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Genome merger: from sequence rearrangements in triticale to their elimination in wheat–rye addition lines

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Abstract Genetic and epigenetic modifications resulting from different genomes adjusting to a common nuclear environment have been observed in polyploids. Sequence restructuring within genomes involving retrotransposon/microsatellite-rich regions has been reported in triticale. The present study uses inter-retrotransposon amplified polymorphisms (IRAP) and retrotransposon microsatellite amplified polymorphisms (REMAP) to assess genome rearrangements in wheat–rye addition lines obtained by the controlled backcrossing of octoploid triticale to hexaploid wheat followed by self-fertilization. The comparative analysis of IRAP and REMAP banding profiles, involving a complete set of wheat–rye addition lines, and their parental species revealed in those lines the presence of wheat-origin bands absent in triticale, and the absence of rye-origin and triticale-specific bands. The presence in triticale × wheat backcrosses (BC) of rye-origin bands that were absent in the addition lines demonstrated that genomic rearrangement events were not a direct consequence of backcrossing, but resulted from further genome structural rearrangements in the BC plant progeny. PCR experiments using primers designed from different rye-origin sequences showed that the absence of a rye-origin band in wheat–rye addition lines results from sequence elimination rather than restrict changes on primer annealing sites, as noted in triticale. The level of genome restructuring events evaluated in all seven wheat–rye addition lines, compared to triticale, indicated that the unbalanced genome merger situation observed in the addition lines induced a new round of genome rearrangement, suggesting that the lesser the amount of rye chromatin introgressed into wheat the larger the outcome of genome reshuffling.

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Introduction

Merging plant genomes is a major evolutionary process, resulting mainly through polyploidization, and has been estimated to have occurred in 30–70% of all plant species. The adjustment of different genomes to a shared nuclear environment can induce genomic and epigenomic variation. Genetic changes include translocations and transpositions as well as sequence deletions and insertions, while epigenetic changes include non-additive gene regulation, transposon transcription, silencing or sub-functionalization of homoeologous genes, and chromatin remodeling (Ma and Gustafson 2008). Several studies in wheat (*Triticum* ssp.), *Arabidopsis*, and *Spartina* polyploids have suggested that the genomic changes that occur during polyploid formation are not random, but rather directed and highly reproducible (Ainouche et al. 2008; Chen et al. 2008; Kashkush et al. 2002; Liu et al. 1998; Madlung et al. 2005; Ozkan et al. 2003; Shaked et al. 2001). The preferential loss of parental-specific bands has been revealed in newly formed polyploids involving *Aegilops* and *Triticum* species using amplified fragment length polymorphism (AFLP) and

restriction fragment length polymorphism (RFLP) (Liu et al. 1998; Ozkan et al. 2003). Similar techniques also disclosed genetic and epigenetic modifications in the synthetic allopolyploid triticale (× *Triticosecale* Wittmack) preferentially affecting rye-origin repeated and low-copy sequences (Ma et al. 2004).

Transposable elements constitute a decisive driving force in genome evolution and are crucial in plant genome adjustment and speciation through sequence modifications and changes in genome size (Charles et al. 2008). Although polyploidy induced retrotransposon transposition was never demonstrated, an increase in retrotransposon-related transcripts has been detected in wheat synthetic neopolyploids (Kashkush et al. 2002, 2003) as well as in *Arabidopsis* polyploid genotypes (Adams and Wendel 2005; Josefsson et al. 2006; Madlung et al. 2005). The analysis of *Spartina* polyploids through sequence specific amplified polymorphism (SSAP), revealed band elimination involving retroelements (Ainouche et al. 2008). Recently, the analysis of triticale through inter-retrotransposon amplified polymorphisms (IRAP), and retrotransposon microsatellite amplified polymorphisms (REMAP) revealed the disappearance of wheat- and rye-origin bands and the appearance of triticale-novel bands in the polyploid genotype. A significant rate of polyploidization-induced rearrangements was thus uncovered in retrotransposon and/or microsatellite-associated sequences, preferentially allocated in major heterochromatic domains, and affecting mainly rye-origin bands (Bento et al. 2008).

Polyploidization is the beginning of alien chromatin introgression in important crops through chromosome engineering. The synthetic intergeneric allopolyploid triticale resulting from the hybridization of wheat (*Triticum* ssp.) and rye (*Secale cereale* L.) has been used in the production of wheat–rye addition, substitution, and translocation lines through initial backcrosses to the wheat parent leading to rye chromosome, miss-segregation in meiosis and their concomitant modification and/or loss. Auto-fertilization of those BC plants therefore leads to plants disomic for wheat chromosomes and with reduced numbers of rye chromosomes, which represent the starting point for the stable introgression of rye chromatin in wheat background thus increasing wheat genome diversity (O'Mara 1940).

