

Chromosome Rearrangements Caused by Double Monosomy in Wheat-Barley Group-7 Substitution Lines

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Abstract

Interspecific or introgressive hybridization is one of the driving forces in plant speciation, producing allopolyploids or diploids with rearranged genomes. The process of karyotype reshaping following homoploid interspecific hybridization has not been studied experimentally. Interspecific hybridization is widely used in plant breeding to increase genetic diversity and introgress new traits. Numerous introgression stocks were developed for hexaploid wheat *Triticum aestivum* L. ($2n = 6x = 42$, genome AABBDD). Double monosomic lines, containing one alien chromosome from the tertiary gene pool of wheat and one homoeologous wheat chromosome, represent a simplified model for studying chromosome rearrangements caused by interspecific hybridization. The pairing of a chromosome from the tertiary gene pool with a wheat homoeologue is restricted by the activity of the wheat *Ph1* gene, thus, rearrangements caused by chromosome breakage followed by the fusion of the broken arms

can be expected. We analyzed chromosome aberrations in 4 sets of lines that originated from double monosomics of barley (*Hordeum vulgare* L.) chromosome 7H and wheat group-7 chromosomes with dicentric or ring chromosomes. The dynamics of wheat-barley dicentric chromosomes during plant development was followed and an increased diversity of rearrangements was observed. Besides the targeted group-7 chromosomes, other wheat chromosomes were involved in rearrangements, as chromosomes broken in the centromeric region fused with other broken chromosomes. In some cells, multi-centric chromosomes were observed. The structure and dosage of the introgressed barley chromatin was changed. The transmission of the rearrangements to the progenies was analyzed. The observed aberrations emphasize the importance of cytogenetic screening in gene introgression projects.

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Interspecific hybridization is recognized as one of the driving forces in speciation [Anderson, 1949; Rieseberg and Wendel, 1993]. Many events of natural introgression through interspecific hybridization are known for a wide range of plants, insects and fish [Baack and Rieseberg,

2007; Mallet, 2007; Gaines et al., 2012; Cornille et al., 2014; Yakimowski and Rieseberg, 2014; Wang et al., 2015]. Homoploid hybrid speciation does not change ploidy, but may change chromosome shape, size and structure. Karyotype alterations caused by interspecific hybridization facilitate speciation, as de novo arising chromosome rearrangements can reproductively isolate a new hybrid lineage from the parents [Grant, 1966; Rieseberg et al., 1995; Rieseberg, 2001; Burke et al., 2004; Lai et al., 2005]. The known types of chromosome rearrangements are peri- and paracentric inversion, intra- and interchromosomal translocation and deletion, and chromosome fusion and fission [Schubert and Lysak, 2011; Salse, 2016]. The mechanisms of karyotype reshaping following homoploid interspecific hybridization have not been studied experimentally. Karyotype alteration may follow several scenarios. If chromosomes of the parent species are colinear and can pair and recombine during meiosis, then mosaic chromosomes with preserved synteny may be formed [Kopecky et al., 2009; reviewed in Gaeta and Pires, 2010]. If pairing occurs between parental chromosomes with disturbed colinearity, rearrangements in a hybrid can result from meiotic crossover between homologous regions of translocations or inversions [Schubert, 2007]. In the absence of meiotic pairing, univalents formed during metaphase I can break at meiotic anaphase and form unstable dicentric and ring chromosomes, which may initiate breakage-fusion-bridge (BFB) cycles in F_1 somatic cells and produce translocations, deletions and duplications [McClintock, 1939, 1941; Lukaszewski, 1995; Gisselsson et al., 2000; Friebe et al., 2005]. In an interspecific hybrid, both homoeologous recombination and chromosome breakage followed by BFB cycles may occur simultaneously affecting multiple chromosomes.

Interspecific hybridization involving crops and their wild relatives is often used in breeding to increase genetic diversity and introgress agronomically important traits [Friebe et al., 1996; Dempewolf et al., 2017]. Numerous introgression stocks have been developed for hexaploid wheat *Triticum aestivum* L. ($2n = 6x = 42$, genome AABBDD), including wheat-alien addition and substitution lines. Wheat double monosomic (dM) lines containing 1 alien chromosome from the tertiary gene pool and 1 genetically related homoeologous wheat chromosome represent a simplified model for studying chromosome rearrangements caused by interspecific hybridization. First, the pairing of chromosomes from the tertiary gene pool with wheat homoeologues is restricted by the activity of the wheat *Ph1* gene [Sears and Okamoto, 1958; Sears,

1977]. Hence, only rearrangements caused by chromosome breakage followed by fusion of the broken ends can be expected. Second, only the targeted alien and wheat chromosomes are expected to be involved in these rearrangements. Third, as a polyploid, wheat has the ability to 'buffer' genome distortions [Feldman and Levy, 2012], i.e., rearranged aberrant chromosomes can be transmitted to the progeny. Finally, in this model system, rearrangements involving an introgressed chromosome can be easily examined cytologically. Alien chromatin can be distinguished in a wheat background by genomic in situ hybridization (GISH), and all wheat chromosomes can be identified by fluorescent in situ hybridization (FISH) with well-developed sets of chromosome-specific cytological markers, including tandem repeats and single-gene probes [Komuro et al., 2013; Danilova et al., 2014].

The study of chromosome rearrangements using this model system has a practical value. In alien gene introgression projects, screening of improved material is often conducted by phenotyping, ignoring chromosome aberrations, which may substantially reduce chances of selecting stably inherited introgressions. In the present study, we analyzed chromosome rearrangements in 4 sets of lines with dicentric and ring chromosomes and their progenies, originated from double monosomics of barley (*Hordeum vulgare* L., $2n = 2x = 14$, genome HH) chromosome 7H and homoeologous wheat group-7 chromosomes. The importance of cytogenetic control in alien gene introgression projects is emphasized.

