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Introgression of A- and B-genome of tetraploid triticale chromatin into tetraploid rye

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Abstract An improvement of rye is one of the mainstream goals of current breeding. Our study is concerned with the introduction of the tetraploid triticale (ABRR) into the 4x rye (RRRR) using classical methods of distant crossing. One hundred fifty BC_1F_9 hybrid plants [(4x rye×4x triticales)× 4x rye] obtained from a backcrossing program were studied. The major aim of this work was to verify the presence of an introgressed A- and B- genome chromatin of triticale in a collection of the 4x rye-tiritcale hybrids and to determine their chromosome compositions. In the present study, karyotypes of the previously reported BC_1F_2s and BC_1F_3s were compared with that of the BC_1F_9 generation as obtained after several subsequent open pollinations. The genomic in situ hybridisation (GISH) allowed us to identify 133 introgression forms in which chromosome numbers ranged between 26 and 32. Using four DNA probes (5S rDNA, 25S rDNA, pSc119.2 and pAs1), the fluorescence in situ hybridisation (FISH) was carried out to facilitate an exact chromosome identification in the hybrid plants. The combination of the multi-colour GISH with the repetitive DNA FISH singled out five types of translocated chromosomes: 2A.2R, 4A.4R, 5A.5R, 5B.5R and 7A.7R among the examined BC_1F_9s. The reported translocation lines could serve as valuable sources of wheat chromatin suitable for further improvements of rye.

Keywords Backcross · Fluorescence and genomic in situ hybridisation · Repetitive DNA probes · Tetraploid rye · Tetraploid triticale · Translocations

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Rye (*Secale cereale* L.) is one of the most important cultivated species in Middle and Eastern Europe. Taking into consideration its adaptation to the variable climate character and tolerance to biotic and abiotic stresses, rye is considered as one of the most desirable cereals in agriculture for farmers in the European regions (Apolinarska 2003). However, in comparison to wheat, rye has a lower feeding value, which is associated with containing anti-nutritional substances. Breeding programs should aim at the improvement of the quality traits of this species using related genepools.

Rye has been reported as a widely-used source of new variation in wheat breeding, however, the opposite gene flow, from wheat to rye is not common (Łapiński and Rafalski 2003). Several attempts were carried out to highlight the genetic diversity of rye, such as distant crossing with wheat (Lukaszewski 2010), triticale (Apolinarska 1993, 1996), *Aegilops* spp. (Simonenko et al. 1998; Wojciechowska and Pudelska 2005; Apolinarska et al. 2010; Kwiatek et al. 2012, 2013) and *Dasypyrum villosum* (syn. *Haynaldia villosa*) (Książczyk et al. 2011; Grasso et al. 2012).

The tetraploid rye (2n=4x=28, RRRR) is an interesting object for cytogenetic studies because of its meiotic pairing mechanisms. The diploid-like behaviour of chromosomes 1R, 2R and 5R in autotetraploid rye were analysed by Naranjo and Orellana (1984). Chromosomes of A- and B-genome of wheat (AABBDD) or triticale (AABBRR) could incorporate chromosome pairing loci, which can be exploited in order to manipulate homoeologous recombination to engineer an alien chromosome segment carrying desirable gene *loci* (Qi et al. 2007). For example, the chromosome 5B is reported as a subject of study concerning the *Ph1* gene, which controls the homologue paring mechanism due to meiosis (Riley and Chapman 1958). It is already proven, the introgression of the chromosome 5B into diploid (Schlegel et al. 1991) and into tetraploid rye reduces the level of chiasmate in first metaphase of homologues paring.

An improvement of cultivated species using interspecific crosses is widely supported by cytogenetic researches. The genomic in situ hybridisation (GISH) is successfully used to discriminate the genome composition of hybrids (Schwarzacher et al. 1989), whereas the fluorescence in situ hybridisation (FISH), with observation of chromosome morphology (Mukai et al. 1993), is a method suitable for chromosome identification as well as observation of an evolution of chromosomal structure and/or its possible modifications using specific probes, such as repetitive DNA sequences like rDNA, telomeres, centromeres, single sequence repeats, transposable elements, retroelements (Heslop–Harrison 2000). The most common repetitive sequences used in physical mapping in the Triticeae are: pSc119.2, pSc200 (derived from rye), pAs1 (wheat), spelt 1 and spelt 52 (*Ae. speltoides*) (Salina et al. 2009). In the present study, chromosome sets of the previously reported 4x rye-tiritcale BC_1F_2 and BC_1F_3 hybrids (Apolinarska 1996) were compared with that of the BC_1F_9 generation as obtained after several subsequent open pollinations. Major aims of the present work were: (1) to verify the presence of introgressed A- and B-genome chromatin from 4x triticale in 4x rye forms using GISH and FISH methods and (2) to evaluate cytogenetic stability of the obtained hybrids.

