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Robertsonian translocations in wheat arise by centric misdivision of univalents at anaphase I and rejoining of broken centromeres during interkinesis of meiosis II

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Abstract. The mechanism of origin of Robertsonian translocations was investigated in plants monosomic for chromosome 1A of wheat and $1H^t$ of *Elymus trachycaulus* by GISH. Chromosomes 1A and $1H^t$ stayed univalent in all metaphase I cells analyzed, suggesting that Robertsonian translocations do not originate from meiotic recombination in centromeric regions with shared DNA sequence homology. At ana-/telophase I, the $1H^t$ and 1A univalents underwent either chromosome or chromatid segregation and misdivided in 6–7% of the pollen mother cells. None of the ana-/telophases I analyzed had Robertsonian translocations, which were only observed in 2% of the "half tetrads" at ana-/telophase II. The frequency of Robertsonian translocations observed at ana-/telophase II corresponds well with the number of Robertsonian translocations (1–4%) detected in progenies derived from plants monosomic for group-1 chromosomes of wheat (1A, 1B, and 1D) and $1H^t$ of *E. trachycaulus.* Our data suggest that Robertsonian translocations arise from centric misdivision of univalents at ana-/telophase I, followed by segregation of the derived telocentric chromosomes to the same nucleus, and fusion of the broken ends during the ensuing interkinesis.

Darlington (1939) reported that chromosome laggards at meiosis have a tendency to misdivide at the centromere in a transverse instead of lengthwise manner, which gives rise to telocentric and isochromosomes. Centric misdivision followed by the fusion of the broken arms from different chromosomes results in whole-arm Robertsonian translocations (Robertson, 1916). Analysis of the B centromere structure in maize (Kaszás and Birchler, 1996) and in wheat-rye Robertsonian translocations derived from consecutive misdivision events (Zhang et al., 2001) indicated that centric breakage-fusion can occur at different positions within the primary constriction without affecting centromere function. These studies showed that centromeres consist of functional subunits that can be divided and reshuffled even between chromosomes of species that belong to different genera without losing their function. Robertsonian translocations also can arise from recombination in the short arms of two acrocentric chromosomes followed by the loss of the acentric fragment (Holmquist and Dancis, 1979; Schubert and Rieger, 1985; Slijepcevic, 1998). The resulting metacentric chromosomes are actually dicentric and usually one of the centromeres is inactivated to ensure mitotic stability. Complete fission-fusion cycles have been reported for faba beans (*Vicia faba* L.) (Schubert et al., 1995) and for wheat-rye translocations (Lukaszewski, 1993, 1994, 1997). Although several steps are required to produce Robertsonian translocations (Badaeva et al., 1995), they are widespread in plants (Jones, 1978), animals (White, 1973), and humans (Page et al., 1996; Sullivan et al.,

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1996) and have played a significant role in karyotype evolution.

Because of the highly buffered polyploid nature of bread wheat *Triticum aestivum* L., 2n = 6x = 42, AABBDD), wheat tolerates aneuploidy to a much larger extent than diploid species. The use of aneuploid stocks allows creating wheat-wheat and wheat-alien Robertsonian translocations in a directed manner by making the appropriate wheat or alien chromosomes in monosomic condition. In such double-monosomic plants, Robertsonian translocations are known to occur at fairly high frequencies, ranging from 4% to almost 20% depending on the chromosomes involved (Lukaszewski and Gustafson, 1983; Davies et al., 1985; Lukaszewski, 1993, 1994, 1997; Marais and Marais, 1994).

Some repetitive DNA sequences at plant centromeres are highly conserved and consist of retrotransposon-like elements (Argon-Alcaide et al., 1996; Jiang et al., 1996; Miller et al., 1998; Presting et al., 1998; Francki, 2001). Sequence homologies between non-homologous centromeres within a species or even between centromeres of related species may provide the structural basis for meiotic chromosome pairing and recombination that also can result in the formation of Robertsonian translocations. This mechanism has been suggested as the most common process for the formation of Robertsonian translocations in humans (Bandyopadhyay et al., 2002). To test this hypothesis and further elucidate the process by which Robertsonian translocations are formed, we produced plants that were double-monosomic for chromosomes of group-1 (1A, 1B, or 1D) of wheat and $1H^t$ of *Elymus trachycaulus* (Link) Gould ex Shinners (2n = 4x = 28, $S^tS^tH^tH^t$), a related perennial grass species. *E. trachycaulus* chromatin can be easily detected in a wheat background by genomic in situ hybridization (GISH). Here we report the meiotic behavior of the double monosomes as univalents and provide evidence that Robertsonian translocations are formed as the result of centromere misdivision of univalents at ana-/telophase I, followed by the fusion of the broken arms in the ensuing interkinesis preceding meiosis II.

