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Wheat chromosome instability in the selfed progeny of the double monosomics 1Rv-1A

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Abstract

Structural alterations of chromosomes are often found in wheat-rye hybrids. In the majority of cases modifications are observed for rye chromosomes, yet chromosome aberration cases are described for wheat, including the progeny of *Triticum aestivum* disomic and monosomic addition lines. Since wheat-rye substitution and translocation lines are the source of rye chromatin in wheat breeding programs, information on possible chromosome changes in the genomes of introgressive forms is important. Chromosome behavior in F_1 meiosis and chromosomal composition of F_2 karyotypes for double monosomics 1Rv-1A were studied by applying C-banding, genomic *in situ* hybridization (GISH) using rye genomic DNA, and sequential *in situ* hybridization using repetitive sequences pAs1, pSc119.2 and centromere specific pAet-06 as probes. The double monosomics 1Rv-1A were obtained by crossing of disomic substitution line with chromosome 1A replaced by *Secale cereale* 1Rv in the bread wheat Saratovskaya 29 (S29) background with S29. The results indicated a high frequency of bipolar chromosome 1Rv orientation, as compared to 1A, at metaphase I (MI) (58.6 and 34.7 % of meiocytes, respectively), and, at anaphase I (AI), chromatid segregation of 1Rv compared to 1A (70.53 and 32.14 % of meiocytes, respectively). In few cases desynapsis of wheat homologues was observed, at AI, the chromosomes randomly distributed between the poles or underwent chromatid segregation. At AI, the two wheat homologues separated onto sister chromatids in 10.89 % of cells. The plant F_2 karyotypes were marked with aneuploidy not only of chromosomes 1A and 1Rv, but also of 1D, 2D, 3D, 3B, 3A, 4A, 6D, 6B, 6A, and 7D. Structural changes were observed for the chromosomes of the first homoeologous group (1Rv, 1A, 1D, 1B), as well as for 2B, 5D, 6B, and 7B. The chromosomes 1Rv and 6B often demonstrated aberrations. The types of aberrations were centromeric break, deletions of various sizes, and a changed repeat pSc119.2 localization pattern.

Additional key words: FISH, GISH, chromosome alterations, karyotype structure, *Secale cereale*, *Triticum aestivum*.

Introduction

Triticum aestivum L. breeding programs face a challenging issue of preserving and increasing the genetic diversity of this culture and its effective use. Species and genera of the *Triticeae* tribe are an important source for expanding wheat genetic potential. Wide hybridization between wheat and rye has been successfully used in wheat breeding programs in the present time (An *et al*. 2013, 2015, Fu *et al*. 2014a). To date, vast experience in creating introgression wheat-rye forms with diverse genome composition has been accumulated. It has been shown that forming genomes with an alien introgression is accompanied by various changes. The process of genome stabilization in wheat-rye hybrids is characterized by instability of chromosome sets, whole-chromosome or whole-genome elimination and structural alterations in the rye and wheat chromosomes

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Abbreviations: AI - anaphase I, FISH - fluorescence *in situ* hybridization; GISH - genomic *in situ* hybridization; MI - metaphase I; PMCs - pollen mother cells; TII - telophase II.
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(Lukaszewski and Gustafson 1983, Badaev *et al*. 1985, Badaeva *et al.* 1986, Bolsheva *et al.* 1986, Dou *et al.* 2006, Zhou *et al.* 2012, Fu *et al.* 2013, Tang *et al.* 2014). The preferential elimination of the D-genome chromosomes occurs in the hexaploid triticale spontaneously formed in the progeny of selfed octoploid triticales (Dou *et al.* 2006, Zhou *et al.* 2012, Li *et al.* 2015). Genomes with different chromosomal compositions are formed in winter and spring hexaploid triticales under the action of natural and/or artificial selection (Oetteler 2005). Selection aiming to preserve the desired traits also affects the chromosomal composition of octoploid triticales (Cheng and Murata 2002). The genome composition of recent triticales is characterized by D(A), D(B), and D(R) substitutions or translocations that are relevant for the improvement of agronomic characteristics (Leonova *et al.* 2005, Dubovets *et al.* 2008, Zhou *et al.* 2012). Translocations occur between wheat and rye chromosomes and between rye chromosomes. The majority of translocations include the whole arms, but small fragments are also observed in some cases. Translocations occur between chromosomes of different homoeologous groups (Lukaszewski and Gustafson 1983, Badaev *et al.* 1985, Badaeva *et al.* 1986, Silkova *et al.* 2006, Hao *et al.* 2013). In the majority of cases, modifications are observed in the rye chromosomes, yet chromosome aberration cases have also been described in wheat chromosomes, including in the progeny of the *T. aestivum* disomic and monosomic addition lines (Fu *et al.* 2013, Tang *et al.* 2014).