The study was carried on 150 plants representative for a collection of introgression forms of rye (4x) derived from materials previously reported by Apolinarska (1996). The tetraploid rye with a triticale chromatin was obtained by crossing a tetraploid rye (derived from twin embryos formerly identified by Prof. S. Sulinowski, Institute of Plant Genetics, PAS, Poznań, Poland) with different tetraploid triticales [RRRR×(AB)(AB)RR] (Apolinarska 1993). Plants of the F_1 generation were backcrossed with the tetraploid rye followed by open pollinations to reach the current status of the examined BC_1F_9 generation. Chromosome preparation was carried out as described by Hasterok et al. (2006) with minor modifications due to specifications of the tetraploid rye hybrids. The DNA isolation from leaves of rye (*S. cereale* L. cv. Dańkowskie Złote) and wheat (*T. aestivum* L. cv. Henika) was done as described by Lombard and Delourme (2001). In the initial stage of this experiment, total genomic DNA of rye was labelled with digoxigenin-11-dUTP (Roche) and the blocking DNA was obtained by autoclaving the total genomic DNA of wheat. Selected lines were analysed using total *Triticum monococcum* L. (AA) genomic DNA probe (labelled with digoxygenin-11-dUTP) and total *Secale cereale* (RR) genomic DNA probe (labelled with tetramethyl-rhodamine-5-dUTP, Roche) which allow to categorise the A- and R- genomes respectively. Unlabelled DNA of *Aegilops speltoides* Tausch (donor of B-genome) was sheared by autoclaving and used as blocking DNA. The 5S rDNA probe was generated from the wheat clone pTa794 (Gerlach and Dyer 1980) by PCR amplification using M13 primers and labelled by PCR with tetramethyl-rhodamine-5-dUTP (Roche) as described by Książczyk et al. (2010). The 25S rDNA probe was made by nick translation of a 2.3-kb *Cla*I sub-clone of the 25S rDNA coding region of *Arabidopsis thaliana* (Unfried and Gruendler 1990) with digoxigenin-11-dUTP for a detection of 45S rDNA loci (Książczyk et al. 2010). The pSc 119.2 sequence was amplified using M13 sequencing primers and labelled by nick translation with digoxygenin-11-dUTP (Roche) according to Koubaláková (pers. comm.). The pAs1 was amplified according to Nagaki et al. (1995). GISH, done according to Kwiatek et al. (2013) with modifications, was used as a screening method in order to detect the wheat chromatin and chromosome rearrangements between parental genomes. After the selection of introgressed lines, three in situ hybridisations on the same chromosome preparations were carried out. First, fluorescence in situ hybridisation (FISH) was made according to Kwiatek et al. (2013) with minor modifications, using 25S and 5S rDNA. During the second FISH, pSc119.2 and pAs1 were used as probes. Mitotic cells were examined with an Olympus XM10 CCD camera attached to an Olympus BX 61 automatic epifluorescence microscope. Image processing was carried out using Olympus Cell-F imaging software (version 3.1; Olympus Soft Imaging Solutions GmbH: Münster, Germany) and Micrographx Picture Publisher software (version 8.1; Micrografx Inc.: Richardson, TX, USA).

The present study was performed to prove the presence of the A- and B-genome chromatin in the BC_1F_9 generation of hybrids [(4x rye×4x triticale)×4x rye], which were obtained by several uncontrolled open pollinations of BC_1F_3 hybrids reported by Apolinarska (1996). Chromosome sets of the BC_1F_4 to BC_1F_8 hybrids were not analysed. An instability in chromosome number was observed among the 150 BC_1F_9 hybrids (Fig. 1). Ninety seven plants (64.67 %) were tetraploids (2n=28), 47 plants (31.33 %) were hyperploids (2n=29–32) and six plants (4 %) were hypoploids (2n=26–27). GISH screening revealed that 133 plants (88.67 %) were introgression forms with fragments of a triticale A- and/or B-genome chromatin. In the three types of translocations (Fig. 1a), we have observed: (t) a single rye chromosome segment terminally localised on a triticale chromosome (124 chromosomes – 81 %), (t_1) two rye chromosome segments terminally localised on triticale chromosome (13–8 %) and (is) a single rye chromosome segment intersitialy localised, near the telomers of triticale chromosome (9–6 %). Root meristems of the 133 introgression forms were then screened by FISH with rDNA probes for detecting *Secale* and triticale rDNA-bearing chromosomes and their possible rearrangements. The rDNA-FISH/GISH in chosen plants of introgression forms revealed that the mean number of *Secale* and triticale chromosomes was 26.65 (24–30) and 2.3 (1–4), respectively. The number of 5S rDNA sites was always 8, while the number of 45S rDNA ones ranged from 2 to 4. The value of *Secale*-genome like chromosomes with 5S rDNA sites ranged from 6 to 8, while the number of A- and B-genome 5S rDNA ones found on rye rearranged chromosomes ranged from