Materials and Methods

Plant Material

The material used in this study included barley cultivar Betzes (WGRC accession TA9001), wheat cultivar Chinese Spring (CS, TA3008), a CS-Betzes 7H chromosome addition line (TA3697) from a set of lines produced by Islam et al. [1978, 1981], and the CS monosomic stocks [Sears, 1954] CSM7A and CSM7D (TA3047 and TA3061, respectively). All lines are maintained at the Wheat Genetics Resource Center at Kansas State University.

The monosomic stocks CSM7A and CSM7D were crossed with the 7H addition line, and 2 sets of double monosomic lines with 42 chromosomes dM7A7H ($2n = 40 + 7A + 7H$) and dM7D7H ($2n = 40 + 7D + 7H$) were selected from the F_1 plants. The progenies of plants from the double-monosomic populations were screened with barley 7H arm-specific KASP markers [Danilova et al., 2018]. Plants with a marker disassociation indicative of the chromosome breakage were analyzed by GISH. In total, 1,065 seedlings were screened. Karyotypes of plants containing cycling dicentric chromosomes and their progenies resulting from self-pollination were analyzed. Roots for cytological analysis were collected from either germinated seeds or plants growing in pots in the greenhouse.

Cytogenetic Analyses

GISH was performed according to Zhang et al. [2001] with modifications [Danilova et al., 2018]. The GISH probe mixture (20 μ L/slide) contained 40 ng of barley gDNA probe labeled with fluorescein-12-dUTP (PerkinElmer, Waltham, MA, USA), 1 ng of Cy5-(GAA)_n oligonucleotide probe (synthesized by Integrated DNA Technologies, Inc., Coralville, IA, USA), and no wheat blocking DNA.

FISH with cDNA probe 7S-4 (tplb0015e09), physically mapped to the short arm of group-7 wheat chromosomes, and oligonucleotide probes FAM-(GAA)_n and FAM-pAs1 or TEX615-pAs1 [Danilova et al., 2014], labeling microsatellite (GAA)_n and D-genome-specific Afa family tandem repeat pAs1 [Nagaki et al., 1995], was used to identify wheat chromosomes and determine the structure of the translocation chromosomes. Chromosome preparations, probe labeling and FISH procedure were as described previously [Kato et al., 2004, 2006] with minor modifications [Danilova et al., 2012]. The cDNA probe was labeled with Texas red-5-dCTP (PerkinElmer). Wheat full-length cDNA clones were obtained from the National BioResource Project-Wheat, Yokohama, Japan. For detecting barley centromere-specific microsatellite [Hudakova et al., 2001; Zeng and Jiang, 2016], the oligonucleotide probe TEX615-(AGGGAG)₄ (Integrated DNA Technologies, Inc.) was used (10 ng per slide). Chromosome preparations were mounted and counterstained with propidium iodide or 4',6-diamidino-2-phenylindole (DAPI) in Vectashield (Vector Laboratories, Burlingame, CA, USA).

Images were captured using a Zeiss Axioplan 2 microscope with a cooled charge-coupled device camera CoolSNAP HQ2 (Photometrics, Tucson, AZ, USA) and AxioVision 4.8 software (Carl Zeiss, Thornwood, NY, USA). Images were processed using the Adobe Photoshop software (Adobe Systems Incorporated, San Jose, CA, USA).

Molecular Marker Analysis

Competitive allele specific PCR (KASP) markers for barley and wheat group-7 chromosomes based on the sequences with determined physical positions were used to assay the presence of barley chromatin [Danilova et al., 2014; 2018]. Two cDNA sequences mapped on the long arms of group-7 chromosomes, 7L-1 (tplb0013b07, relative distance from the centromere 0.2) and 7L-4 (tplb0007o14, relative distance from the centromere 0.9), were used.

Genomic DNA was isolated from leaves of 2-week-old seedlings using a BioSprint 96 workstation according to the Qiagen BioSprint DNA Plant Handbook protocol (QIAGEN Inc., Valencia, CA, USA). KASP was performed as described by Danilova et al. [2018].

Results

Chromosome Constitution of Parents with Cycling Dicentrics

Among the 1,065 progenies of the 2 double monosomics dM7A7H and dM7D7H, the number of seedlings without a barley chromosome (both 7HS and 7HL molecular markers were negative) or with a complete barley chromosome (both 7HS and 7HL molecular markers were positive) was similar, approximately 38 and 42%,

respectively. The 13–18% of plants with a barley chromosome broken at the centromere were represented by telosomes or isochromosomes; 2–3% of the plants had stable Robertsonian whole arm translocations (RobT) with broken barley and wheat arms fused at the centromeric region, and 0.3–0.6% had dicentric chromosomes with a barley arm attached to a wheat chromosome. Karyotypes of plants with different types of cycling dicentric chromosomes, unstable RobTs and a progeny with ring chromosomes were screened.