Since wheat-rye substitution and translocation lines are the source of rye chromatin in wheat breeding programs, the information on possible chromosome changes in the genomes of introgressive forms is significant for breeding, as well as for the understanding of the conditions in which wheat and rye chromosomes co-exist in the same nucleus. The aim of this research was to determine the behavior of chromosomes 1A and 1Rv during the meiosis of double monosomics obtained by crossing the disomic substitution line 1Rv(1A) with the bread wheat Saratovskaya 29. This cross revealed the predominant transfer of chromosome 1A in comparison with 1Rv along with chromosomal composition changes and various chromosome aberrations in wheat subgenomes in karyotypes of the F_2 generation.

Material and methods

Plant materials used for this study included the bread wheat *Triticum aestivum* L. cv. Saratovskaya 29 (S29) and the disomic substitution line with chromosome 1A replaced by *S. cereale* L. cv. Viatka 1Rv in the S29 background (1Rv(1A), 2n=42) (Silkova *et al.* 2007). F_1 hybrids, the double monosomics, were obtained by crossing 1Rv(1A) and cv. S29 (1Rv-1A). F_2 hybrids were obtained from F_1 selfing. During spring 2016, F_1 hybrids were grown in a greenhouse, with day/night temperatures of 24/18 °C, a relative humidity of 80 %, a 16-h photoperiod, and an irradiance of 187 μmol m^{-2} s^{-1}. During summer, they were grown in the field of the Institute of Cytology and Genetics located in Novosibirsk (55°01′00″ N, 82°55′00″E), Russia. One of the F_1 hybrid progeny was grown in the greenhouse during the autumn 2016.

Cytological techniques and *in situ* hybridization: For the meiotic studies, young spikes at the appropriate stages were fixed in a (3:1) mixture of 96 % (v/v) ethanol and ice acetic acid for 24 h and then stored in 70 % ethanol in a refrigerator. For general meiotic analysis in the substitution line 1Rv(1A) and the double monosomic 1Rv-1A, pollen mother cells (PMCs) were stained with and squashed in 3 % (m/v) acetocarmine. A total of 945 meiocytes were examined at metaphase I (MI) in 1Rv(1A). In 1Rv-1A, a total of 1395 meiocytes were examined at MI, including 728 meiocytes in anaphase I (AI), and 1346 meiocytes in telophase II (TII). In order to analyze the 1Rv and wheat chromosome behavior during meiosis in the substitution line 1Rv(1A) and the double monosomic 1Rv-1A, fluorescence *in situ* hybridization (FISH) was used. Anthers were squashed, and slides were frozen in liquid nitrogen, dehydrated through a series of ethanol with increasing concentrations of 70, 90, and 96 %, and stored at -20 °C until needed. An *Aegilops tauschii* pAet6-09 probe specific for rye, wheat, rice, and barley centromere repeats (Zhang *et al.* 2004, Qi *et al.* 2013) and genomic rye DNA were used as probes. The samples of DNA containing the pAet6-09 repeats were kindly provided by Dr. A. Lukaszewcki (University of California, Riverside, CA, USA). The centromere specific probe pAet6-09 was PCR-labeled with biotin 16-dUTP and the total rye DNA was labeled by nick translation with digoxygenin 11-dUTP. Probes were mixed with blocking wheat DNA. The chromatin was stained using 1 mg cm^{-3} 4',6-diamidino-2-phenylindole (DAPI) in *Vectasheild* anti-fade solution (*Vector Laboratories*, Burlingame, CA, USA). A total of 731 meiocytes were examined.

To analyze the karyotypes of F_2 plants (the F_1 progeny grown in the summer of 2016), FISH analysis was also used. Root tips for the karyotyping were collected from plants grown in a hydroponic culture. The samples were then placed in ice water for 24 h, fixed in a solution of ethanol + acetic acid (3:1, v/v) and stored at -20 °C. Slides were frozen in liquid nitrogen and dehydrated in a graded series of ethanol (70, 90, and 96 %) and air dried. The rye tandem repeat pSc119.2, the *Ae. tauschii* clone pAs1, and the *Ae. tauschii* pAet6-09 probe specific for rye, wheat, rice, and barley centromere repeats and genomic rye DNA were used as probes. The centromere specific probe pAet6-09 and the pSc119.2 probe were PCR-labeled with biotin 16-dUTP. The pAs1

was labeled with digoxygenin 11-dUTP. The total DNA from rye was labeled by nick translation with digoxygenin 11-dUTP. Probes were used separately or in combination in different proportions and mixed with blocking wheat DNA. The chromatin was stained using 1 mg cm^{-3} DAPI in *Vectasheild* anti-fade solution. All slides were examined under an *Axio Imager M1* (*Carl Zeiss*, Jena, Germany) microscope. Images were recorded with a *ProgRes MF* camera (*Meta Systems*, *Jenoptic*, Germany) and processed using the *Adobe Photoshop CS2* software.