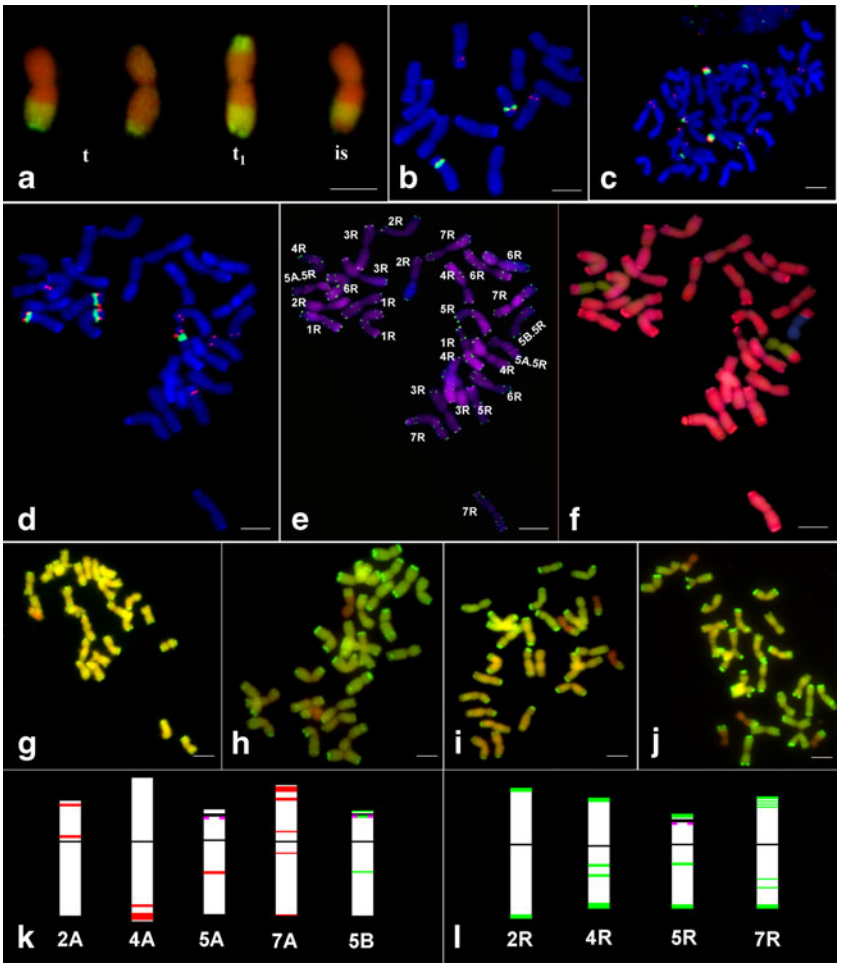

Fig. 1 **a** Types of translocations: *t* - single rye chromosome segment located terminally on a wheat chromosome, t_1 - two rye chromosome segments located terminally on a wheat chromosome and *is* - single rye chromosome segment located intersitialy. GISH with a total genomic DNA from rye as a probe labelled with digoxigenin-11-dUTP (DIG) and detected by anti-digoxigenin (anti-DIG) conjugated with FITC (*green*/*yellow*), with blocking genomic DNA of *T. aestivum* (*orange*/*red*); chromosomes were counterstained with propidium iodide. **b**, **c** FISH with 5S rDNA labelled with tetramethylrhodamine-5-dUTP (*red*) and 25S rDNA labelled with digoxigenin and detected by anti-digoxigenin conjugated with FITC (*green*) showing: *Secale cereale* cv. Dankowskie Złote and *Triticum aestivum* cv. Henika (respectively). **d** FISH with 5S rDNA labelled with ROD (*red*) and 25S rDNA labelled with DIG and detected by anti-DIG conjugated with FITC (*green*), **e** FISH with pAs1 labelled with ROD (*red*) and pSc 119.2 labelled with DIG and detected by anti-DIG conjugated with FITC (*green*). The chromosomes were counterstained with DAPI (*blue*). **f** GISH with a total genomic DNA from rye – R-genome, labelled with ROD (*red*) and total genomic DNA from *Triticum monococcum* – A-genome, labelled with DIG and detected by anti-DIG conjugated with FITC (*green*/*yellow*) with blocking genomic DNA of *Aegilops speltoides* – B-genome (DAPI-*blue*). **g** Root tip mitotic metaphase of tetraploid rye 2n=28 with one, (**h**) two, (**i**) three and (**j**) four translocated A-/B- genome chromosomes. Diagrams of pSc 119.2 (*green bands*), pAs1 (*red bands*) and 5S rDNA (*pink spots*) localisation on (**k**) wheat chromosomes, introgressed into (**l**) rye chromosomes. Scale bar: 10 μm