Plant 913-12 (2n = 41, 7H broken and involved in dicentric), originated from a dM7D7H line, contained barley chromosome arm 7HL according to the marker analysis. In roots collected 3 days after germination, we observed either a dicentric chromosome T7AS·7AL·7HL in some cells or a RobT#1 T7AS·7HL in others in a ratio ~10:1. Chromosome 7A and the structure of the dicentric chromosome were identified by their (GAA)_n pattern. 7A has distal (GAA)_n FISH signals on both arms, the 7AL signal is weaker and located more proximal than the (GAA)_n signal on 7AS in cv Chinese Spring [Danilova et al., 2012]. Earlier, Kynast et al. [2000] studied the dynamics of wheat dicentric chromosomes resulting from chromosome breakage induced by a gametocidal factor on chromosome 4Mg from *Aegilops geniculata* Roth. The authors found that the majority of BFB cycles ceased by 32 days after germination. The longest persistence of a dicentric wheat chromosome was 58 days. Thus, we expected BFB cycles to continue during the first 2 months of plant development. To follow the karyotype changes, we collected roots at 3, 37, 41, 54, and 62 days after germination. In roots collected from germinated seeds, the 7A part of the dicentric chromosome T7AS·7AL·7HL appeared intact (Fig. 1a). At 37–41 days after germination, BFB cycles proceeded, and in addition to the dicentric (40%) and the RobT#1 (24%), other rearrangements were observed, including dicentrics with a shortened 7AL arm (17%), 7HL telocentrics, acrocentric chromosomes with a shortened wheat arm (designated as T#2), isodicentric chromosomes, dicentric chromosomes involving 7A and a shortened 7HL (~5% each), and wheat telocentrics (Fig. 1). Based on the changed (GAA)_n position from distal to interstitial, the 7AL arm appeared rearranged in some dicentrics, possibly through a paracentric inversion (Fig. 1b). As distinct from 3-day-old roots, cells containing 2 rearranged chromosomes involving 7HL were observed (Fig. 1g–j). At 54 days, we analyzed cells at the late anaphase–early telophase and observed that out of 78 cells counted, the BFB cycles continued in one-third of the cells, 57 cells (73.1%) proceeded through division normally, and

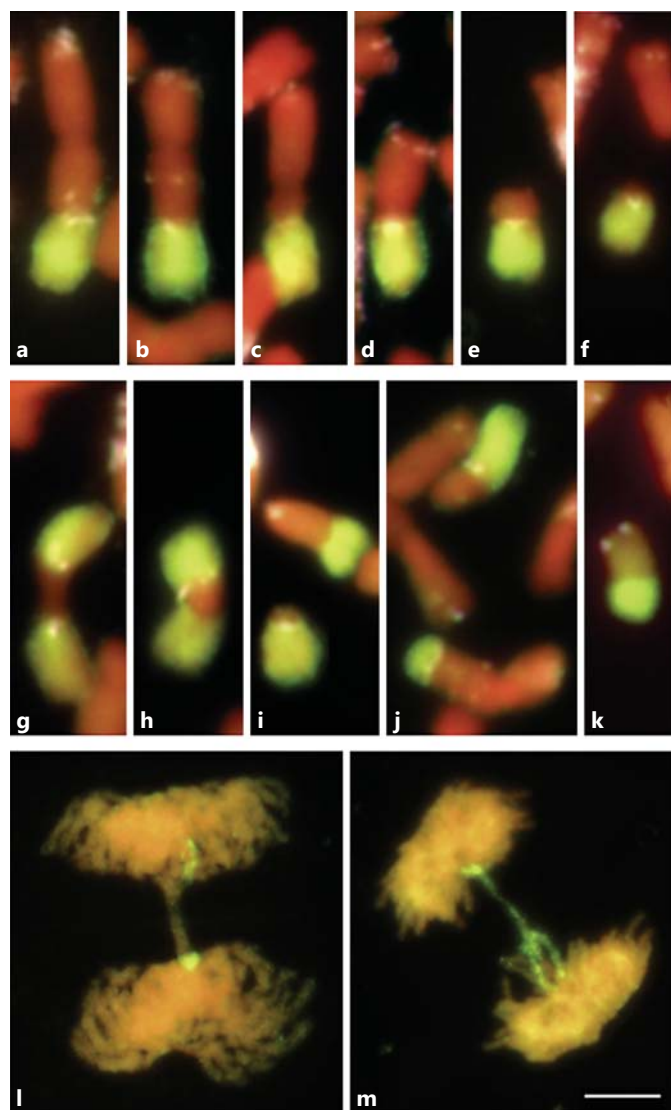


Fig. 1. Rearrangements observed during breakage-fusion-bridge cycles in root somatic tissue involving chromosome 7A and 7HL in plant 913-12 at 37-41 days after germination. **a-k** Metaphase chromosomes. **b-f** Reduction and rearrangement of arm 7AL. **g-j** Duplication of barley chromatin; **i-k** Shortening of barley arm 7HL. **l-m** Anaphase-telophase, 54 days after germination showing bridge formation of wheat (**l**) and barley (**m**) chromatin. Barley chromatin is labeled in green, (GAA)_n repeats are white, and chromosomes counterstained with propidium iodide are visualized in red. Scale bar, 10 μ m.

21 (26.9%) had bridges consisting of either wheat or barley chromatin (Fig. 1l, m). At 62 days (stem elongation stage) finding roots with meristems suitable for cytological analysis was difficult. Among 16 metaphases with barley chromatin present, we observed 10 RobTs, 3 dicentrics, and 3 7HL telo- or acrocentrics with very short wheat arms.