The present work analyses DNA sequence restructuring assessed through microsatellite and retrotransposon PCR-based molecular marker techniques of an entire set of wheat lines containing the addition of each individual rye chromosome pair. In all wheat–rye addition lines, IRAP and REMAP analyses revealed the absence of rye-origin and triticale-novel sequences and the presence of wheat-origin bands absent in the triticale parent. Moreover, we demonstrated that rye-origin band loss occurring in triticale corresponded to sequence rearrangements only involving primer annealing sites, whereas rye-specific band losses observed in the addition lines result from massive sequence elimination events.

Materials and methods

Plant material and DNA isolation

The following plant materials were used: hexaploid wheat *T. aestivum* L. 'Chinese Spring' ($2n = 6x = 42$, AABBDD), diploid rye *S. cereale* L. 'Imperial' ($2n = 2x = 14$, RR), the correspondent synthetic octoploid triticale (*T. aestivum* 'Chinese Spring' × *S. cereale* 'Imperial'; $2n = 8x = 56$, AABBDDRR), and the set of seven wheat–rye addition lines, each composed of the entire hexaploid wheat genome plus a single pair of rye homologous chromosomes. The rye cultivar 'Imperial' used is highly inbred and the octoploid triticale and their corresponding wheat–rye addition lines are at least 35 generations old (Ma et al. 2004). All seeds used in the present study were from the original E.R. Sears seed stocks and were obtained from the USDA–Sears collection, Columbia, Missouri. We have also analyzed the BC triticale × wheat F_1 (AABBDDR) produced using 'Chinese Spring' wheat.

Seeds from all genotypes were germinated and grown in controlled conditions at a 16 h light (20°C)/8 h dark (20°C) cycle. Genomic DNA was isolated from fresh young leaves of 8-week-old plants using modified cetyltrimethylammonium bromide (CTAB) method (Saghaimaroof et al. 1984).

For all cytogenetic analyses, three plants of each accession were analyzed separately. Root tips were collected from 1-week-old plants, cold treated for 24 h, fixed in ethanol/acetic acid (3:1 v/v) for 24 h at room temperature, and stored at −20°C until use.

PCR amplification, electrophoresis and data analysis

PCR was performed for IRAP and REMAP analyses and to amplify rye sequences, using the primers presented in Table 1. IRAP and REMAP PCR reactions were performed as previously detailed (Kalendar et al. 1999). A total of five combinations of primers for the LTR regions of three barley (*Hordeum vulgare* L.) retrotransposons and for two-anchored microsatellite were used (Bento et al. 2008). The selected retrotransposons (Nikita, Sabrina, and Sukkula) have proven to be useful in DNA fingerprinting and evolutionary studies, not only in barley but also in related taxa (Leigh et al. 2003).

To test the presence of characteristic rye sub-telomeric sequences primers were designed for pSc200, a 521 bp clone of an *S. cereale* DNA sub-telomeric tandem repeat (accession number Z50039) (Vershinin et al. 1996).

Table 1 Primers used for PCR analysis

Primer	Sequence
pSc200	
Forward	5′-TCTTTGATCACCGTTTCTTCG
Reverse	5′-CCCCACCCATGTATGGATAA
LTR	
C0699 (Nikita)	5′-CGCTCCAGCGGTACTGCC
C0945 (Sabrina)	5′-GCAAGCTTCCGTTTCCGC
9900 (Sukkula)	5′-GATAGGGTCGCATCTTGGGCGTGAC
SSR	
(CT)9G	5′-CTCTCTCTCTCTCTCTCTG
(CA)9G	5′-CACACACACACACACACAG
MoB-111-1000R [1–3]	
Forward	5′-ATT AGT ATG CTG CCG TCG TG
Reverse	5′-ATT ACC TTC AAA CCC TCC
MoB-111-1000R [1]	
Forward	5′-ATT AGT ATG CTG CCG TCG TG
Reverse	5′-ACC ACT TTG CTG CTT CCT TC
MoB-111-1000R [2]	
Forward	5′-AGT GTC CTT AGG ATG GAC AAC TG
Reverse	5′-GTG GTT CCA ACT CCT GTA
MoB-111-1000R [3]	
Forward	5′-GTG GTT CCA ACT CCT GTA
Reverse	5′-ATT ACC TTC AAA CCC TCC

To characterize rearranged DNA sequences previously observed in (Bento et al. 2008), primers were designed to amplify several internal segments of the rye-origin sequence MoB-111-1000R (accession number EF486521) obtained with REMAP Nikita/(CA)9G.

PCR amplification of all sequences was performed in 10 μl reactions containing: 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl$_2$, 0.25 mM dNTP's, 1 mM each primer, 0.5 U *Taq* polymerase and 25 ng DNA template; using the following program: 4 min at 94°C; 30 cycles of 30 s at 94°C, 30 s at 54°C, 30 s at 72°C; termination by 4 min of final extension at 72°C. PCR products were run on 1% agarose gels for 2–3 h at 110 V, detected by ethidium bromide staining, and photographed using Bio-Rad GEL DOC 2000. Selected bands were gel isolated, purified, cloned, and finally sequenced following the procedures described in Rocheta et al. (2006). Homology between internal fragments and original rye-origin bands were verified using BioEdit version 7.0.5.3 sequence alignment editor.