For the analysis of chromosome composition and the chromosome translocations involving the A-subgenome of F_2 plants (the F_1 progeny grown in the spring of 2016), C-banding was used (Badaeva *et al.* 1994). Preparations were analyzed under an *Amplival* microscope (*Carl Zeiss*). Images were recorded with a *Leica DC 300* camera and processed using the *Adobe Photoshop 5.0* software.

Results

The chromosome behavior in meiosis of the substitution line 1Rv(1A) did not differ from that in normal meiosis. Chromosomes formed 19.16 ± 0.05 ring bivalents, 1.7 ± 0.05 rod bivalents, and 0.28 ± 0.02 univalents per cell on average (Table 1). Chromosome 1Rv formed either a ring or rod bivalent (Fig. 1 Suppl.). At metaphase I (MI) of meiosis in the double monosomic 1Rv-1A, 2.15 ± 0.03 univalents were formed; at anaphase I (AI), 1.7 ± 0.11 univalents divided into chromatids; at telophase II (TII), 1.2 ± 0.09 micronuclei per cell were formed (Table 2).

Each meiocyte always had two univalents, 1Rv and

Table 1. Chromosome pairing at metaphase I in disomic substitution line 1Rv(1A) (acetocarmine staining).

Plants	Cells	Bivalents		Univalents
		ring	rod	
9	945	19.16 ± 0.05	1.7 ± 0.05	0.28 ± 0.02

Table 2. Univalent chromosome behavior in meiosis of double monosomics 1Rv-1A (acetocarmine staining).

Metaphase I			Anaphase I			Telophase II		
plants	cells	univalents	plants	cells	sister chromatid segregation	plants	cells	micronuclei
10	1395	2.15 ± 0.03	8	728	1.7 ± 0.11	8	1346	1.2 ± 0.09

1A (Fig. 2 Suppl.). In a few cases, additional unlabeled (wheat) univalents were present. In order to analyze the univalent chromosome segregation, the centromere specific probe pAet06 was used. Based on the localization pattern of pAet06, the monopolar/bipolar orientation of univalent chromosomes was identified. The presence of a diffuse and stretched signal indicated a bipolar orientation, whereas dot and dense signals showed a monopolar orientation. It should be noted that the pAet06 probe localization pattern of chromosome 1Rv, which localized to the equatorial plate, was different (Fig. 2 Suppl.). The hybridization signal appeared as either a diffuse and stretched signal or a dot localized on one side of the primary constriction. At the diakinesis stage preceding the bipolar attachment of the chromosome to the spindle, the localization of the pAet06 probe at centromere of 1Rv was characterized by a diffuse stretched signal (Fig. 2 Suppl.).

The comparison of the two chromosomes showed that chromosome 1Rv was bipolar oriented more often – in 58.6 % of the PMCs (Table 3). In chromosome 1A, there was a bipolar orientation in 34.7 % of the PMCs. At AI, the bipolar oriented univalents separated onto sister chromatids (Fig. 1). Chromosome 1A underwent chromatid segregation in 32.14 % of the PMCs, however, the number of meiocytes with chromatid segregation in 1Rv increased to up to 70.53 % of the PMCs (Table 3). Separation of univalent sister chromatids ended at the arms, when the centromeric regions had separated and were extended between the poles (Fig. 1). The rye chromosome also had centromere breaks (misdivisions) in 8.9 % of the meiocytes (Table 3, Fig. 1); wheat chromosome breaks were also observed (in 9.9 % of meiocytes; Fig. 1).

In a few cases, bivalent segregation failed (Fig. 2*A*,*B*). Homologous wheat chromosomes that underwent desynapsis were distributed between the poles (Fig. 2*C*,*D*) or underwent chromatid segregation (Fig. 2*E*,*F*) without complete separation of the whole sister chromatid (Fig. 2*A*,*B*). At the AI stage, the two

homologues separated onto sister chromatids in addition to 1A and 1Rv in 10.89 % of the meiocytes.

During the second division, the sister chromatids lagged at the equator. Later, they either distributed between the poles or broke. Thus, univalents could stay unincluded in microspores or included as a chromatid or an arm (Fig. 3 Suppl.). For example, chromosome 1R formed micronuclei in 14.23 % of microspores, included chromatids in 27.69 % and arms in 23.65 % of microspores (Table 3).