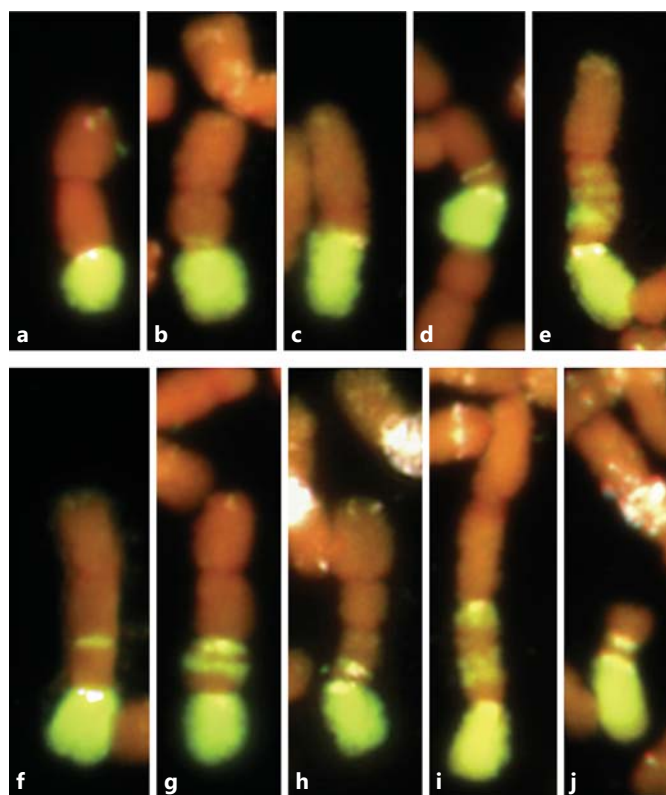


Fig. 2. Rearrangements observed during breakage-fusion-bridge cycles involving chromosome 7A and the 7HL arm in plant 913-588 at 3 days after germination. **a-e** Dicentrics. **f-i** Multicentrics. **j** Dicentric with shortened wheat segments. Barley chromatin is labeled in green, (GAA)_n repeats are white, and chromosomes counterstained with propidium iodide are shown in red.

The chromosome constitution of 3 other plants was analyzed in roots collected 3 days after germination. Plant 913-588 ($2n = 41$, 7H broken and involved in dicentric), a progeny of a dM7D7H line, also had 7A and barley arm 7HL involved in BFB cycles, but as distinct from plant 913-12, besides dicentric T7AS·7AL·7HL, RobT#1 T7AS·7HL and acrocentrics, barley chromatin was involved in multicentric chromosomes. Moreover, barley and wheat chromatin underwent multiple breakages and fusions in sites other than the centromere, resulting in chromosomes with striped, zebra-like arms (Fig. 2).

The third plant 914-300 ($2n = 41$ or 42 , 7A absent), originated from dM7A7H, had unstable RobT#1 T7AS·7HL present in three-fourths of the cells and 7HL telocentrics or acrocentrics with very short wheat arms in one-fourth of the cells. One cell containing the dicentric with shortened 7AL arm was observed.

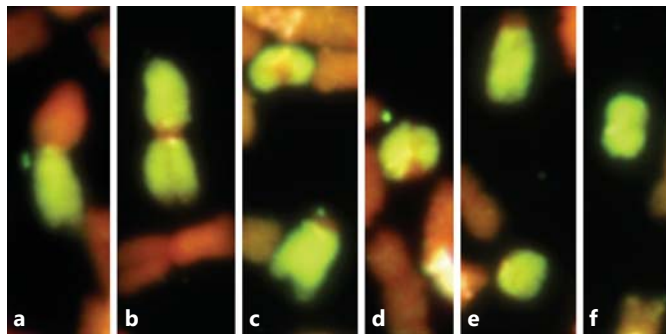


Fig. 3. Rearrangements observed in plant 628–91. **a** RobT. **b** Isochromosome. **c, d, f** Ring chromosomes. **e** Acrocentric and a mini-chromosome. Barley chromatin is labeled in green, (GAA)_n repeats are white, and chromosomes counterstained with propidium iodide are shown in red.

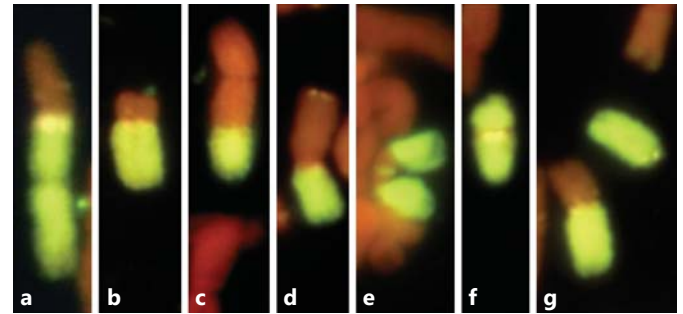


Fig. 4. Chromosome aberrations in progenies of plant 913–12. Each panel represents a different plant. Barley chromatin is labeled in green, (GAA)_n repeats are white, and chromosomes counterstained with propidium iodide are visualized in red.

Table 1. Chromosome constitution in progenies of plants with cycling dicentric or ring chromosomes

