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Further manipulation by centric misdivision of the 1RS.1BL translocation in wheat

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Summary

Complete chromosomes 1R and 1B were reconstructed in wheat from the centric wheat-rye translocation 1RS.1BL. Three substitutions: 1R(1A), 1R(1B), 1R(1D), and three new centric translocations: 1RS.1AL, 1RS.1BL, 1RS.1DL were produced from the reconstructed chromosome 1R. Each one of these has the same rye chromosome arm 1RS which was present in the original translocation 1RS.1BL of ’Kavkaz’ wheat. Reconstructed chromosome 1B and a normal chromosome 1R were used to produce a new 1RS.1BL translocation. This translocation has the long arm from the original 1RS.1BL translocation of ‘Kavkaz’, but a different 1RS arm. The third generation centric translocations were mitotically stable and were normally transmitted to progeny. Misdivision frequency of the reconstructed chromosomes 1R did not change relative to normal 1R, whereas the misdivision frequency of the two reconstructed chromosomes 1B tested was significantly higher relative to normal 1B. These experiments demonstrate that repeated cycles of centric breakage and fusion do not impair the function of centromeres in wheat and rye but may change chromosome’s susceptibility to misdivision.

Introduction

Translocations of the short arm of rye (*Secale cereale* L.) chromosome 1R to the long arm of wheat (*Triticum aestivum* L.) chromosome 1B appeared independently in several breeding programs in Europe (Zeller & Hsam, 1984). Of those, the translocation 1RS.1BL from wheat cultivars ‘Aurora’ and ‘Kavkaz’ has spread to wheat breeding programs and commercial wheat cultivars throughout the world (Lukaszewski, 1990 and references therein). Originally, the popularity of this 1RS.1BL translocation in wheat breeding might have been due to the presence on the 1RS arm of several genes for resistance to pathogens and pests of wheat (Zeller & Hsam, 1984). However, as the resistance started breaking down it has become clear that the translocation also has a positive effect on yield in at least some genetic backgrounds (Rajaram et al. 1983; Carver & Rayburn, 1994; Moreno-Sevilla et al. 1995; McKendry et al. 1996). Unfortunately, the presence of the translocation is associated with breadmaking quality defects (Zeller et al. 1982; Burnett et al. 1995).

In a previous study (Lukaszewski, 1993), complete chromosomes 1R and 1B have been reconstructed from the 1RS.1BL translocation. This was accomplished by centric breakage and fusion in plants double monosomic for complementary translocations 1RS.1BL and 1BS.1RL. Reconstruction of the complete chromosomes 1B and 1R from two centric translocations demonstrated that two cycles of centric fission and fusion resulted in stable chromosomes capable of normal behavior in cell divisions. The experiments presented in this note were undertaken to test whether further centric breakage and fusion events were possible and whether they would affect chromosome’s behavior, and to extend the range of genetic stocks for studies of the yield-increasing factors in the 1RS.1BL translocation.

Materials and methods

All experiments were performed in hexaploid wheat ‘Pavon’. All cytogenetic stocks of ‘Pavon’ used were essentially the same as described earlier (Lukaszewski, 1993). To keep track of the origin of chromosome arms in successive generations of centric translocations, the arms were designated by subscripts: p for ‘Pavon’, v for ‘Veery’ (= ‘Kavkaz’) and e for line E12165. Line E12165 is a hexaploid wheat with a 1R(1D) substitution, selected at the International Maize and Wheat Improvement Center (CIMMYT) from a cross involving triticale ‘Panda’ and a wheat line cmH72A.429 (R. Rodriguez, pers. comm.). It would appear that chromosome 1R in E12165 is from ‘Panda’ but line cmH72A.429 has never been karyotyped.

The reconstructed chromosome 1R, in effect a Robertsonian translocation, will be referred to as $1RS_v.1RL_e$ while the reconstructed chromosome 1B as $1BS_p.1BL_v$. Of the three chromosomes $1BS_p.1BL_v$ identified, two were recovered in backcrosses to Pavon and were denoted -1 and -2. Both chromosomes $1RS_v.1RL_e$ were recovered in backcrosses and these are also denoted -1 and -2.

Cytological analyses of mitotic chromosomes were based on C-banding, as described previously (Lukaszewski, 1993). Plants with the desired chromosomes were identified, grown in the greenhouse and either backcrossed or self-pollinated. All heads from which seed was taken for the next generation were bagged to avoid any possibility of cross-pollination.

Results

The identity of the chromosome arms in the reconstructed chromosomes $1RS_v.1RL_e$ and $1BS_p.1BL_v$ was verified by Acid-PAGE (polyacrylamide gel electrophoresis) and SDS-PAGE of the endosperm storage proteins (data not shown) which confirmed that all stocks of ‘Pavon’ with chromosomes $1RS_v.1RL_e$ had the same allele of the rye storage protein gene *Sec-1* as the original translocation 1RS.1BL, and that this allele differed from the one present in chromosome 1R of line E12165. All stocks with the chromosomes $1RS_v.1RL_e$ also had the same allele of the rye storage protein gene *Sec-3* (located on 1RL) as the chromosome 1R from wheat line E12165.