0 to 2. Different chromosome complements in *Secale* introgression forms have been found and examples of those observed with one rye/triticale translocation with no rDNA site, three rye/triticale translocations with one 5S rDNA site or two 5S rDNA ones are presented in Fig. 1d–f. The number of *Secale*-genome like chromosomes with 45S rDNA sites ranged from 2 to 4. Therein, 2 to 4 pairs of rDNA-bearing chromosomes with co-localisation of 5S and 45S rDNA sites were found (Fig. 1d). No triticale 45S rDNA-bearing chromosomes were observed as individuals or in part as rearranged triticale chromosomes with a region of *Secale* chromatin. The number of *Secale*/triticale recombinant chromosomes found among the introgression forms ranged from 1 to 5, and in nine plants, also triticale-genome like 5S rDNA sites were observed. It suggests the presence of regions of wheat chromosomes 5A and 5B in the rearranged chromosomes, based on distribution patterns of 5S rDNA loci in *T. aestivum* chromosomes. Nevertheless, a possibility of 5A.5R and 5B.5R translocated chromosomes in the genome of *Secale* introgression forms cannot be excluded, but rDNA-FISH/GISH is not able to detect them. When used quadruple-FISH connected with GISH, the pAs and pSc signals allowed a reevaluation of rye/wheat chromosomes in the *Secale* (4×) introgression forms by rDNA-FISH for 5A.R and 5B.R (Fig. 1d, e), and other translocations (2A.2R, 4A.4R, 7A.7R)

(Fig. 1k, l). The compilation of four probes based on repetitive DNA resulted in specific FISH patterns, that allow us to identify particular chromosomes. Obtained patterns of the tetraploid rye with introgressions of wheat chromatin were compared with FISH patterns of the A-, B- and R-genome chromosomes of triticale (Cuadrado and Jouve 1994) and the A- and B- genome chromosomes of wheat (Schneider et al. 2003; Sepsi et al. 2008). In the tetraploids, we have distinguished 16 (16.49 %) plants without introgressed wheat chromatin and 25 (25.77 %) plants with one, 44 (45.36 %) plants with two, nine (9.28 %) plants with three and three (3.09 %) plants with four substituted chromosomes (Fig. 1g–j, respectively). Using combined FISH and GISH methods, 2A.2R (in three plants, 3.09 %), 4A.4R (three plants, 3.09 %), 5A.5R (74 plants, 76.29 %), 5B.5R (six plants, 6.19 %) and 7A.7R (25 plants, 25.77 %) translocations were singled out. Among the six hypoploid plants, five plants (83.33 %) possessed 27 chromosomes, where 2A.2R (one plant, 16.66 %), 5A.5R (three plants, 50 %) and 7A.7R (four plants, 66.67 %) translocation chromosomes were found. Only a single rye chromosome segment terminally localised on wheat chromosome was observed. In one hypoploid plant (2n=26) (16.67 %) we have observed a deletion of 5R chromosome pair. In hyperploids (47 plants), 5A.5R (38 plants, 80.85 %), 5B.5R (three plants, 6.38 %) and 7A.7R (16 plants, 34.04 %) translocation chromosomes were found and all types of translocation were observed. Moreover, GISH allowed us an accurate determination of the breakpoints in the translocations, and of the amount of rye chromatin that has been incorporated into A- and B-genome chromosomes. The breakpoint localisation, with regard to the size of an introgressed alien chromosome fragment, was diversified. The translocated fragments of rye chromatin into the A- or B-genome chromosomes presented in this study are mainly terminally localised (89 % of all translocated chromosomes, Fig. 1a). Such type of translocations were extensively described by Lukaszewski and Gustafson (1983). The intersitialy localised, single rye chromosome segments (6 % of the translocated chromosomes), were also found near the telomers of triticale chromosomes. This type of translocation is impossible to identify using quadruple FISH. Schlegel and Korzun (2008) reported a similar breakpoint localisation in the distal region of the chromosome arm 4BL. On the other hand, Carvalho et al. (2009) identified a spontaneous translocation of whole chromosome arm as being the 7BS/7RL. The small size of an introgressed rye chromosome segments might be correlated with a consecutive alien chromatin elimination during successive pollinations. Ribeiro-Carvalho et al. (1997) studied two independent accessions which included terminal rye segments, and a further accession had one chromosome pair with a terminal segment and a second pair with an intercalary chromosome segment of rye. Besides that, there are some reports regarding translocations of chromosome segment of different homologous group, i.e. 4BL.5RL (Lukaszewski and Gustafson 1983; Schlegel and Korzun 2008). It should also be mentioned that in 2011, about 1050 cultivars carried the 1RS.1BL translocation, about 100 cultivars - the 1RS.1AL translocation, and about 30 cultivars - a 1R(1B) substitution (Schlegel 2012).