913-12			913-588			914-300			628-91		
chromosome constitution	<i>n</i>	%	chromosome constitution	<i>n</i>	%	chromosome constitution	<i>n</i>	%	chromosome constitution	<i>n</i>	%
No 7HL ^a chromatin	67	73.6	no 7HL ^a chromatin	53	55.2	no 7HL ^a chromatin	87	78.4	no 7HL ^a chromatin	88	77.8
acro T#2	11	12.1	heterozygous T#3	31	32.3	RobT#1 7AS·7HL	7	6.3	telo 7HL	7	6.2
RobT#1 7AS·7HL	6	6.6	T7AS·7AL·7HL ^b	8	8.3	dic	2	1.8	different RobTs ^c	4	3.5
iso 7HL·7HL	3	3.3	homozygous T#3	8	8.3	telo 7HL	5	4.5	rearranged iso 7HL·7HL	4	3.5
telo 7HL	2	2.2	T7AS·7AL·7HL ^b	8	8.3	iso 7HL·7HL	3	2.7	iso 7HL·7HL	3	2.7
dic	2	2.2	RobT#1 7AS·7HL	1	1	unstable iso-telo	4	3.6	acro	3	2.7
			telo 7HL	2	2.1	acro	2	1.8	iso+telo 7HL/cell	2	1.8
			RobT#4 7DS·7HL	1	1	mini, ring	1	0.9	small telo/iso + iso 7HL·7HL	1	0.9
									telo or iso	1	0.9
Total	91		total	96		total	111		total	113	

iso, isochromosome; telo, telocentric; dic, dicentric; acro, acrocentric chromosomes.

^a According to the marker screening. ^b Barley centromere inactive. ^c T2BS·7HL, T5BL·7HL, T5DL·7HL, T6BS·7HL.

The fourth plant 628–91 ($2n = 41$ or 42) was a progeny of selfed 914–300 and had variable structures involving 7HL, such as ring chromosomes of different sizes, acrocentrics with shortened wheat arms, isochromosomes arose from the acrocentrics, RobT#1 T7AS·7HL and their combinations ($2n = 40 + t7HL + r7HL$, $40 + i7HL$ or $40 + RobT#1$) (Fig. 3). To verify the transmission of different types of rearrangements and the level of chromosome aberrations, the karyotypes of the progenies from these 4 plants were analyzed.

Chromosome Constitution of Progenies from Plants with Dicentric and Ring Chromosomes

The progenies were first screened with 7HL molecular markers, and plants with barley chromatin detected were analyzed cytologically.

In 73.6% of the progenies of plant 913–12, barley chromatin was not detected by markers. In 25.4% of progenies, barley chromatin was present in form of an acrocentric translocation T#2 (12.1%) (Fig. 4b) or a compensating RobT#1 T7AS·7HL (6.6%) (Fig. 4d), telosomes,

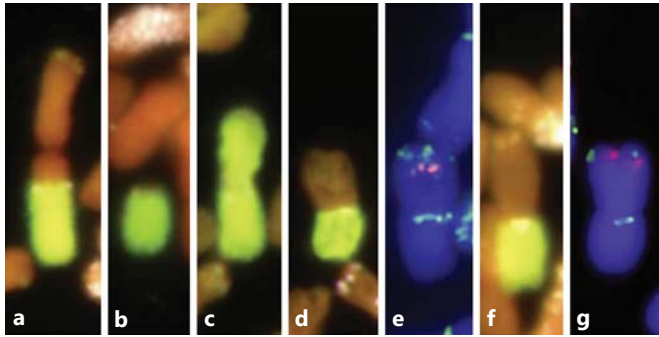


Fig. 5. Chromosome aberrations observed in progenies of plant 913-588. Panels **a-d** and **f** represent different plants. The structure of translocation chromosomes **d, e** and **f, g** was determined by FISH. In GISH images barley chromatin is labeled in green, (GAA)_n repeats are white, and chromosomes counterstained with propidium iodide are shown in red. In FISH images (**e, g**) (GAA)_n and pAs1 repeats are green, cDNA 7S-4 signals are red, and chromosomes counterstained with DAPI are visualized in blue.

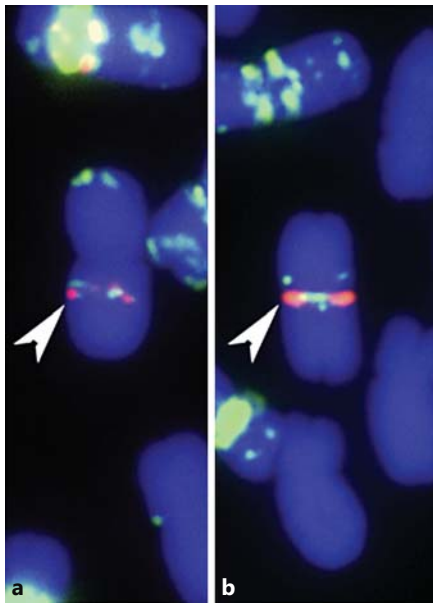


Fig. 6. a Translocation chromosome T7AS·7AL-7HL has barley centromere-specific repeats in inactive centromere (arrowhead). **b** Barley chromosome 7H. Barley centromere is shown by arrowhead. (GAA)_n repeat is labelled in green, barley centromere-specific microsatellite is red, and chromosomes counterstained with DAPI are shown in blue.

isochromosomes and dicentrics involving 7HL (3.3, 2.2 and 2.2%, respectively) (Fig. 4, Table 1). The percentage of dicentrics increased compared to the parent plant (2.2% vs. 0.3%), and a new type of dicentric T7AL·7HL·7HL was found (Fig. 4a). It seemed that all stable translocati-