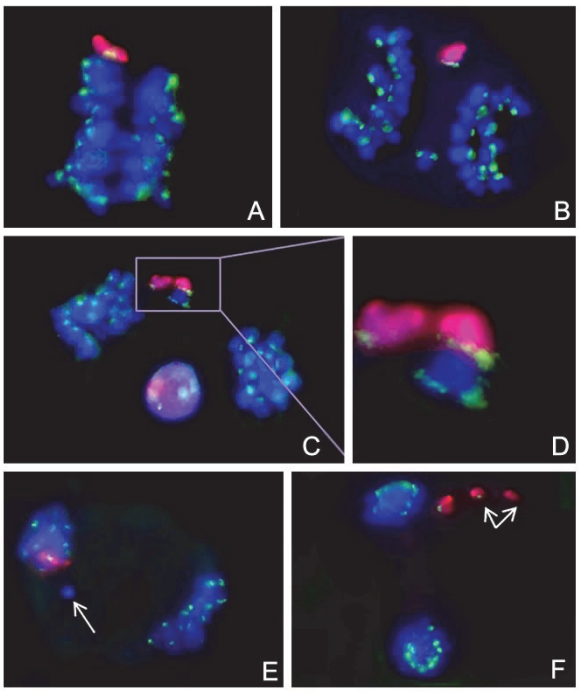

Fig. 1. 1Rv and 1A chromosome segregation at anaphase I: *A* - reductional distribution of 1Rv and 1A; *B* - lagging of 1Rv and 1A at the equatorial plate; *C* - 1Rv and 1A sister chromatid segregation; *D* - enlarged detail from *C*; *E* - reductional distribution of 1Rv, with an acentric wheat chromosome fragment (indicated with an *arrow*); *F* - 1Rv sister chromatid segregation with one broken chromatid (indicated with an *arrow*). Genome and sequential *in situ* hybridization, with the rye chromosome labeled in *red*, and the centromere specific probe pAet-06 labeled in *green.*

The analysis of chromosome behavior in the first and second mitosis during pollen grain maturation did not show any abnormalities. However, the micronuclei remained unchanged and did not include in nuclei, which predetermined the elimination of chromosomes (Fig. 3 Suppl.).

Karyotypes of F_2 plants were analyzed using C-banding (Table 1 Suppl.) and FISH (Table 2 Suppl.). The results of the C-banding revealed an advantage of chromosome 1A transfer compared to 1Rv. Chromosome 1A was present in 19 of the 27 analyzed karyotypes. Nine of them had it in the disomic state, and 10 in the monosomic state. The rye chromosome was identified in 14 out of 27 karyotypes. Four karyotypes had it in the disomic state, while 7 had it in the monosomic state. Two karyotypes had telocentric 1RS and 1RL and one had telocentric in addition to chromosomal 1R: 1R +t1RL. In addition to the expected structural changes of chromosomes 1R and 1A and their elimination, we observed changes in the chromosomal composition and chromosome aberrations in the wheat subgenomes A and D. Two plants had monosomy of chromosome 3D, and several plants had monosomy of chromosomes 1D and 4A. Two plants had an additional chromosome 7D. In the karyotype of one plant, a telocentric 1DL was found (Fig. 3*A*). The karyotype of the other plant underwent several changes, including long arm deletion 5D, 5DS.5DL-del, short arm deletion 1A, 1AL.1AS-del, and 6A monosomy (Fig. 3*B*). As a result, 8 plants had a modified karyotype that affected wheat subgenomes A and D.

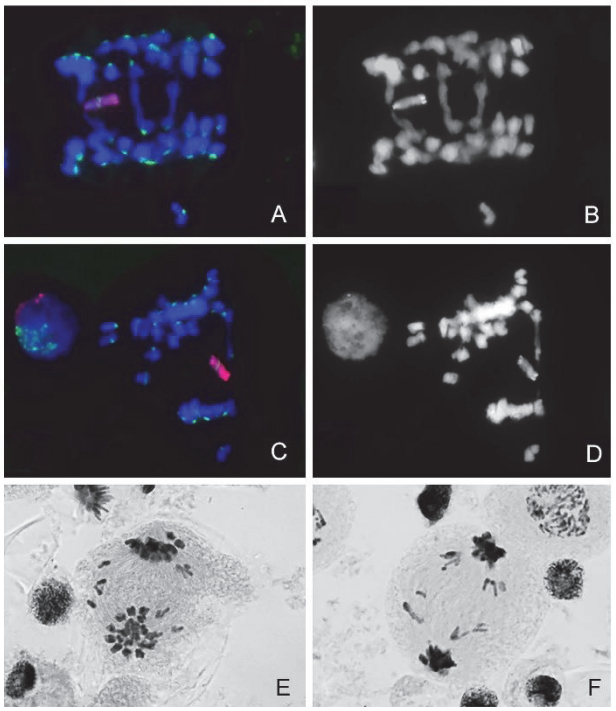

Fig. 2. Failed distribution of wheat homologues in meiosis of the double monosomic 1Rv-1A: *A* - delayed arm separation; *B* - the same cell as *A*, with DAPI counterstaining. *C* - unequal wheat chromosome distribution between the poles; *D* - the same cell as *C*, with DAPI counterstaining. *E, F* - four chromosomes, including wheat homologues, underwent chromatid segregation; *F* - broken chromatids. *A-D* - genome and sequential *in situ* hybridization, with the rye chromosome labeled in *red* and the centromere specific probe pAet-06 labeled in *green. C-F* - acetocarmine staining.