Materials and methods

Disomic chromosome addition plants (DA1H^t, 2n = 44) that had chromosome $1H^t$ derived from *E. trachycaulus* (Morris et al., 1990; Jiang et al., 1994) added to the chromosome complement of common wheat were crossed with the Wichita monosomic stocks for chromosomes 1A, 1B, and 1D. F_1 plants with 2n = 42 chromosomes that were double-monosomic for chromosomes 1A/$1H^t$, 1B/$1H^t$, and 1D/$1H^t$ were identified. The chromosomal constitution of the derived progenies was determined by N-banding analysis according to Gill et al. (1991).

Meiosis of double-monosomic 1A/$1H^t$ plants was analyzed in pollen mother cells (PMCs) after GISH. GISH was done according to Zhang et al. (2001) using FITC-labeled, total genomic *E. trachycaulus* DNA as a probe and unlabeled total genomic wheat DNA as a blocker. The probe to blocker ratio was 1:40, the hybridization stringency was between 77 and 79%, and post hybridization washes were at 80–82%. Slides were counterstained with propidium iodide (Molecular Probes, Eugene, OR, USA) mounted in Vectashield (Vector Laboratories, Burlingame, CA, USA), and analyzed with an epifluorescence Zeiss Axioplan 2 microscope. Images were captured with a SPOT CCD (charge-coupled device) camera operated with Spot 2.1 software (Diagnostic Instruments, Inc., Sterling Heights, MI, USA) and processed with Photoshop v5.5 (Adobe Systems, San Jose, CA, USA) software. Images were printed on a Kodak ds8650 PS Color Printer.

