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Introgression of Wheat Chromosomes into Diploid Rye by Use of a Hexaploid Triticale with an ABRRRR Genome

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The objective of this study was to continue attempts to introduce wheat chromosomes, particularly those from the B genome, into diploid rye. An allohexaploid having 2 wheat mixogenomes (1B, 2A, 3B, 4B, 5B, 6A and 7B) and 4 rye genomes (RRRR) was crossed with substitution 2× rye containing the chromosomes of the wheat A genome except 3A, and next backcrossed with substitution rye. Karyotypes were analysed by C-banding in the produced plants of the generations F_1, BC_1–F_1, and BC_1–F_2. In nearly all plants of the F_1 generation (except one), 4–12 wheat chromosomes were found, mainly those of the B genome. A comparison of 2 successive generations indicates that both the mean and range of numbers of wheat chromosomes in the offspring of substitution plants and substitution-addition plants changed sometimes to the advantage of wheat chromosomes but sometimes to their disadvantage. A decline was observed in the contribution of B chromosomes and of chromosomes 2A and 6A, but pollen introduced some wheat chromosomes from the male parent: 1A, 4A, 5A and 7A. Wheat B chromosomes contributed to disturbances in plant development (lack of the spike emergence stage), but usually caused spike sterility, and even the single grains produced were usually unable to germinate. As a result, wheat chromosomes of the B genome were finally completely eliminated from the analysed material. The presence of wheat chromosomes of the A genome in fertile 2× rye plants, as well as their transfer to the next generations, indicate that the A genome is more closely related to the rye genome than the B genome. Positive introgression of wheat chromatin from the A genome into 2× rye depends to a large extent on chromosome engineering by means of appropriate crossing combinations, as A chromosomes from the male parent were much better tolerated than those from the female parent.

Keywords: diploid rye, genome, wheat chromosome, substitution, addition

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Introduction

Diploid rye is cultivated mainly in Europe, and used mainly in the baking industry, mostly for production of bread (from mixed flour), but also for animal forage and as initial material in triticale breeding. This plant species is characterized by resistance to diseases, low sensitivity to water deficit, high tolerance to soil acidity, salinity, and low temperatures as well as frost hardiness. However, rye is much less suitable for animal feeding than other cereal crops are. That is due to the presence of antinutritive compounds in rye grain, which limit the growth of young animals (Rakowska 1994). Rye is applied as a source of variation in numerous wheat breeding programmes, whereas beneficial genes of wheat are not used for rye breeding. Thus rye breeding should aim to improve its qualitative traits by use of alien chromatin. However, research conducted by Boros et al. (2001) showed that genes controlling the synthesis of soluble arabinoxylans and soluble dietary fibre in rye are scattered throughout the genome, and therefore may not be easily manipulated in breeding. Anyway, Lukaszewski et al. (2000) succeeded to transfer the *Glu-D1* locus encoding high molecular weight glutenin subunits 5+10 from bread wheat to diploid rye. Interspecific or intergeneric transfers of chromatin into diploids are inherently more complicated than into polyploids because diploid species are less tolerant of alien chromatin (Duvick 1990 after Lukaszewski et al. 2000). However, attempts are made to introduce alien chromatin into diploids, also into 2× rye, through chromosome manipulations by means of crossing combinations at various ploidy levels. Lukaszewski et al. (2000) transferred wheat chromatin from 6× triticale into 2× rye by repeated backcrossing of 6× triticale with 2× rye. In contrast, Łapiński and Rafalski (2001) introduced wheat DNA into 2× rye in the form of translocations and substitutions as a result of crossing of 4× triticale with 2× rye. Also from 4× triticale 2× rye hybrids, some addition rye lines with various wheat chromosomes have been derived (Schlegel 1982, Thiele et al. 1988, Baum 1991, Melz et al. 1991). Apolinarska (1996b, c, 1998) used 4× triticale in crosses with 4× rye to introduce wheat chromatin into 4× rye. The resultant substitution-translocation 4× rye lines with wheat chromosomes were used as the intermediate form for introgression of wheat chromosomes into 2× rye. By crossing 2× rye with substitution-translocation 4× rye, already in the F_1 generation, Apolinarska and Wojciechowska (2003) as well as Apolinarska (2003a) obtained fertile 2× rye plants with substitution and/or addition wheat chromosomes from the A genome. The addition lines of 2× rye developed by Schlegel (1982), Thiele et al. (1988), Baum (1991), Melz et al. (1991) contained wheat chromosomes of both A and B genomes. In earlier research by Apolinarska and Wojciechowska (2003) as well as Apolinarska