According to the FISH results (Table 2 Suppl.), the rye chromosome was not found in just 7 karyotypes of the 28. However, monosomic 1R plants prevailed (14 plants), and only 4 disomic 1R plants were found. Two pairs of plants had telocentric in addition to chromosomal 1R: 1R+t1RS and 1R+t1RL. One plant had

only t1RS. Chromosome 1A was found in 24 karyotypes. In 16 plants, 1A was found in the disomic state, while in 8 it was found in the monosomic state. Seven plants were characterized by modified karyotypes with the participation of wheat subgenomes. One of them underwent significant changes, including the monosomy of chromosome 3D and an unidentified chromosome and subtelomere deletion of the short arm T1DL.1DS-del (Fig. 4). In the second karyotype, the monosomy of chromosomes 1A, 3A, and 2D and the trisomy of chromosomes 3B and 6B, two of which had significant deletions of long arms and a telocentric t1RS, were observed (Fig. 4). In the third karyotype, monosomy of chromosomes 1A and 6D was observed, and two chromosomes were not identified (Fig. 4). In the other four karyotypes, the following chromosomal alterations were observed: *1*) two telocentrics t1BL and t6BL (Fig. 5), *2*) trisomy for chromosome 7B, one of which was Robertsonian translocation T7BS.W, and a different pattern of repeated pSc119.2 localization in interstitial region of the 1B chromosome long arm (Fig. 5), *3*) telocentrics t7BL and t6BL (Fig. 5), and *4*) telocentric t2BS (Fig. 5). Chromosome 1B was characterized by different repeat pSc119.2 localization patterns in the interstitial region of the long arm of the other karyotypes as well (Fig. 4).

Thus, the analysis of 55 karyotypes based on two methods showed that the F_2 plants demonstrated aneuploidy not only of chromosomes 1A and 1R but also of 1D, 2D, 3D, 6D, 7D, 3B, 6B, 3A, 4A, and 6A. In the above cases, 2 plants had an aneuploid 7D, three had an aneuploid 3D, two had an aneuploid 4A, and only a few plants had aneuploidy of the rest of the chromosomes. Structural changes were observed in chromosomes of the first group of rye genome R and wheat subgenomes A, B and D, as well as in chromosomes 2B, 5D, 6B, and 7B. Aberrations were observed more often in chromosomes 1R (6 cases) and 6B (4 cases). The most prevalent type of chromosome aberrations was the centromere break (13), followed by deletions (6) and an altered repeat pSc119.2 localization pattern (4).

Table 3. 1R and 1A chromosome behavior in meiosis of double monosomics 1Rv-1A (FISH); chrom. - chromosome, equ. - equational, red. - reductional.

Chrom.	Cells	Orientation at metaphase		Cells	Type of division			Cells	Telophase II		
		bipolar	monopolar		equ.	red.	breaks		micronuclei	chromatids	arms
1R	199	58.6	41.4	112	70.53	20.5	8.9	420	14.23	27.69	23.65
1A	199	34.7	62.3	112	32.14	67.8	9.9	-	-	-	-