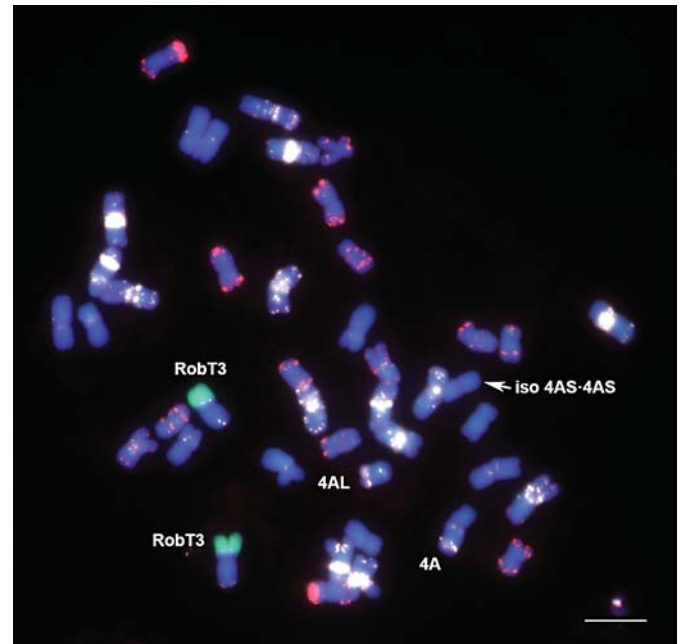
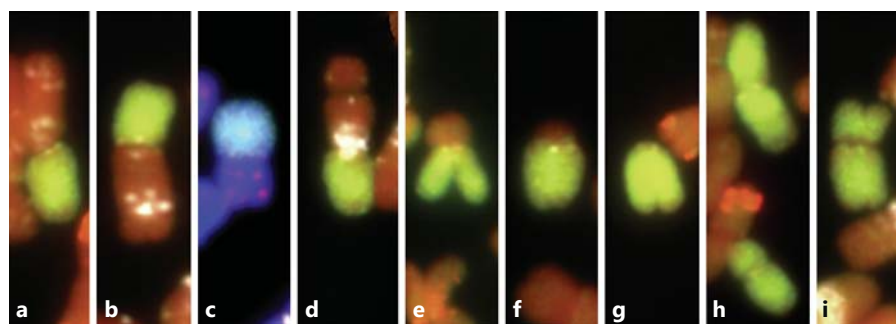


Fig. 7. F3 progeny of 913-588 (2n = 43) with stably transmitted T3 T7AS·7AL-7HL and 4A involved in rearrangements. Barley chromatin is labelled in green, (GAA)_n repeats are white, pAs1 repeat is red, and chromosomes counterstained with DAPI are shown in blue. Scale bar, 10 μ m.

tions involving 7HL in plant 913-12 observed at 37-41 days after germination were transmitted to the progenies. Some translocations were possibly new, considering the monosomy of 913-12, where 7D was present in one copy and some translocation chromosomes had a rearranged structure, which may prevent meiotic pairing.

In 39% of the progenies of plant 913-588 the translocation chromosome T7AS·7AL-7HL (T#3) containing complete wheat arm 7AS, a wheat centromere, the proximal part of 7AL, and the complete barley arm 7HL was present (Fig. 5a, 6). Compensating RobT#1 T7AS·7HL and T7DS·7HL (designated as RobT#4) were found in one plant each, and telocentric 7HL was observed in 2.1% of the plants (Table 1). The structure of 2 compensating RobTs was confirmed by FISH with the cDNA probe 7S-4 and repeats (GAA)_n and pAs1 (Fig. 5e, g). The translocation T#3 was stably transmitted through both male and female gametes, because it was present in a homozygous condition in 8.3% of the progenies. The long arm of the translocation T#3 lacks the primary constriction in the barley centromeric region, although it has a 7H centromeric band (GAA)_n and barley centromere-specific tandem repeat [Hudakova et al., 2001; Zeng and Jiang, 2016],

Fig. 8. Chromosome aberrations in progenies of plant 628–91. Each panel represents a different plant. **a–e** RobTs T2BS · 7HL, T7HL · 5BL, T7HL · 5DL, T6BS · 7HL; T?W · 7HL. **f, g** Acro- and telocentrics. **h, i** Rearranged 7HL isochromosomes. The structure of translocation chromosomes was determined by (GAA)_n or pAs1 pattern. Barley chromatin is labeled in green, (GAA)_n repeats are white. In GISH images chromosomes counterstained with propidium iodide are shown in red. In FISH image (**c**) pAs1 repeat is red, and chromosomes counterstained with DAPI are shown in blue.



but this repeat is smaller in size than in the intact chromosome 7H (Fig. 6). We used FISH to analyze 16 F₃ progenies of plant 913–588 that originated from 2 plants homozygous for the translocation T#3 (8 each), and found a T#3 pair present in all plants. Based on the transmission stability of T#3, the barley centromere in this chromosome is inactive. Fifteen F₃ plants had a chromosome number 2n = 42 or 41, if one chromosome 7D was absent. One F₃ plant had 2n = 43, because of a broken chromosome 4A (2n = 38 + 2T#3 + 4A + telo4AL + iso4AS) (Fig. 7). Most aberrations observed in plant 913–588, including the zebra chromatin, were not found in the progenies. Because plants with dicentrics were selected and planted as the screening proceeded, they were grown under different conditions. Plant 913–588 was selected later and grown under hot conditions unfavorable for wheat (Kansas late spring–summer greenhouse season), and a large portion of the earlier emerged tillers were sterile. Seeds were collected from a subset of fertile tillers that developed later in fall. It was shown earlier, that the stability of an alien chromosome may be affected by environmental conditions [reviewed in Khush, 1973].

In 78.4% of the progeny of plant 914–300, barley chromatin was not detected. In 9.9%, 7HL was involved in a compensating RobT#1 T7AS · 7HL, in a dicentric or acrocentric chromosomes with a shortened wheat arm (6.3, 1.8, and 1.8%, respectively). In 10.8% of the plants, 7HL was present as a telocentric or isochromosome or a combination of both (Table 1). In one progeny (plant 628–91), barley ring chromosomes, telocentrics, RobTs, acrocentrics and isochromosomes were observed in different cells (Fig. 3).