GISH

Genomic in situ hybridization (GISH) was performed on root tip meristematic cells prepared as previously described

(Silva et al. 2008). Fixed root tips were digested with pectinase/cellulase in 1 × EB for 2 h 15 min at 37°C, and squashes were performed in 45% glacial acetic acid. Rye total genomic DNA was used as a probe labeled by nick translation with biotin-dUTP (with wheat total genomic blocking DNA) and detected with streptavidin Cy3-conjugated. Nuclei and chromosomes were counterstained with 4′,6-diamidino-2-phenylindole hydrochloride (DAPI) in Citifluor antifade mounting medium (AF1; Agar Scientific). Samples were examined using a Zeiss Axioskop 2 epifluorescence microscope and images were obtained using a Zeiss AxioCam digital camera. Digital images were processed using PHOTOSHOP (Adobe Systems).

Results

Cytological and molecular identification of rye chromosomes in wheat–rye addition lines

The wheat–rye addition lines were produced through controlled BC of the octoploid triticale to the wheat parent, followed by successive generations of self-fertilization and subsequent rye chromosome selection. The first backcross (BC) generation yielded plants with the hexaploid wheat genome plus the rye genome in a haploid condition. Selfed BC plants contained the complete wheat complement and variable numbers of rye chromosomes, from one to seven. Selected BC plants which were monosomic addition lines, contained the complete wheat complement and one rye chromosome, produced disomic addition lines (O'Mara 1940). To confirm their genomic constitution, both cytological and molecular methodologies were used.

The presence of a pair of rye chromosomes in each wheat–rye addition line was confirmed using GISH with rye total genomic DNA in both metaphase and interphase cells (Fig. 1a, b, respectively). In interphase nuclei GISH allowed for the detection of the characteristic nuclear Rabl organization since rye chromosomes are observed as thin linear "strings" throughout the nucleus, with DAPI-positive sub-telomeric heterochromatic domains of both homologous localized in the same nuclear hemisphere.

The presence of rye chromosomes in the addition lines was further confirmed by PCR amplification of pSc200, a rye-origin sub-telomeric tandem repeat (accession number Z50039) that contains a 381 bp repeat unit. Primers amplified a 446 bp fragment, as a result of the amplification from the forward primer of one repeat to the reverse primer in the next repeat unit (Electronic supplementary material, Figure S1). The pSc200 banding profiles produced from wheat, rye, triticale and the seven wheat–rye addition lines are presented in Fig. 2a. The band amplified from rye genomic DNA was ~450 bp, matching the referred fragment size
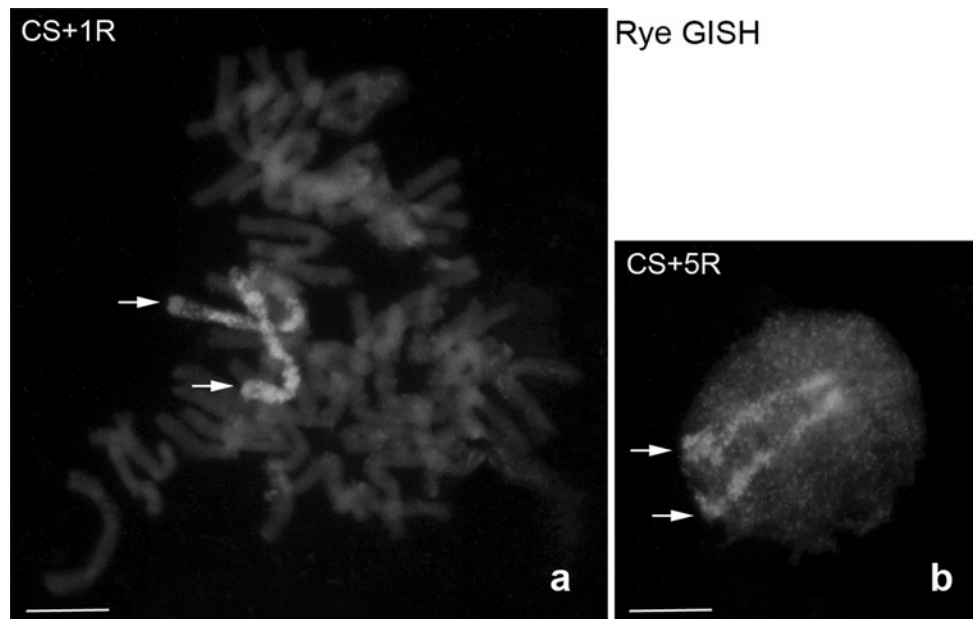
Fig. 1 GISH on meristematic root-tip cells using rye total genomic DNA as a probe for the confirmation of rye chromosomes in wheat–rye addition lines. **a** Metaphase cell of CS + 1R wheat–rye addition line; hybridization signal revealing the pair of 1R homologous chromo-somes (*arrows*). **b** Interphase nucleus of CS + 5R wheat–rye addition line, hybridization signal revealing the pair of 5R homologous chromo-somes as string domains (*arrows*). *Bar* 5 μm
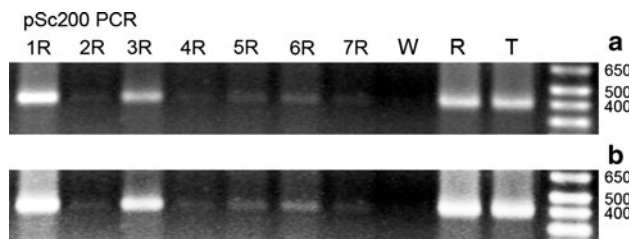