(2003a), no fertile 2× rye plants with wheat chromosomes from the B genome were produced, so in this study further attempts were made to reach this goal. For this purpose, we used a 6× triticale with 2 wheat mixogenomes dominated by B chromosomes.

The objective of this study was to continue the attempts to introduce wheat chromosomes, particularly those from the B genome, into diploid rye.

Material and methods

An allohexaploid form with ABRRRR genome, produced by crossing 4× rye with 4× triticale (Apolinarska 1996a), was pollinated with a mixture of substitution 2× rye with all chromosomes of the wheat A genome except 3A (Apolinarska and Wojciechowska 2003, Apolinarska 2003a). Plants of the F_1 generation were subjected to open pollination and backcrossing with substitution 2× rye, while plants of the BC_1–F_1 generation were subjected only to open pollination. Plants of the generations F_1, BC_1–F_1, and BC_1–F_2 were grown in the same greenhouse as the parent plants. The C-banding method (Lukaszewski and Gustafson 1983) was used to compare karyotypes of all germinated grains of the generations F_1 and BC_1–F_1. For research on the BC_1–F_2 generation, we used all the grains that were set by the substitution-addition rye plants with participation of wheat chromosomes from the B genome or from both A and B, and 3 grains from each of the most fertile plants with substitution and/or addition chromosomes from the A genome.

Results

F_1 hybrids

From the crossing combinations of the 6× form containing 2 wheat mixogenomes (1B, 2A, 3B, 4B, 5B, 6A and 7B) and 4 rye genomes (RRRR) (Fig. 1) with substitution 2× rye, a total of 175 grains developed from 8017 pollinated florets (so seed set reached 2.17%), and 23.42% of the grains germinated. Karyotypes were analysed by C-banding in all the produced 41 plants of the F_1 generation. Their chromosome numbers varied from 14 to 35 (Table 1), but in most cases from 20, as only 1 plant proved to be 2× rye with 14 chromosomes, while all the remaining 40 plants had also wheat chromosomes. Numbers of rye chromosomes in the studied F_1 plants ranged from 14 to 28, while numbers of wheat chromosomes among the 40 rye plants ranged from 4 to 12. In 17 plants the 7-chromosome wheat mixogenome from the initial form was found. In 2 plants, 12 wheat

Figure 1. C-banded mitotic chromosomes of hexaploid triticale with 2 wheat mixogenomes: 1B, 2A, 3B, 4B, 5B, 6A, 7B and 4 rye genomes (RRRR)

chromosomes were found, as the wheat mixogenome provided pairs of chromosomes 1B, 3B, 4B, 5B and 7B, while 2A and 6A where monosomic. In 1 plant with 9 wheat chromosomes and in 2 plants with 8 chromosomes, pairs of chromosomes 1B and 5B, and only 5B, respectively, were present. In plants with 4, 5 and 6 wheat chromosomes, some wheat chromosomes from the mixogenomes of the initial allohexaploid were eliminated from both A and B genomes, except 1B. Most frequently, the F_1 hybrids lacked wheat chromosomes 4B and 5B, or – with decreasing frequency – 2A and 6A, 7B or 3B. Five plants had wheat chromosome 5A and 1 plant had 7A, which were introduced by pollen from the male parent. In all the analysed plants of the F_1 generation, a total of 74 wheat chromosomes from the A genome and 177 from the B genome were found (Table 2). Results of the analysis of karyotypes and the range of chromosome numbers in F_1 plants indicate that female gametes and pollen differed in chromosome number and genome composition. Among the 41 plants analyzed cytologically, only 12 open-pollinated plants produced 21 grains, but none of them germinated.