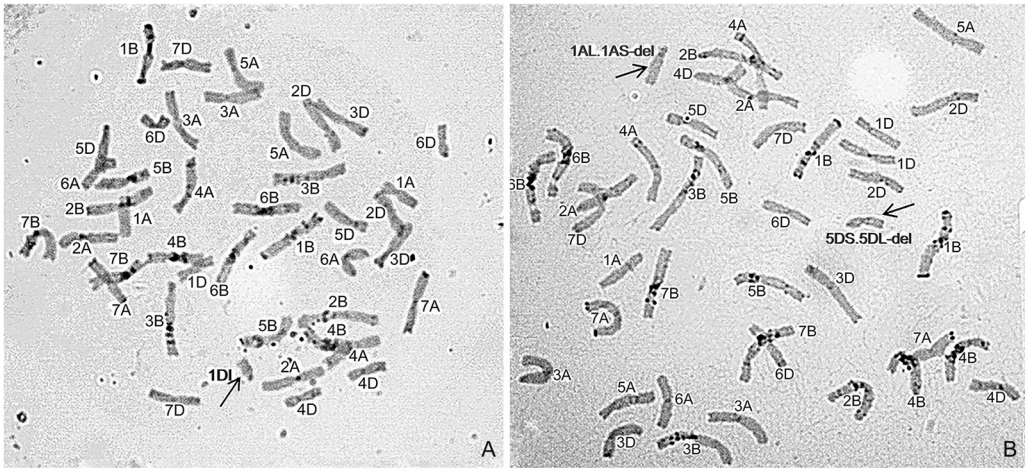

Fig. 3. Alterations in wheat chromosomes: *A* - changed 1D (indicated with an *arrow*); *B* - structural alterations of 1A and 5D (indicated with an *arrow*; C - banding staining).

Discussion

Normally, chromosome pairing and recombination are observed during meiosis. Chiasmata, cohesion that joins chromosome arms, the specific side-by-side geometry of centromeric regions, and the protection of centromeric cohesin from cutting by separase together control the segregation of homologues to the poles during the first

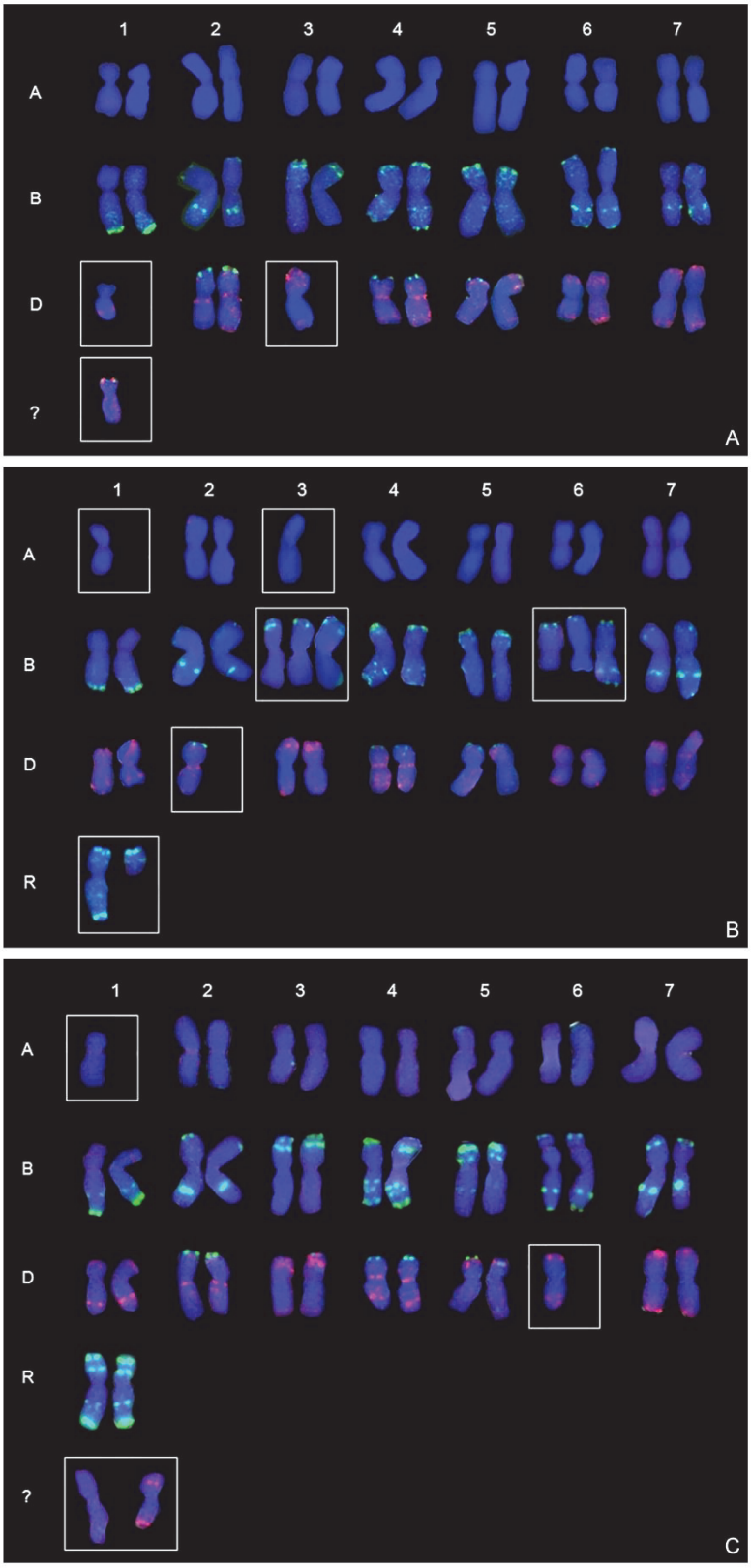

Fig. 4. Examples of changed karyotypes of the F_2 double monosomic 1Rv-1A: *A* - monosomy T1DL.1DSdel, monosomy 3D and one unidentified chromosome; *B* - monosomy 1A, 3A, 2D, trisomy 3B, trisomy 6B+T6BS.6BLdel+T6BS.6BLdel, and 1Rv+t1RvS; *C* - monosomy 1A, monosomy 6D and two unidentified chromosomes. In sequential *in situ* hybridization, the probe pSc119.2 is labeled in *green,* while the probe pAs1 is labeled in *red*. *Frames* mark chromosome alterations.