Fig. 2 pSc200 PCR banding profiles of wheat (*W*), rye (*R*), triticale (*T*) and seven wheat–rye addition lines (numbers correspond to rye homologous chromosomes pair). A rye-origin band with ∼450 bp is detected in triticale and wheat–rye addition lines. No amplification in wheat was observed. **b** An overexposed copy of **a**, reveling pSc200 bands in all wheat–rye addition lines. Molecular weight marker: 1 kb⁺

and was also present in triticale. As no amplification product was detected from wheat DNA, pSc200 PCR experiments were used to confirm the presence of rye chromosomes in a wheat background. Higher exposure of the gel presented in Fig. 2a, revealed seven wheat–rye addition lines bands similar to the ones observed in rye and triticale (Fig. 2b). Thus, the pSc200 sequence identified all genotypes containing rye chromatin.

Triticale- and rye-origin bands are absent in wheat–rye addition lines and present in triticale × wheat backcross

Five IRAP and REMAP primer combinations were used in the analysis of wheat–rye addition lines, namely the primer for the LTR sequence of the barley retrotransposon Nikita

by itself and in combination with other LTR primers from the barley retrotransposons Sabrina and Sukkula, and with the (CT)9G and (CA)9G anchored microsatellite primers. The banding profiles yielded reproducible arrays of distinct bands for the three replicates performed for all primer combinations analyzed. Only bands between 100 and 1,650 bp were scored, as this gel region produced the highest quality profiles. Banding profiles from wheat, rye, triticale and the seven wheat–rye addition lines obtained with two of the five primer combinations studied are shown in Figs. 3, 4, and 5 and the results obtained from all primer combinations are presented in Table 2.

IRAP and REMAP banding profiles comparing wheat–rye addition lines profiles with wheat and triticale progenitors were analyzed. The results revealed that banding patterns of all wheat–rye addition lines were identical to wheat progenitor banding profiles for all the primer combinations evaluated. All wheat-origin bands were present in the addition lines, even those absent in the triticale. However, a contrasting situation was observed for rye-origin bands present in the triticale, and for triticale-specific bands. In wheat–rye addition lines, we observed the absence of parental triticale bands with the following origins:

- Rye-origin DNA bands observed in rye and triticale;
- triticale-origin DNA bands absent in wheat and rye.

The seven addition line IRAP banding profiles obtained using the Nikita primer (Fig. 3) revealed one wheat-origin band absent in triticale, the loss of two rye-origin bands
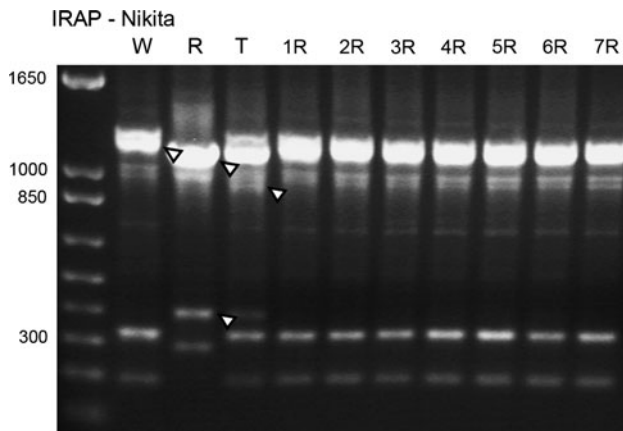
Fig. 3 IRAP banding profiles of wheat (*W*), rye (*R*), triticale (*T*) and the seven wheat–rye addition lines, obtained with primer Nikita (numbers correspond to rye homologous chromosomes pair). *Arrowheads* indicate a wheat band absent in triticale and present in all the addition lines and three bands absent in all the addition lines: two rye-origin bands and one triticale-origin band. Molecular weight marker: 1 kb[+]
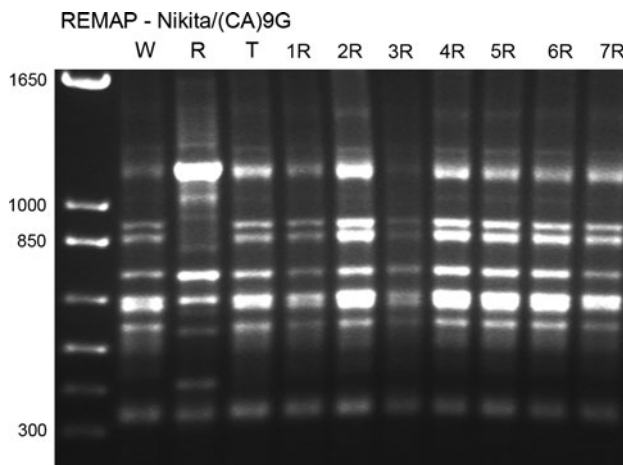