Results

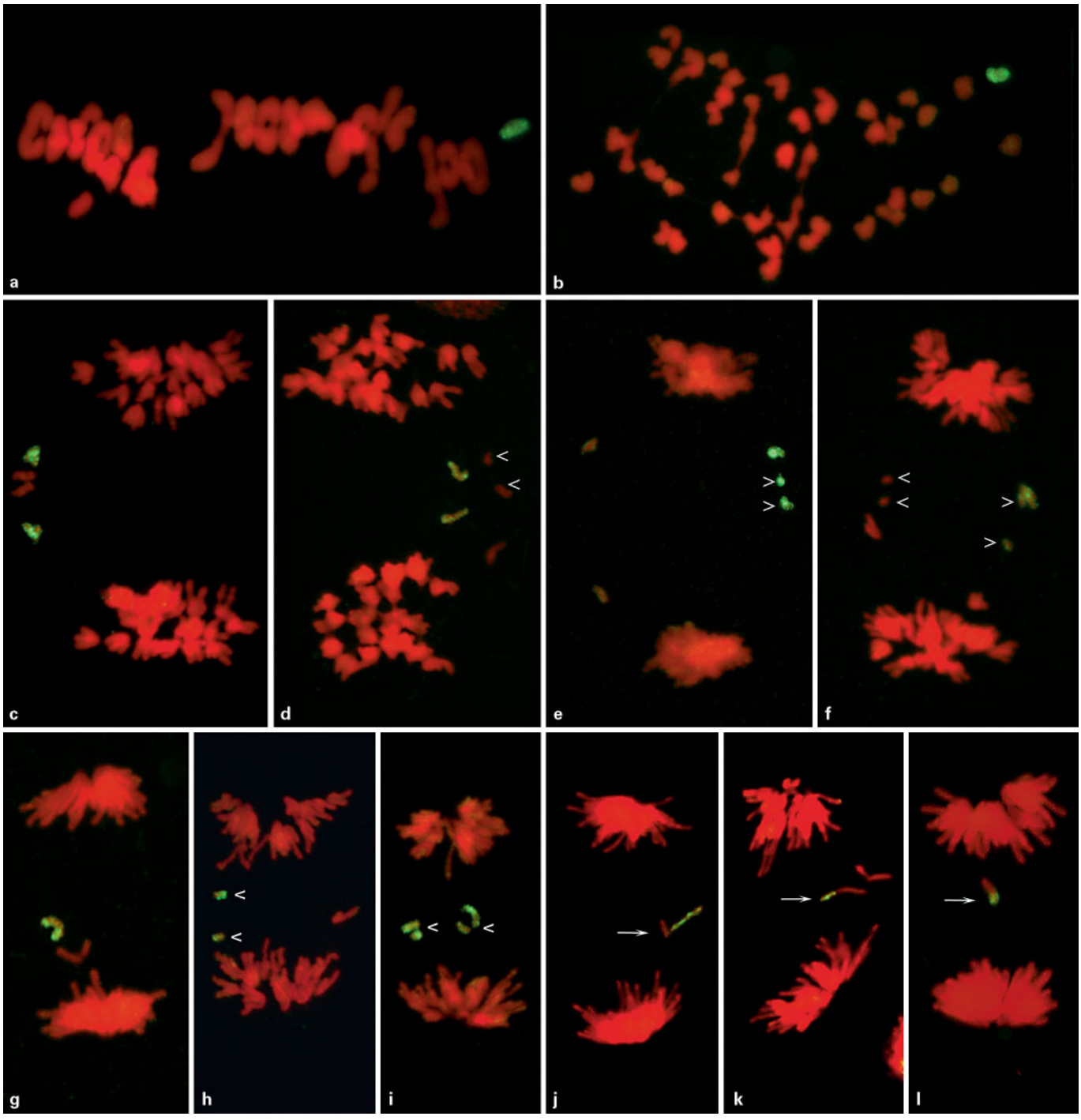
GISH using total genomic *E. trachycaulus* DNA as a probe painted chromosome $1H^t$ over its entire length, which allowed studying its behavior at the first and second meiotic division. A total of 307 PMCs at metaphase I were analyzed and chromosomes $1H^t$ and 1A stayed as univalents in all cells (Fig. 1a). The ana-/telophase I segregation data of chromosomes $1H^t$ and 1A are summarized in Fig. 2. Chromosome $1H^t$ underwent chromosome segregation in 147 PMCs (43%) (Fig. 1b) and chromatid segregation in 194 PMCs (57%) (Fig. 1c–f), with misdivision in 19 PMCs (6%) (Fig. 1e, f). The corresponding values for chromosome 1A are chromosome segregation in 138 PMCs (40%) and chromatid segregation in 203 PMCs (60%) with misdivision in 25 PMCs (7%). Misdivision of both chromosomes $1H^t$ and 1A was observed in four PMCs (1%) (Fig. 1f).

Squash preparation, of PMCs at ana-/telophase II resulted in a breakdown of the outer cell wall and, thus, only "half tetrads" stayed together and could be analyzed (Fig. 1g–l). A total of 166 "half tetrads" were analyzed. Chromosomes $1H^t$ and 1A often lagged behind the rest of the chromosome complement and were at equatorial plate when the rest of the chromosomes were already at the spindle poles. Wheat-*E. trachycaulus* Robertsonian translocations were observed in three "half tetrads" (2%). The arm ratios and GISH patterns of the translocation chromosomes identified them as T1AS·$1H^t$L (Fig. 1j), T1H^tS·1AL (Fig. 1k), and T1AS·$1H^t$S (Fig. 1l), respectively.