Table 1. Distribution of chromosome number in hexaploid form (ABRRRR) × substitution diploid rye hybrids, generations F_1, BC_1–F_1, BC_1–F_2.

Generation	No. of analyzed plants	Chromosome number																			Range rye chromosome number	Range wheat chromosome number
		14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	32	33	35		
F1	41	1						2	2	1		2	5	9	6	6	1	1	3	2	14–28	0–12
BC_1–F_1	56	15	10	12	4	2	2	2	4	1	2	1	1								12–22	0–8
BC_1–F_2	59	50	7	2																	12–15	0–2

Table 2. Frequency of A and B wheat genome chromosomes in hexaploid form (ABRRRR) × substitution diploid rye hybrids, generations F_1, BC_1–F_1, BC_1–F_2

Generation	No. of analyzed plants	A wheat genome chromosome							B wheat genome chromosome					
		1A	2A	4A	5A	6A	7A	Sum	1B	3B	4B	5B	7B	Sum
F_1	41		34		5	34	1	74	44	39	29	29	36	177
BC_1–F_1	56	1	18		16	7	8	50	10	22	9	10	13	64
BC_1–F_2	59		2	2	27	2	20	53		1	1	1		3

BC_1–F_1 generation

To obtain the BC_1–F_1 generation, 6077 florets of F_1 hybrids of the 6× form with substitution 2× rye were pollinated with a mixture of pollen from substitution 2× rye. In total, 175 grains were produced, so seed set reached 2.87%. Germination rate amounted to 32%. Karyotypes were analyzed in all the produced 56 plants. Chromosome numbers of the analysed plants ranged from 14 to 25 (Table 1), including 0–8 wheat chromosomes and 12–22 rye chromosomes. In karyotypes of 12 plants no wheat chromosomes were found: 8 of them were 14-chromosome diploid rye plants, while 4 plants (1 with 15 chromosomes and 3 with 16 chromosomes each) had additional rye chromosomes. In the remaining 44 plants, wheat chromosomes were detected; among them, 10 plants had 1–3 wheat chromosomes only from the B genome, while 20 plants had chromosomes from both genomes A and B (Fig. 2a), and 14 had only A chromosomes. The most frequent chromosome from the B genome was 3B, whereas less frequent were 7B, 1B and 5B, 4B. The most frequent chromosome from the A genome was 2A, while less frequent were 5A, 7A, 6A and 1A (Table 2). In comparison with the F_1 generation, the frequency of chromosomes from the B genome markedly decreased. In all the analyzed plants of the generation BC_1–F_1, a total of 64 chromosomes of the B genome were identified, and 50 chromosomes from the A genome. The 2× rye plants that had only substitution and/or addition chromosomes of the wheat B genome did not set any seed, and some of them did not even reach the spike emergence stage. In contrast, the open-pollinated plants with substitution and/or addition wheat chromosomes from both A and B genomes produced single grains, and those with A chromosomes varied greatly in fertility, as they produced from 0 to 58.2 grains per spike (the highest value in a plant with substitution chromosome 5A).

BC_1–F_2 generation

Among the 20 tested grains of plants from the BC_1–F_1 generation, whose karyotypes included B chromosomes, 8 germinated, but only 3 of the BC_1–F_2 plants contained single chromosomes from the B genome: addition 3B, 5B, or substitution 4B (Fig. 2b). In the BC_1–F_2 generation, a total of 59 plants were analysed, whose chromosome numbers varied from 14 to 16, including 14–15 rye chromosomes and 0–2 wheat chromosomes (Table 1). In 19.64% of the plants, the genetic material of wheat was eliminated. Among chromosomes of the A genome, most often chromosomes 5A and 7A were detected, while chromosomes 2A, 4A and 6A only twice each. The plant with addition chromosome 3B did not set any seed, while that with 5B produced 6 grains and that with substitution chromosome

4B produced 15 grains, but none of them germinated. In the BC_1–F_2 generation, the fertility of 2× rye plants with substitution wheat chromosomes from the A genome also varied greatly, and ranged from 0 to 68.2 grains per spike (the highest value in a plant with 2 substitution chromosomes: 5A and 7A).