Fig. 4 REMAP banding profiles of wheat (*W*), rye (*R*), triticale (*T*) and the seven wheat–rye addition lines, obtained with primer combination Nikita/(CA)9G (numbers correspond to rye homologous chromosomes pair). Banding patterns observed in all the addition lines corresponds to the sum of banding profiles detected in the parental genotypes triticale and wheat. Molecular weight marker: 1 kb[+]

(present in triticale), and the loss of one triticale-origin band. The Nikita/Sabrina and Nikita/Sukkula IRAP primer combination analysis revealed the presence of all wheat-origin bands including one absent in triticale, and the absence of the four rye-origin bands present in the triticale. REMAP Nikita/(CT)9G (Fig. 4) detected the presence, in addition lines, of one wheat-origin band (absent in triticale) and the absence of the three rye- and two triticale-origin bands. Nikita/(CA)9G REMAP (Fig. 5) was used as a control experiment since the banding profiles obtained for wheat and triticale and the wheat–rye addition lines banding profiles were identical.

Since all rye-origin bands present in triticale and all triticale-origin bands were absent in all wheat–rye addition
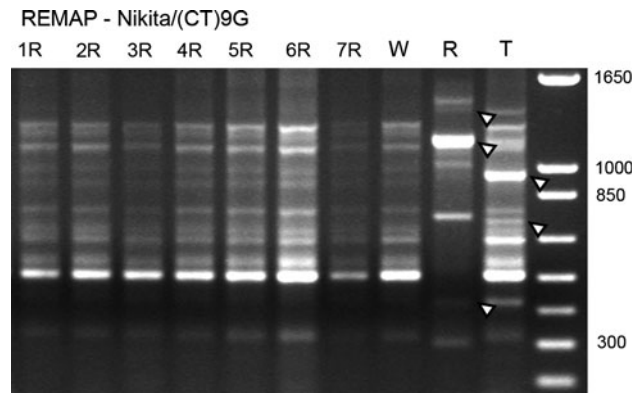

Fig. 5 REMAP banding profiles of wheat (*W*), rye (*R*), triticale (*T*) and the seven wheat–rye addition lines, obtained with primer combination Nikita/(CT)9G (numbers correspond to rye homologous chromosomes pair). *Arrowheads* indicate five bands absent in all the addition lines: three rye-origin bands and two triticale-origin bands. Molecular weight marker: 1 kb[+]

lines, detailed experiments, to evaluate the accuracy of the techniques used, were performed to consider the possibility that the loss of rye/triticale origin bands in the addition lines resulted from rye sequence-reduction in comparison to wheat-origin sequences. Several IRAP and REMAP experiments were performed using a DNA template mixture containing a fixed quantity of wheat DNA and decreasing quantities of rye DNA until 1:1/7 of wheat:rye ratio, which corresponds to the addition of one rye chromosome to the wheat genome in comparison with rye genomic DNA present in triticale. Fig. S2 of Electronic supplementary material corresponds to the IRAP experiment obtained with primer Nikita using decreasing concentrations of rye DNA mixed with wheat DNA, showing the presence of two rye-origin bands in all rye dilutions. This experiment proved that the absence of rye bands in the wheat–rye addition lines was not the result of a technical artifact.

To assess the timing course of genome rearrangements detected in wheat–rye addition lines, Nikita IRAP experiments were performed on four plants that resulted from the triticale × wheat BC. Banding profiles from backcross plants, wheat, rye, triticale and two wheat–rye addition lines (2R and 5R) (Fig. 6) revealed the presence of all wheat-origin, one rye-origin (∼400 bp) and one triticale-origin (∼900 bp) bands in the four BC plants analyzed.

The overall characterization of wheat–rye addition lines using IRAP and REMAP demonstrated that in these genotypes rye- and triticale-origin bands were absent. Moreover, all bands from wheat-origin were present, including those absent in triticale. To confirm that wheat-origin bands absent in triticale and present in the addition lines corresponded to the same wheat sequence, we further analyzed the MoB-11-1200W wheat-origin sequence (accession number EF486520), which was gel-isolated from the Nikita IRAP banding profiles of wheat–rye addition lines CS + 1R and CS + 7R, purified

Table 2 IRAP and REMAP analysis in wheat lines with the addition of rye chromosomes comparing with triticale and wheat progenitors

Primer combined with Nikita	Number of bands with different primer combinations					Total
	IRAP			REMAP		
	Nikita	Sabrina	Sukkula	(CT)9G	(CA)9G	
Wheat[a]	7	7	11	9	12	46
Triticale[a]	10	10	14	13	12	59
Addition lines	7	7	11	9	12	46
Addition lines lost bands[b]	3	4	4	5	0	16
Rye-origin	2	4	4	3	–	13
Triticale-origin	1	–	–	2	–	3

[a] Results published in (Bento et al. 2008)