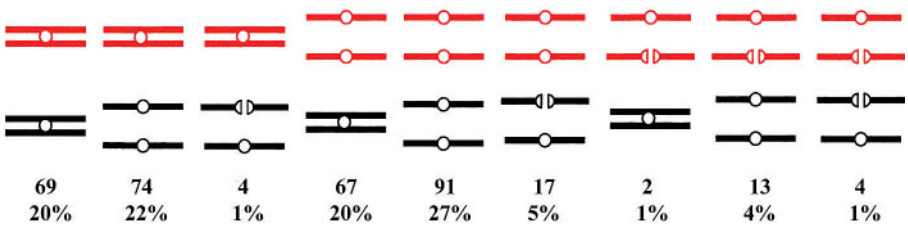
Chromosome constitutions of progenies derived from double-monosomic 1A/$1H^t$, 1B/$1H^t$, and 1D/$1H^t$ plants were determined by N-banding analysis. Chromosome $1H^t$ has a prominent proximal N-band in the short arm and prominent proximal and distal N-bands in the long arm (Fig. 3). In progenies derived from double-monosomic 1A/$1H^t$ plants, misdivision products were observed in 10% of the plants including telocentrics (8%), isochromosomes (1%), and Robertsonian translocations (1%) (Table 1, Fig. 3). The total number of misdivision products observed in progenies derived from double-monosomic 1B/$1H^t$ plants was 19% and consisted of 15% telocentrics and 4% Robertsonian translocations. In 1D/$1H^t$-derived progenies 16% misdivision products were observed consisting

Fig. 1. Meiotic segregation patterns of *E. trachycaulus* chromosome $1H^t$ and 1A of wheat in double-monosomic 1A/$1H^t$ plants after GISH using total genomic *E. trachycaulus* DNA as a probe. *E. trachycaulus* chromatin is detected by green FITC fluorescence and wheat chromosomes are counterstained with PI and fluoresce red. (**a**) Metaphase I with $1H^t$ and 1A staying univalent; (**b–f**) ana-/telophases I showing $1H^t$ and 1A undergoing chromosome segregation (**b**) and chromatid segregation (**c–f**) with misdivision of 1A (**d**), misdivision of $1H^t$ (**e**), and misdivision of both $1H^t$ and 1A (**f**). (**g–l**) "Half tetrads" at ana-/telophase II with misdivision of $1H^t$ (**h**, **i**) and Robertsonian translocations (**j–l**). (**j**) T1AS·$1H^t$L. (**k**) T1H^tS·1AL. (**l**) T1AS·$1H^t$S. Arrowheads indicate the misdivision products and arrows point to the Robertsonian translocations.

Fig. 2. Ana-/telophase I segregation of *E. trachycaulus* chromosome $1H^t$ (red) and 1A of wheat (black) in double-monosomic 1A/$1H^t$ plants.



1



2

Table 1. Chromosome constitutions observed in progenies of double-monosomic $1H^t/1A$, $1H^t/1B$, and $1H^t/1D$ plants

Chromosome constitution	No. of plants	1× $1H^t$	2× $1H^t$	telo $1H^tS$	telo $1H^tL$	iso $1H^tS$	Wheat	Robertsonian translocation
$1H^t/1A$	86	41 (48%)	1 (1%)	3 (3%)	4 (5%)	1 (1%)	35 (41%)	1 (1%)
$1H^t/1B$	45	5 (11%)	–	5 (11%)	2 (4%)	–	31 (69%)	2 (4%)
$1H^t/1D$	80	25 (31%)	4 (5%)	1 (1%)	5 (6%)	5 (6%)	38 (48%)	2 (3%)

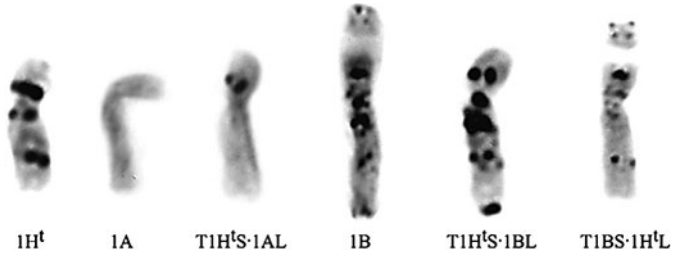


Fig. 3. N-banding patterns of *E. trachycaulis* chromosomes $1H^t$, 1A, 1B of wheat and derived Robertsonian translocation chromosomes recovered in the offspring of double-monosomic $1H^t/1A$ plants.

of 7% telocentrics, 6% isochromosomes, and 3% Robertsonian translocations. Robertsonian translocations involving chromosomes $1H^t$ and either 1A, 1B, or 1D of wheat were observed in 2.7% of the plants analyzed (Table 1, Fig. 3).