In 77.8% of the progeny of plant 628–91 with 7HL rings and other aberrations, barley chromatin was not detected. In 11.6% plants 7HL was present as a telosome or

isochromosome or combinations thereof and in 2.7% as an acrocentric chromosome. The RobTs found in 4 progenies involved wheat chromosomes other than 7A; they consisted of 7HL and either 2BS, 5BL, 5DL, or 6BS (Fig. 8a–d). A rearranged isochromosome consisting of a complete and a half shortened 7HL was present in 3.5% of the plants, and short 7HL telocentric or small isochromosome was found in one plant (Fig. 8h, i; Table 1). No ring chromosomes were observed.

Discussion

We observed chromosome rearrangements in wheat-barley double monosomic plants, which followed a univalent centric misdivision at meiotic anaphase I and II resulting from the absence of meiotic pairing between an alien chromosome and a wheat homoeologue [Friebe et al., 2005; Lukaszewski, 2010]. Barley chromosome 7H, when broken at the centromere, either stabilized as a telosome or fused with a broken wheat arm and formed a RobT, or fused with a distal end of an untargeted chromosome and formed a dicentric chromosome. The dicentric chromosome with this structure may be described as a primary dicentric entering BFB cycles. Dicentric chromosomes with a similar structure were observed in wheat lines that originated from double monosomics involving other species from the tertiary gene pool. Chromosomes of *Dasypyrum villosum* (L.) Candargy and *Thinopyrum intermedium* (Host) Barkworth and D.R. Dewey, broken at the centromere, formed dicentrics consisting of an alien arm attached to the distal end of an untargeted wheat chromosome [Liu C et al., 2011; Liu W et al., 2011, 2013]. Secondary rearranged dicentrics and multacentrics (Fig. 1b, c; Fig. 2e–i) were formed in somatic tissues un-

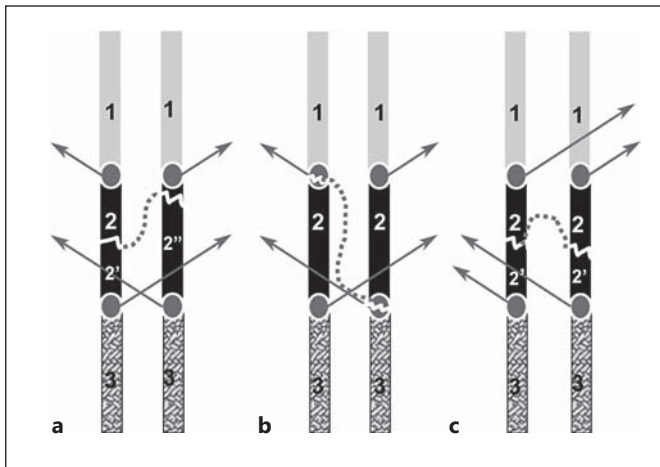


Fig. 9. Scenarios of spindle attachment and breakage-fusion bridge cycles of dicentric chromosomes. **a** Different arms of sister chromatids fuse, arm 2 has a deletion or duplication in resulted dicentrics 1-2'-3 or 1-2-2'-3. **b** Both sister chromatids broke and fused at different centromeres resulting in RobT 1-3 and multicentric chromosome 3-2-2'-1. **c** Sister halves of broken chromosomes fused, arm 3 and part of arm 2 duplicated in isodicentric 3-2'-2'-3. Dotted lines show fusion.

dergoing BFB cycles. The breakage and shortening of barley chromatin (Fig. 1i-k, m; Fig. 8h, i) and the formation of zebra-like segments with alternating wheat and barley chromatin (Fig. 2e-i) indicates that at some point of the BFB cycle, a broken centromere of a detached segment fused with the distal end of 7HL (for example, Fig. 4a). The detection of RobTs consisting of 7HL and non-targeted chromosome arms 2BS, 5BL, 5DL, and 6BS (Fig. 8a-d) indicates that a distal end of any chromosome can be involved in fusion, dicentric and BFB cycles.

It was shown earlier [McClintock 1939; Lukaszewski, 1995; Friebe et al. 2000; Kynast et al., 2000] that newly broken chromosome ends tend to fuse. Kynast et al. [2000] used FISH to analyze wheat multicentric and ring chromosomes that arose from chromosome breakage, induced by a gametocidal factor. They detected telomeric repeats at 2 terminal sites in multicentrics but not at interstitial sites. No telomeric repeats were detected on ring chromosomes. In cancer cells, the fusion of degraded telomeres was described as the most common mechanism initiating primary dicentric chromosomes. Reduction in telomere-specific repeats resulted in sticky ends and caused the fusion. As residual telomeric and subtelomeric sequences retained at the site of telomere fusion, the distal part of chromosomes involved in the dicentrics were not lost [reviewed by MacKinnon and Campbell,

2011]. In our experiment, it was not clear whether a chromosome broken at centromeric area fused with an intact telomere of a random chromosome and formed a dicentric, or if it fused with a broken or degraded telomeric end and what may cause telomere damage.