The initial research conducted on BC_1F_2 (60 plants) and BC_1F_3 (235 plants) generations of hybrids showed that the 1A, 2A, 3A, 4A, 5A, 6A, 7A, 2B, 3B, 5B and 7B chromosomes were introgressed (Apolinarska 1996). The chromosome number ranged from 21 to 35 chromosomes and the substitution per plant extended between 1 and 5 chromosomes. Substitution plants were characterised by a significantly higher fertility than hypo- and hyperploids plants (Apolinarska 1996), which indicates that stability of chromosome number resulted in fertility. Moreover, the chromosome number changes that occur in addition forms were probably a crucial reason for accelerated rearrangements accompanied mostly by the loss of alien genetic material (Alkhimova et al. 1999). Among the BC_1F_3s the most frequently transmitted alien

Table 1 Number of plants with proper chromosome number and different translocation types in the examined BC_1F_9 generation

Chromosome number	Number of plants with:											∑
	Lack of A- or B-genome chromatin	7A.7R	2A.2R	5A.5R	5A.5R 5A.5R	5A.5R 7A.7R	5A.5R 5B.5R	5A.5R 2A.2R	5A.5R 7A.7R	4A.R 7A.R	4A.R 7A.7R	5A.5R 7A.7R 4A.4R	
2n=26	1												1
2n=27			1					3		1			5
2n=28	16	7		18	32	12	6	3			3		97
2n=29		9		28									37
2n=30						3	2						5
2n=31							1						1
2n=32											4		4
∑	17	16	1	46	32	15	9	6		1	7		150

chromosomes were 7A and 5A (Apolinarska 1996), which is reflected by the frequency of 5A.5R and 7A.7R translocations in the examined BC_1F_9 generation after an open pollination (Table 1).

It can be supposed that the fifth homologous group is one of the most structurally conservative in Triticeae (Sarma et al. 2000), taking into consideration that 115 plants (76.7 %) carried the 5A.5R translocation and the structure of fifth group chromosomes with NOR localisation in the majority of Triticeae species. The introgression of A- and B-genome chromatin in the presented material was revealed by translocations only. In comparison with 23 different translocations, reported in the initial research among BC_1F_3 hybrids (Apolinarska 1996), only five translocations (2A.2R, 4A.4R, 5A.5R, 7A.7R and 5B.5R; Fig. 1) were found in the present progeny plant material (BC_1F_9). Additionally, in a confrontation with previous studies, translocations between A- and B-genome have not been verified. According to fertility data (number of seeds per spike) of the BC_1F_3 plants, it was reported that substitution and addition forms with the 6A chromosome had the highest average fertility (22 and 17.67 % respectively), while the substitution and addition plants with the chromosome 2A had the lowest (6.46 and 5.0 % respectively) (Apolinarska 1996). However, in the BC_1F_9 generation, segments of the chromosome 2A were present (in translocation form) in four plants, whereas chromosome 6A has been completely eliminated due to subsequent pollinations.

The present study was treated as a screening research for selecting given forms suitable for further breeding. For example, the 5B.5R translocation forms with *Ph1* loci could be used for rye breeding as there is the potential to create recombinant chromosomes composed of part rye and part alien chromatin, allowing the introgression of a genetic material from wild relatives. Moreover, an application of molecular cytogenetics in plant breeding could be an essential tool in selection. A utilisation of the FISH/GISH methods might be an essential selection tool in breeders point of view. The combination of multi-color GISH with repetitive DNA FISH allowed to single out five types of terminally localised translocation chromosomes: 2A.2R, 4A.4R, 5A.5R, 5B.5R and 7A.7R. Because of the highest frequency of the 5A.5R translocation, it can be assumed that the fifth homologous group of chromosomes is one of the most structurally conserved in Triticeae.

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