division; sister centromeres separate only during the second division. In meiosis of common wheat hybrids, the Ph1 locus suppresses the recombination of homoeologous chromosomes (Sears 1976, Martin *et al.* 2014), and univalent chromosomes randomly distribute between the poles or separate whole onto sister chromatids, at their arms and in centromeric regions (Sears 1952, Friebe *et al.* 2005). Such behaviour was observed for homoeologous 1A and 1R, which never formed bivalents. A characteristic feature of bipolar oriented univalents is lagging at the equator after bivalent segregation, which indicates cohesion retention. After that cohesin is sequentially removed from the centromeres and arms, and sister chromatids segregate to the poles. Retention of centromeric cohesion caused chromosome break across the centromere – misdivision. In 8.9 % of cells, the chromosome 1R underwent misdivision. Wheat chromosomes also underwent breaks in 9.9 % of meiocytes, but we did not observe the wheat chromosomes in detail.

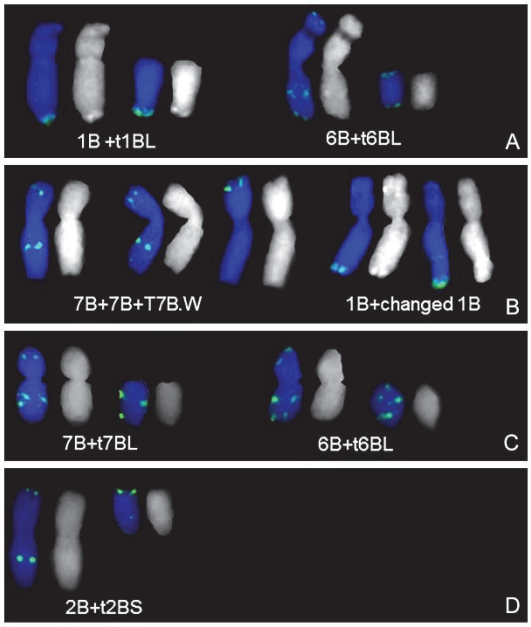

Fig. 5. Chromosome alterations of the B-subgenome in four plant karyotypes (*A* - *D*). Sequential *in situ* hybridization. The probe pSc119.2 is labeled in *green.*

Since centromeric regions define the type of chromosome orientation (monopolar or bipolar) and their subsequent segregation, we analyzed centromere organization using DNA probes. At MI, there were two types of site localization of the specific centromere probe pAet-06. It appeared as either a dense dot signal or a diffuse and stretched signal. It has been shown previously that the first type of signal corresponds with a monopolar-oriented chromosome, whereas the second type of signal corresponds to a bipolar oriented chromosome (Lukaszewski 2010, Silkova and Loginova, 2016). The rye chromosome 1R was bipolar-oriented more often than 1A (58.6 and 34.7 % of meiocytes, respectively). Chromosomes with monopolar-oriented centromeres were expected to move to one of the poles in AI. However, surprisingly, 1R showed an increase in the number of separated sister chromatids in AI compared to the number of bipolar oriented centromeres (70.53 and 58.6 % of meiocytes, respectively). As a consequence, the number of 1R chromosomes that moved to the pole in AI without dividing into sister chromatids was half as large as the number of monopolar-oriented ones in MI (20.5 and 41.4 % of the meiocytes, respectively). This shows the possibility of 1R centromeric region co-orientation, which was not observed for the chromosome 1A centromeric region. Eventually, 1R sister chromatids segregated to the poles almost twice as often as 1A sister chromatids (70.53 and 32.14, respectively).