Discussion

GISH analysis in double-monosomic $1A/1H^t$ plants showed that chromosomes 1A and $1H^t$ stayed univalent in all the 307 PMCs analyzed. Although the centromeres of non-homologous chromosomes in grasses share a high degree of sequence homology, the present study shows that these regions do not form chiasmate associations or if they occasionally do, they do not persist until meiotic metaphase I. However, if crossing over had occurred at the centromeres between the univalents during early prophase, we should have observed Robertsonian translocations by metaphase I, but none were observed. Thus, crossing over in shared DNA sequences that are present in non-homologous centromeres can be ruled out as a mechanism by which Robertsonian translocations are formed in wheat.

Chromosome segregation at ana-/telophase I for chromosomes $1H^t$ and 1A, was 43 and 40%, respectively, and chromatid segregation was observed in 57% of the PMCs for $1H^t$ and 60% of the PMCs for 1A. Sanchez-Monge and Mac Key (1948) and Sears (1952) analyzed the meiotic segregation of wheat chromosome 5A in monosomic condition and observed chromatid segregation in more than 95% of the PMCs at ana-/telophase I analyzed. We observed a lower frequency of chromatid segregation, most likely because we also included early anaphases I where both sister chromatids were still attached to the same centromere, although they may separate and segregate to opposite spindle poles at later stages.

Misdivision of $1H^t$ and 1A was observed in 6 and 7% of the PMCs, respectively, and misdivision of both chromosomes 1A and $1H^t$ occurred in 1% of the PMCs at ana-/telophase I. Misdivision frequencies in wheat varied depending on the chromosome and genetic background ranging from 3 to about 51% (Sears, 1952, 1973; Steinitz-Sears, 1966; Morris et al., 1969; Vega and Feldman, 1998).

The frequency of Robertsonian translocations observed in progenies derived from double-monosomic $1H^t/1A$ plants (1%) is similar to the frequency of centric fusions observed in PMCs at ana-/telophase II (4 per "half tetrad" corresponding to 2% per gametophyte). The frequency of induced Robertsonian translocations observed in this study is relatively low compared to those reported previously in wheat. Depending on the chromosomes involved, the frequency of centric fusions in wheat can range from 4 to about 20% and also is affected by environmental conditions (Davies et al., 1985; Lukaszewski, 1993, 1994, 1997; Marais and Marais, 1994).

All Robertsonian translocations detected at ana-/telophase II were still at the equatorial plate, when the rest of the chromosome complement already was at the spindle poles, suggesting that the centric fusion products lacked a functional centromere. However, this is highly unlikely at least for the $T1AS{\cdot}1H^tL$ translocation chromosome shown in Fig. 1j because its V-shaped appearance indicated the attachment of microtubules and initiated movement to one spindle pole.

We did not observe centric fusion of the misdivision products in any of the 341 PMCs analyzed at ana-/telophase I. We only observed Robertsonian translocations at ana-/telophase II indicating that centric fusion occurred during interkinesis. The data further suggest that the broken chromosome ends were not fully stabilized by telomeric repeats although telomerase activity is known to be present in anthers (Heller et al., 1996). Friebe et al. (2001) have shown that chromosome healing by the de novo addition of telomeric repeats is a gradual process and that cells have to pass through several cell divisions to acquire the complete repeat length. Thus, it is possible that the newly broken chromosome ends have not acquired a fully functional telomere, which makes them vulnerable to fusion with other broken ends present in the same nucleus. In animals and yeast, broken chromosome ends are censored by checkpoint proteins, which leads to cell arrest (Chin et al., 1999). A similar meiotic checkpoint was not detected in *Arabidopsis* (Couteau et al., 1999) suggesting that plants in general may lack a meiotic double-strand break checkpoint (Preuss and Britt, 2003). Based on the results, we hypothesize that Robertsonian translocations arise as a result of the following sequence of events: a) the participating chromosomes must be in the univalent condition,

which triggers centromere breaks, b) the broken chromosomes must be included in the same nucleus where repair/reunion of broken chromosomes occurs at the centromeres during interkinesis, and c) the process may be repeated in meiosis II.

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