[b] Addition lines lost bands: rye-origin lost bands - bands observed in rye profiles as well as in triticale profiles, and absent in all the wheat–rye addition lines; triticale-origin bands - bands observed in triticale considered as novel, since they are not observed in wheat and rye progenitors profiles, and absent in all the wheat–rye addition lines
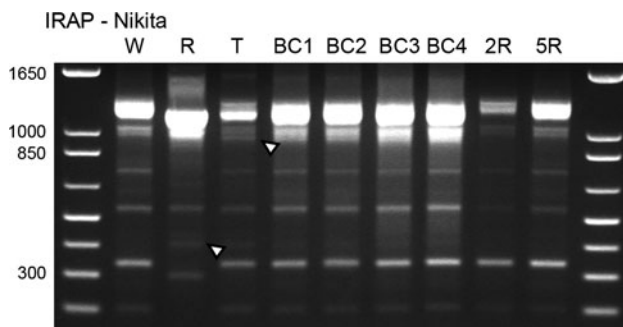

Fig. 6 IRAP banding profiles obtained with primer Nikita of wheat (*W*), rye (*R*), triticale (*T*), four different individual plants from triticale-wheat backcross (*BC1*–*BC4*) and two wheat–rye addition lines (CS + 2R and CS + 5R). One rye-origin band (∼400 bp) observed in triticale and one triticale-origin band (∼900 bp) are both observed in the four BC individual plants and are absente in the two wheat–rye addition lines (*arrowheads*). Molecular weight marker: 1 kb⁺

Fig. 7 Dimensions (bp) of fragments expected from the amplification of four internal segments of the rye-origin sequence MoB-111-1000R (accession number EF486521): MoB-111-1000R [1–3], MoB-111-1000R [1], MoB-111-1000R [2], and MoB-111-1000R [3]

and cloned for sequencing. Sequencing analyses of these bands (Electronic supplementary material, Fig. S3) revealed that they share 84 and 83% homology, respectively, with the wheat sequence previously characterized.

Triticale rye-origin sequences are eliminated in wheat–rye addition lines

The absence of rye- and triticale-specific bands in the addition lines raises questions about the processes underlying such absences, such as sequence rearrangements or deletions. Primers to amplify four internal segments of the REMAP rye-origin 964 bp sequence MoB-111-1000R (accession number EF486521) were designed (Fig. 7). The results obtained in rye confirmed the amplification of four bands with the expected sizes:

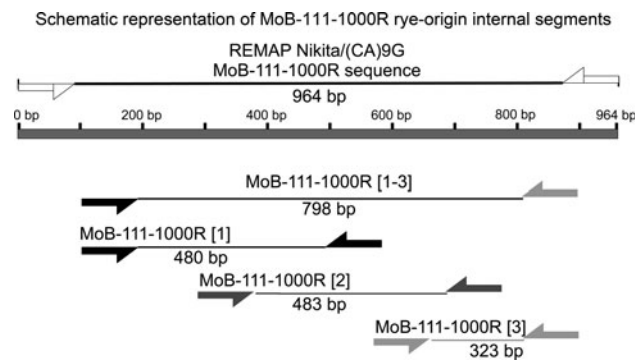- one whole internal fragment: MoB-111-1000R [1–3] with 798 bp;

- three partial internal fragments: MoB-111-1000R [1] with 480 bp, MoB-111-1000R [2] with 483 bp and MoB-111-1000R [3] with 323 bp.

Unlike the original MoB-111-1000R rye band, the whole internal segment with the expected 798 bp was detected in rye and in triticale (Fig. 8). The ∼800 bp bands amplified from rye and triticale DNA were gel-isolated, purified and cloned for sequencing, revealing high homology between the original MoB-111-1000R sequence and MoB-111-1000R [1–3] internal segment amplified from both rye (100%) and triticale (98%).

The amplification with primers designed to MoB-111-1000R [1] revealed one ∼450 bp band in wheat, distinct from the 480 bp band obtained in rye with both being present in triticale (Fig. 9). Further sequencing of the 480 bp rye-origin sequence amplified from triticale showed that, as expected, it shared 97% homology with the sequence amplified from rye.
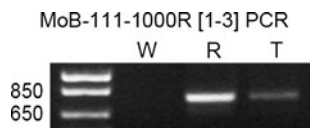
Fig. 8 PCR banding profiles of wheat (*W*), rye (*R*), triticale (*T*) obtained with primers designed to amplify the whole internal fragment of the sequence MoB-111-1000R (MoB-111-1000R [1–3]). As expected, only one band with ~800 bp was amplified from rye. A similar band was amplified from triticale and no amplification products were detected in wheat. Molecular weight marker: 1 kb⁺
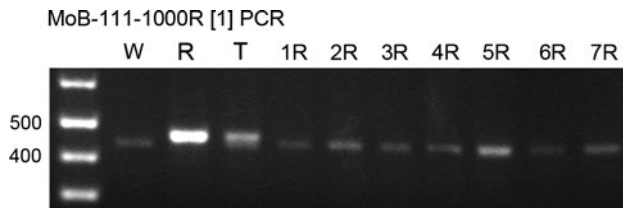