In the other cases, wheat chromosomes and alien homoeologous chromosomes in double monosomics did not differ in the frequency of the sister chromatids segregation. The analysis of meiosis in monosomic plants by chromosome 1A of wheat and $1H^t$ of *Elymus trachycaulus* did not show any differences in the frequency of chromosome segregation (Friebe *et al.* 2005). Univalents underwent either chromosome or chromatid segregation and misdivided in 6 - 7 % of the pollen mother cells. Chromosome $1H^t$ underwent chromosome segregation in 43 % of PMCs and chromatid segregation in 57 % of PMCs, with misdivision in 6 % of PMCs. The corresponding values for chromosome 1A are chromosome segregation in 40 % of PMCs and chromatid segregation in of 60 % PMCs, with misdivision in 7 % of PMCs. The analysis of sister centromere behavior was performed for two univalents, 2R and reconstructed 2B (2B rec), with the entire centromere from 2R. The results did not show a prevalence of any behavior type for these chromosomes: both chromosomes segregated to the poles in 32 % of meiocytes, and sister chromatids separated in 49 % of meiocytes. In 19 % of meiocytes, they broke across the centromere (Lukaszewski 2010). However, it was observed that the proportion of univalents in bipolar attachment increases over time and that most of this increase is caused by the separation of the originally fused sister centromeres (Lukaszewski 2010). Indeed, this was demonstrated in our experiment with chromosome 1R.

At the moment, it is hard to provide a definite explanation why sister centromeres of univalent chromosomes become bipolar oriented and why univalent sister chromatids segregate to the poles in the first meiotic division of plants with aneuploidy.

Monosomy of two chromosomes, one of which is alien, allows centric breaks to be obtained from two univalent chromosomes, and, in the case of possible telocentric joining, it allows forms with alien translocations. However, in this study, changes were observed not only for univalent chromosomes 1R and 1A but also for chromosomes A, B, and D of the wheat subgenomes. The prevailing type of aberration was a centromere break (13). Deletions were observed less

often (6), as were changes in the repeat pSc119.2 localization pattern (4). Among the F_2 progeny aneuploids of chromosomes 1D, 2D, 3D, 6D, 7D, 3B, 6B, 3A, 4A, and 6A were also observed.

Studies conducted by other groups have reported changes in wheat chromosomes structure, as well as in karyotype chromosomal composition after analyzing the progeny of three monosomic addition lines: 4R, 6R, and 7R (Fu *et al.* 2013). The disappearance of a fragment detected by the pSc119.2 FISH signal in the telomeric region of the 3DS chromosome in the 4R monosomic addition line and 4A trisomy and chromosome 2D deletion in the selfed progeny of the 7R monosomic addition line were observed. Furthermore, chromosomes 1A and 4B were eliminated from some of the progeny of the 6R monosomic addition line. The presence of chromosome 1R in the wheat-rye addition line (3-8-20-1R-2) caused lagging chromosomes, micronuclei, chromosomal bridges, and the one pole segregation of 1R chromosome during mitosis. In some progenies of the line, chromosome 4B was absent (Fu *et al.* 2014b).

In our previous study, the C-banding of the 1Rv(1A) line chromosomal composition did not reveal chromosome alterations in karyotypes compared to karyotypes of the background cv Saratovskaya 29 (Silkova *et al.* 2007). However, the univalent state of the double monosomic 1Rv-1A chromosomes caused desynapsis of homologues with low frequency, which could consequently lead to chromosomal composition instability and aberrations in the wheat subgenome chromosomes. Furthermore, a later disjunction of the chromosome arms, which occurs after the centromeric regions, can maintain cohesion on arms at AI. Due to a delayed dissociation from the arms of the sister chromatids, breakages in the arm area leading to deletions are possible, and acentric fragments may emerge.

Analysis of the F_2 generation karyotypes also revealed low 1R transfer competitiveness compared to that of the wheat 1A homologue. The rye chromosome was eliminated in the whole or chromosome arms after centric breaks. In contrast, 1A transferred more often and underwent changes very rarely. As was shown previously, the rye chromosome 5R of dimonosomic 5R-5D was also less competitive than 5D and underwent changes. In addition to telocentric T5RS, it formed isochromosomes T5RS.5RS and chromosomes with the long arm deletion T5RS.5RL-del. (Silkova *et al.* 2011). The rye chromosomes were likely distinct in the double monosomic cells.

In some cases, alien chromosomes compete better than wheat chromosomes. Chromosome 1D elimination was observed in 5 out of 24 disomic addition lines of common wheat (2n=44), carrying alien chromosomes *Agropyron intermedium*, *Ag. elongatum*, *Aegilops longissima*, *Ae. peregrine*, which belong to the 1st homoeologous group (Garg *et al.* 2007). This fact can be explained by wheat evolution history, as genome D was likely the most recently attached and was not integrated into the wheat genome during an evolutionary short period (Garg *et al.* 2007).

To summarize, the results of our work show that double monosomy, emerging when the wheat-rye substituted line 1Rv(1A) is intercrossed with the cultivar, creates conditions for changes in the structure and composition of wheat subgenome chromosomes in progeny. According to our data, one of the causes is desynapsis of chromosome homologues that leads to distortions in chromosome separation, their elimination, and breaks.

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