Discussion

The analysis of karyotypes of 3 generations of hybrids of the 6× form ABRRRR with 2× rye showed that numbers and composition of wheat chromosomes changed markedly from generation to generation in the offspring of individual substitution plants or substitution-addition plants, sometimes to the advantage of wheat chromosomes but sometimes to their disadvantage. Because of the chromosomal composition of the wheat mixogenomes of the female parent, wheat chromosomes from the B genome dominated in the F_1 generation. The pairs of chromosomes from the B genome as well as the lack of chromosome 2A or 6A and of some B chromosomes in the analyzed plants indicate that in female gametes the course of meiosis is disturbed, resulting in variable chromosome composition. The analysis of karyotypes of the next generations (BC_1–F_1 and BC_1–F_2) attests to elimination of wheat chromosomes from female gametes. The dramatically declining contribution of B chromosomes in both successive generations attests to a much lower tolerance of the rye genome to their presence than to the presence of A chromosomes. Also in earlier studies, during introgression of substitution-addition 4× rye from backcross hybrids of 4× rye with 4× triticale into 4× rye, in all the analyzed generations the A chromosomes were 4-fold more frequent than B chromosomes, and the former also participated in wheat-rye translocations much more frequently (Apolinarska 1996c, 1998). In contrast, the substitution-addition 2× rye originating from crosses between 2× rye and substitution 4× rye (with participation of all A chromosomes except 3A, and chromosomes 3B, 5B, 7B) completely eliminated B chromosomes and tolerated 1–3 chromosomes of the A genome per plant (Apolinarska and Wojciechowska 2003; Apolinarska 2003a). Chromosomes 1A, 4A, 5A and 7A, present in the analyzed generations of hybrids of the form ABRRRR with substitution-addition 2× rye, indicate that wheat chromatin was transferred by pollen from the male parent. In the last analyzed generation BC_1–F_2, the most frequent wheat chromosomes transferred from the male parent were 5A and 7A, while the frequency of A chromosomes from the female parent, i.e. 2A and 6A, was markedly reduced (Table 2). Selection pressure eliminated not only B chromosomes, but also chromosomes 2A and 6A, due to plant sterility or a remarkable decrease in fertility. In contrast, chromosomes from the wheat A genome originating from the male parent did not reduce