Following the third backcross of the reconstructed chromosome $1RS_v.1RL_e$-1 to ‘Pavon’, double monosomics 20" + 1B’ + $1RS_v.1RL_e$-1’ were selected, grown, and allowed to self-pollinate. A sample of 103 progeny were screened by C-banding and two substitutions $1RS_v.1RL_e$-1(1B) and three centric translocations $1RS_v.1BL_p$ were recovered. The new centric translocations $1RS_v.1BL_p$ differ from the original translocation 1RS.1BL of ‘Kavkaz’ by the 1BL arm.

The reconstructed chromosome $1RS_v.1RL_e$ was moved by monosomic shift from its original position in substitution for chromosome 1B to substitutions for chromosomes 1A and 1D. To accomplish this, double monosomics 20" + $1RS_v.1RL_e$-2’ + 1B’ were crossed as male to ’Pavon’ monosomics 1A and 1D, triple monosomics 19" + 1A’ + 1B’ + $1RS_v.1RL_e$-2’ and 19" + $1RS_v$. $1RL_e$-2’ + 1B’ + 1D’ were selected, grown, and backcrossed as male to ’Pavon’. A triple monosomic 19" + 1A’ + 1B’ + 1R’ is expected to produce three types of 21-chromosome pollen: 19 + 1A + 1B, 19 + 1A + 1R, and 19 + 1B + 1R, with frequencies dependent on the relative compensating ability of chromosomes 1A, 1B and 1R.

Among 20 karyotyped progeny from the cross ‘Pavon’ x 19" + 1A’ + 1B’ + $1RS_v.1RL_e$-2’, two were double monosomics 20" + 1A’ + $1RS_v.1RL_e$-2’, and among 20 karyotyped progeny from the cross ’Pavon’ x 19" + 1B’ + 1D’ + $1RS_v.1RL_e$-2’, three were double monosomics 20" + 1D’ + $1RS_v.1RL_e$-2’. These double monosomics were grown and allowed to self-pollinate. Among 382 progeny from the double monosomic 20" + 1A’ + $1RS_v.1RL_e$-2’ screened, 13 substitutions $1RS_v.1RL_e$-2(1A) and two translocations $1RS_v.1AL_p$ were identified. Among 212 progeny of the double monosomics 20" + 1D’ + $1RS_v.1RL_e$-2’ screened two substitutions $1RS_v.1RL_e$-2(1D) and three translocations $1RS_v.1DL_p$ were isolated. These new centric translocations $1RS_v.1AL_p$ and $1RS_v.1DL_p$ have the same 1RS arm as that originally present in the 1RS.1BL translocation of ‘Kavkaz’; the 1AL and 1DL arms are of ‘Pavon’ wheat.

Plants of ‘Pavon’ with the reconstructed chromosomes $1BS_p.1BL_v$-1 and -2, were backcrossed as male to ‘Pavon’ monosomic 1B. Following the third backcross, plants monosomic for the $1BS_p.1BL_v$-1 chromosome were selected, grown and self-pollinated. Among the progeny, disomics 20" + $1BS_p.1BL_v$-1" (= translocation homozygotes) were selected. These disomics are, in effect, single chromosome arm substitutions where the long arm of chromosome 1B of ‘Pavon’ has been replaced by the long arm from the 1RS.1BL ‘Kavkaz’ translocation as it was present in ‘Veery’ (Figure 1).

To complete the set of all possible centric translocations involving chromosome arms 1RS and 1BL of the original translocation 1RS.1BL of ‘Kavkaz’ with chromosomes of ‘Pavon’, Pavon $1BS_p.1BL_v$-1 line was

Figure 1. Reconstruction of complete chromosomes 1R and 1B from the 1RS.1BL translocation and construction of new centric translocations from the reconstructed chromosomes. From left to right, upper row: 1RS.1BL and 1BS.1RL; middle row: reconstructed 1R (= $1RS_v.1RL_e$) and 1B (=$1BS_p.1BL_v$), bottom row: new centric translocations $1RS_v.1AL_p$, $1RS_v.1BL_p$, $1RS_v.1DL_p$, $1RS_e.1BL_v$.

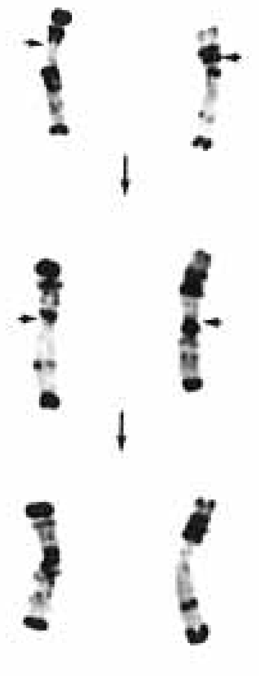

Figure 2. Exchange of chromosome arms by centric breakage and fusion between chromosomes $1R_i$ and $1B_i$. From left to right, upper row: translocations 1RS.1BL and 1BS.1RL, after non-centric break at short arrows chromosomes $1R_i$ and $1B_i$ (middle row) are produced. Following centric breakage and fusion (short arrows) with exchange of arms, chromosome 1B with a distal rye segment on the short arm and chromosome 1R with a distal segment of 1BS on the short arm are obtained (bottom row).

crossed to a ‘Pavon’ $1R_e(1B)$ substitution line. Among 116 progeny of double monosomics 20" + $1BS_p.1BL_v$-1’ + $1R_e$’ screened, three translocations $1RS_e.1BL_v$ were identified. These translocations differ from the original translocation 1RS.1BL of ’Kavkaz’ by the 1RS arm.