Fig. 9 PCR banding profiles of wheat (*W*), rye (*R*), triticale (*T*) and seven wheat–rye addition lines (numbers correspond to rye homologous chromosomes pair), obtained with primers designed to amplify the first internal fragment of the sequence MoB-111-1000R (MoB-111-1000R [1]). In triticale a double band was observed, corresponding to the wheat and rye parental bands. In the addition lines, only the wheat-specific band is observed. Molecular weight marker: 1 kb⁺

The amplification obtained using primers designed to MoB-111-1000R [2] revealed two different bands in wheat (approximately ~400 and ~450 bp) and one in rye (483 bp). The banding profile obtained in triticale showed the three bands resulting from the addition of the parental wheat and rye banding profiles (Fig. 10). The sequencing of the MoB-111-1000R [2] sequences amplified from wheat, rye, and triticale revealed that they were all analogous sequences (Electronic supplementary material, Fig. S4), and that both rye- and wheat-origin bands amplified from triticale shared extremely high levels of homology (more than 98%) with the ones amplified from the parental species. On the other hand, ~400 and ~450 bp wheat amplified sequences also shared a high level of homology (80 and 91%, respectively) with the sequence amplified from rye.

The band correspondent to MoB-111-1000R [3] was present in all three genotypes with a strong intensity in rye, a faint one in wheat and an intermediate one in triticale.

The amplification of DNA from the wheat–rye addition lines with primers to MoB-111-1000R [1] and MoB-111-1000R [2] only revealed the wheat-origin bands, whereas the rye-origin bands were missing on all seven wheat–rye addition line banding profiles (Figs. 9, 10, respectively). It was, therefore, demonstrated that the rye MoB-111-1000R internal sequences maintained in triticale and in the triticale × wheat BC were absent in addition lines.
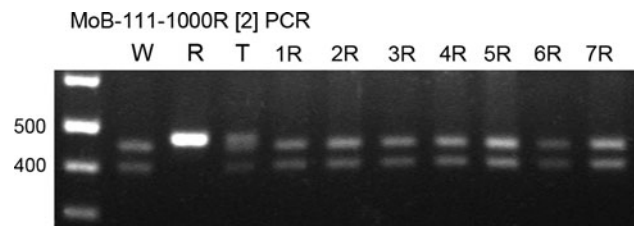

Fig. 10 PCR banding profiles of wheat (*W*), rye (*R*), triticale (*T*) and seven wheat–rye addition lines (numbers correspond to rye homologous chromosomes pair), obtained with primers designed to amplify the second internal fragment of the sequence MoB-111-1000R (MoB-111-1000R [2]). In triticale, three bands were observed, corresponding to two wheat-origin bands plus one rye-origin band. In the addition lines, only the two wheat-specific bands were observed. Molecular weight marker: 1 kb⁺

Discussion

A high rate of induced DNA sequence rearrangements was recently demonstrated to occur due to polyploidization namely in triticale and those events are associated with retrotransposon and/or microsatellite-rich genomic regions (Bento et al. 2008) as they were disclosed through the utilization of IRAP, REMAP and ISSR techniques (Kalendar et al. 1999). Detection of DNA sequence rearrangements in triticale were feasible due to parental genomic diversity unraveled by those important molecular tools, largely used also to characterize intraspecific variability in barley, *Vitis vinifera*, and *Pisum* ssp. (Kalendar et al. 1999; Pereira et al. 2005; Smykal 2006).

If no rearrangement events had resulted during the addition line creation, the presence of all bands observed in wheat plus those of triticale-origin would be expected in the global profiles of the seven wheat–rye addition lines. However, the genomic screening of wheat–rye addition lines compared to the triticale and wheat parents, accomplished through IRAP and REMAP using five different primer combinations, revealed the absence of all rye- and triticale-origin bands. Concerning the wheat genome, all wheat-origin bands absent in triticale were present in all wheat–rye addition lines, probably resulting from the BC of triticale to wheat. Moreover, the detailed analysis of one IRAP wheat-origin copia-like retrotransposon related sequence (MoB-11-1200W) amplified with Nikita IRAP (Bento et al. 2008), confirmed that the wheat-origin sequence amplified in the addition lines share high homology with the original characterized wheat band.

Besides the substantial elimination of rye-origin sequences in wheat–rye addition lines, our results also disclosed another novel restructuring event where the triticale-origin bands that emerged from wheat–rye polyploidization were eliminated. The directed and controlled loss of non-coding and coding sequences has been suggested to overcome
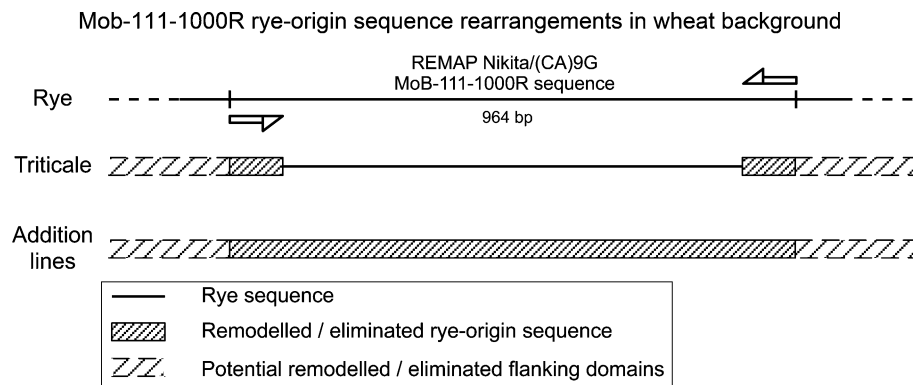
Mob-111-1000R rye-origin sequence rearrangements in wheat background