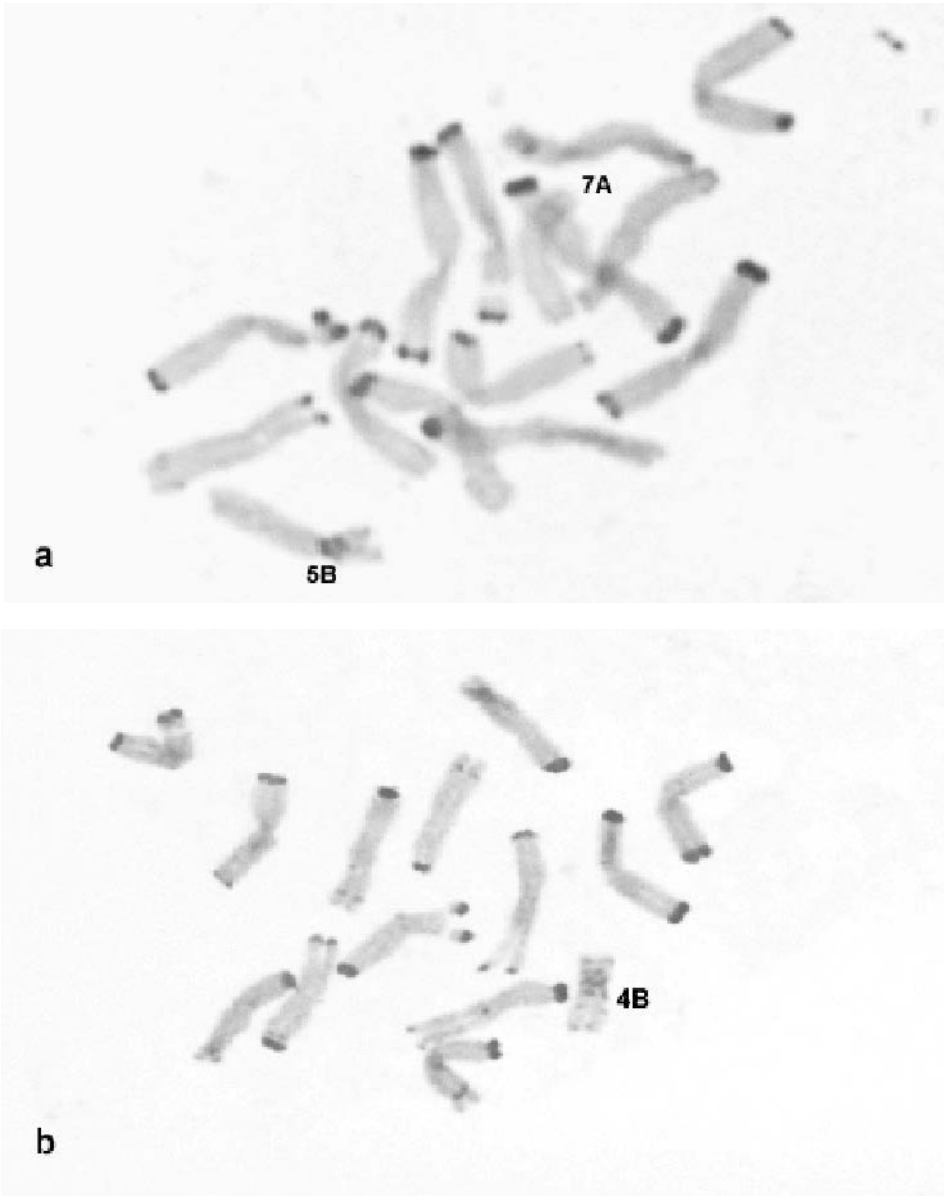

Figure 2. C-banded mitotic chromosomes of diploid rye with wheat chromosome(s): (a) addition and/or substitution chromosomes 7A, 5B; (b) substitution chromosome 4B

fertility, because they derive from substitution 4× rye, from which they were transferred into substitution-addition 2× rye (Apolinarska and Wojciechowska 2003, Apolinarska 2003a). Metaphase I of meiosis was analyzed in the substitution rye, both 4× (Apolinarska 2003b) and 2× (Apolinarska, 2006). The analyzes revealed exceptionally high homeologous wheat-rye pairing. Thus wheat chromosomes in pollen from the male parent probably contain some sequences homologous to rye chromosomes. In contrast, the wheat chromosomes from the female parent did not participate in wheat-rye pairing in the intermediate forms, i.e. 4× and 2× substitution rye, so they are characterized by a much lower homeology to rye chromosomes, as compared to the male parent. Wheat B chromosomes in the analyzed material contributed to disturbances in plant development (lack of the spike emergence stage), but they usually caused spike sterility, and even if single grains were produced, they were usually unable to germinate. The complete elimination of B wheat chromosomes from the last analyzed generation of hybrids between the hexaploid ABRRRR and substitution 2× rye, is confirmed by earlier research and conclusions of Apolinarska and Wojciechowska (2003) and Apolinarska (2003a), that the wheat B genome is not tolerated at the diploid level in rye. Probably because of the much lower homeology between the wheat B genome and the rye genome (Apolinarska 2003b), the seed and/or embryo development is disturbed. The presence of wheat chromosomes of the A genome in fertile 2× rye plants, and their transfer to the next generations confirm previous conclusions of Apolinarska (2003a, b) that the A genome is more closely related to the rye genome than the B genome. The above investigations also show that positive introgression of wheat chromatin from the A genome into 2× rye depends to a large extent on chromosome engineering by means of appropriate crossing combinations, as A chromosomes from the male parent were much better tolerated than those from the female parent.