Considering the different scenarios of spindle attachment and bridge formation and alternations of fusion modes of broken chromosome ends during BFB cycles, a variety of rearrangements, including secondary dicentrics and multicentrics, can arise from a primary dicentric, resulting in mosaic tissue with different permutations in different cells [Friebe et al., 2000]. Dicentrics with a rearranged or shortened wheat arm (Fig. 1c, d; Fig. 2b, d) and multicentrics can arise after chromosome type breakage, when sister chromatids broken at different sites and different arms of sister chromatids fuse (Fig. 9a, b). Changes in dosage of barley chromatin resulted from formation of isodicentrics (Fig. 1g, h; 4e), and 7HL isochromosomes (Fig. 3b; 5c) arose after chromosome breakage followed by fusion of the sister halves of a broken chromosome (Fig. 9c). Sears [1952] observed the instability of wheat telocentrics in somatic tissues, where they were either lost or gave rise to an isochromosome. Active BFB cycles can induce breakage in other chromosomes and result in additional rearrangements, as was shown in maize [McClintock, 1951] and wheat [Lukaszewski, 1995]. Dicentric, isodicentric, multicentric, and ring chromosomes involved in BFB cycles were observed in cancer tissues. In cancer cells, the BFB cycle is recognized as one of the mechanisms that causes genome instability producing aberrations, such as deletions, inversions, segment amplifications, interchromosomal translocations, and aneuploidy [Gisselsson et al., 2000; MacKinnon and Campbell 2011].

We observed that BFB cycles ceased in roots at 8 weeks after germination, which is in agreement with other observations [Friebe et al., 2000, 2001; Kynast et al., 2000]. The healing of the broken chromosome ends in wheat occurs by either the gradual addition of telomeric repeats at the broken termini or fusion with other broken ends [Friebe et al., 2001]. The most common mechanism of stabilization of dicentric chromosomes observed in cancer cells is centromere deletion (loss of centromere-specific α -satellite DNA). Less frequent functional centromere inactivation, when α -satellite DNA was still present but the centromeric constriction was absent, was observed in dicentric derivatives with a greatly reduced intercentromeric distance. The splitting of a dicentric chromosome into monocentrics and stabilization of broken chromosome ends by the addition of new telomeres has

also been described [reviewed in MacKinnon and Campbell, 2011]. We found one translocation chromosome T#3 T7AS·7AL·7HL with a barley centromere inactivated (Fig. 5a; 6), possibly because of reduced intercentromeric distance or a reduced amount of barley centromeric repeats. We mostly observed such types of stabilization of dicentric chromosomes as the healing of broken chromosome ends (telocentrics) and fusion of broken ends (translocation chromosomes involving complete or shortened wheat and barley arms).

Aberrations involving barley chromosome 7H and several wheat chromosomes were observed in plants with dicentric chromosomes originating from double monosomics and included inter- and intrachromosomal translocations of whole arms or smaller segments, duplications and deletions. Many of these aberrations were transmitted to the progenies (Fig. 4, 5, 8). We observed a very low ratio of progenies with dicentric chromosomes in F₁ of dM involving barley chromosome 7H. Similar ratios were reported for wheat dMs, involving chromosomes of *D. villosum* and *Th. intermedium* [Liu C et al., 2011; Liu W et al., 2011, 2013]. But we observed the increased ratio in F₂ originating from plants with dicentrics. Unlike our model dMs, in an interspecific hybrid more than one chromosome can form univalents and breaks during meiosis, resulting in higher ratio of dicentrics and rings. Thus, our observations may reflect a beginning of a karyotype reshaping process in homoploid interspecific hybrids with limited chromosome pairing. A well-studied example of homoploid hybrid speciation are annual sunflower species [Ungerer et al., 1998; Lai et al., 2005; Rieseberg et al., 1995; Rieseberg, 2006]. As was established by comparative genetic mapping and cytogenetic analysis, the chromosome structure is not completely preserved within the genus; multiple translocations and inversions were found [Chandler et al., 1986; Rieseberg et al., 1995; Burke et al., 2004; Lai et al., 2005]. In interspecific sunflower hybrids most chromosomes can pair, but meiotic abnormalities were observed [Chandler et al. 1986]. Thus, some chromosome aberrations present in hybrid sunflower species may arise from meiotic crossover between homologous regions of translocations or inversions of rearranged parental chromosomes [Rieseberg, 2001]. However, chromosomes colinear in the parental species were also found rearranged in hybrids, hence, other mechanisms besides recombination were involved, possibly univalent centric misdivision followed by BFB cycles. Most grass species from the tribe Triticeae are known for a conserved chromosome macrostructure [Devos and Gale, 2000]. But colinear homoeologues do not pair dur-

ing meiosis in hybrids of evolutionary diverse Triticeae, which conforms the existence of numerous allopolyploids [Tsunewaki, 2009; Feldman and Levy, 2012]. Thus, chromosome alterations, described in diploid grasses, known for homoploid hybrid origin and rearranged genomes, such as rye *Secale cereale* L. [Martis et al., 2013] and *Aegilops markgrafii* (Greuter) Hammer [Danilova et al., 2017], may arise through BFB cycles.

Introgressing new traits into wheat from wild and cultivated relatives is possible by interspecific hybridization followed by directed chromosome engineering. Robertsonian translocations can be produced by the centric breakage-fusion of univalents in double monosomic plants, and the alien chromosome arms can be shortened by induced homoeologous recombination [Sears, 1952; reviewed in Friebe et al., 1996; Qi et al., 2007; Zhang et al., 2015]. Among the progenies that originated from double monosomic plants, we observed chromosome aberrations involving untargeted wheat chromosomes. In addition, the structure and dosage of the introgressed barley chromatin was changed in somatic cells of plants with BFB cycles and in their progenies. Although in some plants the barley chromatin was lost, in others its dosage was duplicated, triplicated, or quadruplicated through the formation of RobTs, telosomes, isochromosomes and their combinations. The observed aberrations emphasize the importance of cytogenetic screening in gene introgression projects.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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