Fig. 11 Schematic representation of REMAP Nikita/(CA)9G rye-origin sequence (MoB-111-1000R) and its rearrangements when introgressed in wheat background. Although the original REMAP band is absent in triticale, internal fragments were confirmed by amplification and sequencing, revealing that polyploidization probably induced changes in flanking domains of primer annealing sites. In wheat–rye addition lines, a major rearrangement of that sequence occurred as neither the original REMAP rye sequence, nor its internal segments are amplified, disclosing the occurrence of extensive alien chromatin elimination

genome redundancy induced by different genomes merging (Ma and Gustafson 2005) and a preferential occurrence of restructuring events involving rye parental genome was observed in wheat–rye hybrids and triticale (Bento et al. 2008; Ma et al. 2004; Ma and Gustafson 2006; Tang et al. 2008). This tendency was also observed on AFLP banding profiles of wheat–rye addition lines (Ma et al. 2004) which showed that the rye-origin triticale bands were absent in the addition lines.

Differences in genome size, chromatin organization patterns and cell cycle duration are well known to cause genomic conflicts in newly formed hybrids. Genomic differences have been cytologically revealed in triticale as dense rye heterochromatic sub-telomeric domains not present in wheat (Bennett 1977; Neves et al. 1997), as we confirmed in wheat–rye addition lines through DAPI-positive sub-telomeric heterochromatic bands on rye chromosomes and through PCR analysis of pSc200 rye-origin sequence. Wheat–rye addition line chromosome instability has been well documented (Alkhimova et al. 1999; Riley 1960) and seems to suggest a new round of genomic adjustment due to a greater genome composition unbalance. However, major genomic restructuring events are not an immediate result of triticale-wheat BCs, since bands absent in the addition lines are yet present in plants resulting from triticale × wheat BC. Thus, rye-origin sequence elimination must occur throughout self-fertilization of selected lines from the progeny of triticale × wheat BC. Accordingly, Gustafson et al. (1983) found rye-chromosome alterations involving sub-telomeric heterochromatic bands, but only in a F_3 generation of a triticale × wheat BC.

Transcriptional activation of retrotransposons has been previously reported in newly synthesized wheat amphiploids (Kashkush et al. 2002, 2003) and *Arabidopsis* (Adams and Wendel 2005; Josefsson et al. 2006; Madlung et al. 2005),

On the other hand, the analysis of wheat–rye hybrids using wheat SSRs (Tang et al. 2008), and tobacco (*Nicotiana* ssp.) allopolyploids (Petit et al. 2007) and *Spartina* hybrids (Ainouche et al. 2008) through retrotransposon sequence-specific amplified polymorphism (SSAP), revealed only low frequent parental band disappearance events. A crucial question arises about the extension of the restructuring events disclose by IRAP and REMAP as genomic diversity, assessed by those techniques, is based on banding profiles. Band loss can result either from minor changes in primer annealing sites or from gross elimination of DNA sequences, and both are indiscriminately described as genome rearrangements. DNA sequence analysis of a rye-origin band absent in both triticale and wheat–rye addition lines largely confirmed the distinct levels of genome rearrangements resulting from merging genomes or chromosome introgression. Although the original rye REMAP hydroxyproline-rich glycoproteins (HRGP) coding related sequence (MoB-111-1000R) was absent in triticale, most of its internal sequence was present in triticale, demonstrating that polyploidization rearrangements could involve primer annealing sites. Conversely, in wheat–rye addition lines rearranged sequences present in triticale and its internal segments were eliminated. The disappearance of the MoB-111-1000R rye-origin band in triticale resulted from a small sequence rearrangement markedly contrasting with the deletion of an entire rye sequence demonstrated to occur in the wheat–rye addition lines (Fig. 11). Genomic rearrangements are, therefore, much more drastic in wheat–rye addition lines than in triticale, indicating a massive elimination of rye sequences, when restricted amounts of rye chromatin were introgressed into a hexaploid wheat background.

The high level of rearrangements concerning microsatellite and/or retrotransposon-rich genome fractions identified in the wheat–rye addition lines demonstrated that genome

reshuffling not only entailed polyploid induced genome adjustments, but enhanced more restricted chromatin introgression (i.e. chromosome pair additions, substitutions or translocations, involving the transfer of DNA from one species to another) frequently involved in plant breeding approaches.

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