References

Apolinarska, B. 1996a. Different chromosome combinations on tetra- and hexaploid level from hybrids of tetraploid rye × tetraploid triticale. In: Guedes-Pinto, H., Darvey, N., Carnide, P.V. (eds), Triticale: Today and Tomorrow. Developments in Plant Breeding. Kluwer Acad. Publ., Netherlands, pp. 189–194.

Apolinarska, B. 1996b. Transfer of chromosomes of the A and B genomes of wheat to tetraploid rye. J. Appl. Genet. **37**:345–356.

Apolinarska, B. 1996c. Additions, substitutions and translocations of wheat chromosomes in tetraploid rye. Inter. Symp. Rye Breed. Genet. 27–29 June, Stuttgart, Germany. Vortr. Pflanzenzüchtung. **35**:304–305.

Apolinarska, B. 1998: Tetraploid rye with wheat genetic material. In: Maluszyńska, J. (ed.), Plant Cytogenetics. Spring Symp., Cieszyn 19–22 May 1997, Silesian Univ. Katowice, Poland, pp. 206–209.

Apolinarska, B. 2003a. Substitutions, additions and translocations of wheat chromosomes into diploid rye. Bull. Plant Breed. and Acclim. Inst. **230**:195–203.

Apolinarska, B. 2003b. Chromosome pairing in tetraploid rye with monosomic-substitution wheat chromosomes. J. Appl. Genet. **44**:119–128.

Apolinarska, B. 2006. Chromosome pairing in diploid substitution rye and addition rye with wheat chromosomes. Cereal Res. Comm. **34**:1223–1229.

Apolinarska, B., Wojciechowska, B. 2003. Production of diploid rye/wheat chromosome additions and substitutions. Cereal Res. Comm. **31**:73–79.

Baum, M. 1991: Rye-wheat hybrids: the production of wheat chromosome additions to rye. Genome **34**:840–844.

Boros, D., Lukaszewski, A.J., Anioł, A. 2001. Location of genes controlling the content of dietary fibre and arabinoxylans in rye. Proc. Eucarpia Rye Meet. July 4–7, 2001, Radzików, Poland, p. 285.

Łapiński, B., Rafalski, A. 2001. Tetraploid triticale as a source of new variation for rye breeding. Proc. Eucarpia Rye Meet. July 4–7, 2001, Radzików, Poland, pp. 157–164.

Lukaszewski, A.J., Brzeziński, W., Klockiewicz-Kamińka, E. 2000: Transfer of the Glu-D1 locus encoding high molecular weight glutenin subunits 5+10 from breadwheat to diplod rye. Euphytica **115**:49–57.

Lukaszewski, A.J., Gustafson, P.J. 1983: Translocations and modifications of chromosomes in triticale × wheat hybrids. Theor. Appl. Genet. **64**:239–248.

Melz, G., Thiele, V., Seidel, A., Buschbeck, R. 1991. Rye-cytoplasmic rye-wheat additions – a new material for breeding. Genet. Polonica **32**:89–93.

Rakowska, M. 1994. Antinutritive compounds in rye grain. Plant Breed. Acclimat. and Seed Prod. **38**:21–42.

Schlegel, R. 1982: First evidence for rye – wheat additions. Biol. Zbl. **101**:641–646.

Thiele, V., Buschbeck, R., Seidel, A., Melz, G. 1988: Identification of the first rye-cytoplasmic rye-wheat-addition using LAP-isozymes. Plant Breed. **101**:250–252.