cultivars 'Mv Magdaléna' and 'Mv Béres', thus making it possible to detect the presence of the 'Kriszta' 1RS chromosome arm in wheat × rye hybrid plants and to monitor the presence of 'Kriszta' chromatin in backcross derivatives.

Several studies demonstrated that *Sr31*, *Lr26* and *Yr9* are separate, closely linked loci within the 1RS chromosome arm (Mago et al. 2005). These resistance genes were localized to the terminal region of the 1RS chromosome arm in rye cultivar 'Petkus', approximately 5 cM distal to the *Sec-1* locus (Singh et al. 1990; Lukaszewski 2000). The SSR marker RMS13 was mapped to the terminal region of 1RS, while SCM9 was located proximally to the *Sec-1* locus (Nagy et al. 2003). For this reason, recombinant plants selected by these markers are likely to carry the chromosome region characterized by the *Sr31*, *Lr26* and *Yr9* resistance genes, making it possible to replace the resistance genes of 'Petkus' rye by that of 'Kriszta'. The progenies of the recombinant plants need to be tested for disease resistance. New microsatellite markers developed for the 1RS

chromosome arm (Kofler et al. 2008) could be useful in detecting more detailed rearrangements between the 1RS chromosome arms originating from 'Petkus' and the pollinator in the new wheat–rye hybrids.

Single chromosome recombinant lines are valuable tools for the genetic analysis of different traits in wheat (Snape et al. 1985; Sutka and Snape 1989). The method for developing single chromosome recombinant lines was elaborated by Law (1966), who reported that crossing an intervarietal substitution line to the recipient wheat variety led to the production of a single-chromosome heterozygote in a constant genetic background, from which recombinant lines could be developed. The idea of replacing the 1RS chromosome arm in the 1BL.1RS translocation in wheat cultivars by crossing with a new rye cultivar is based on homologous chromosome pairing between the two 1RS arms, so recombinants can only be expected on one chromosome arm, as in the earlier method for the development of single chromosome recombinant lines (Law 1966). The effect of the new 1RS arm can only be studied in exactly the same genetic background, so several backcrosses to the original wheat cultivars containing the 1BL.1RS translocation are needed and the presence of the 1RS recombinant chromosomes in the progenies must be monitored using molecular markers.

The method described here has the advantage that a large number of wheat–rye hybrid seeds can be produced using the new recipient wheat genotypes, as they carry both the *kr1kr1kr2kr2* recessive crossability genes and the 1BL.1RS translocation. The wheat × rye hybrid analysed with GISH, three-colour FISH and an SSR marker in the present experiment was developed with the rye cultivar 'Kriszta', shown by our own observations and by Kruppa (2001) to have good resistance to leaf rust. 'Kriszta' is a perennial rye cultivar originating from a *S. cereale* × *S. montanum* hybrid, which could explain its good resistance. Furthermore, hundreds of hybrid seeds were produced using other rye cultivars, so it is expected that when the F_1 hybrids are backcrossed with translocated wheat a high number of recombinations will take place in F_1 meiosis, leading to new allelic variation in the 1RS arm of translocated wheats, allowing selection to be made for disease resistance, breadmaking quality or agronomic traits, such as yield and adaptability.

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