In the process of reconstruction of complete chromosomes 1R and 1B (Lukaszewski 1993), two chromosomes of ambiguous origin were recovered: chromosome 1R with a proximal segment of 1BS in the short arm (designated $1R_i$), and chromosome 1B with a proximal segment of 1RS in the short arm (designated $1B_i$). Originally, it was speculated that these two chromosomes might have originated by homoeologous recombination between chromosome arms 1RS and 1BS.

Plants with the chromosomes $1R_i$ and $1B_i$ were crossed and backcrossed three times to ‘Pavon’ monosomics 1B. Following self pollination of the BC_3 progeny, disomics 20" + $1B_i(1B)$ and 20" + $1R_i(1B)$ were selected and intercrossed. Among 84 karyotyped progeny of a double monosomic 20" + $1R_i$’ + $1B_i$’, six chromosomes were identified which must have resulted from centric breakage and fusion (Figure 2). Four of these were chromosomes 1B with a distal segment of the short arm replaced by a segment of 1RS. These are designated $1RS_v.1BS_p.1BL_p$. The remaining two were chromosomes 1R with distal segments of the short arm replaced by segments of 1BS. These are designated $1BS_p.1RS_v.1RL_e$.

Considerable differences in the misdivision frequencies of the individual chromosomes used in this study were observed (Table 1). Of the three group-1 chromosomes of ‘Pavon’, 1A and 1D had low and 1B high misdivision frequency. The original chromosome $1R_e$ misdivided twice as often as $1B_p$. On the other hand, reconstructed chromosomes $1BS_p.1BL_v$ misdivided with a significantly ($p < 0.05$) higher frequency than the original chromosome 1B of ‘Pavon’, whereas the misdivision frequency of the reconstructed chromosomes $1RS_v.1RL_e$ remained unchanged relative to that of the original chromosome $1R_e$. Increased misdivision frequency of the reconstructed chromosomes

Table 1. Misdivision frequencies of normal chromosomes 1A, 1B, 1D and 1R and reconstructed chromosomes 1BS$_p$.1BL$_v$ and 1RS$_v$.1RL$_e$ in Pavon wheat

Chromosome	Number of plants analyzed	Number of misdivision products*	Misdivision frequency
Normal chromosomes:			
1A$_p$	445	22	4.9%
1B$_p$	189	17	9.0%
1D$_p$	212	9	4.2%
1R$_e$	246	45	18.3%
Reconstructed chromosomes:			
1BS$_p$.1BL$_v$-1	134	30	22.4%
1BS$_p$.1BL$_v$-2	90	15	16.7%
1RS$_v$.1RL$_e$-1	315	58	18.4%
1RS$_v$.1RL$_e$-2	382	56	15.7%

* Telocentrics, isochromosomes and centric translocations. Some iso-chromosomes of 1A and 1D might not have been identified. Isochromosomes constituted 16.6% of the misdivision products of chromosomes 1B and 1R.

1BS$_p$.1BL$_v$ was not associated with somatic instability and the chromosomes were normally transmitted to progeny.

Discussion

Centric breakage of univalents in wheat is relatively common - a complete set of 42 telocentric chromosomes has been isolated without a concentrated effort (Sears & Sears, 1978). Isolation of telocentrics in the progenies of isosomics (Sears and Sears, 1978) and successful reconstruction of complete chromosomes from two centric translocations (Lukaszewski, 1993) have proven that two consecutive cycles of centric breakage in wheat were possible. Two consecutive cycles of centric fission-fusion were observed in *Vicia faba* (Schubert et al. 1995). The experiments described here demonstrate that three cycles of centric breakage and fusion do not impair the function of the kinetochores of wheat and rye chromosomes. Normal function of the kinetochores is demonstrated by the normal behavior of the reconstructed chromosomes in mitosis, and in meiosis when paired. On the other hand, increased susceptibility to centric breakage of some reconstructed chromosomes suggests that perhaps they have higher number of active centromeric units present. Presence of multiple active units (centrogenes) in the centromeres of plant chromosomes was postulated by Darlington (1939). In maize, up to four consecutive rounds of centric misdivision were performed and while in some cases this reduced the structural complexity of the centromere, it also increased the number of copies of the individual units present (Birchler, 1994; E. Kaszas & J. Birchler, personal communication.).

The experiments with repeated centric fission-fusion cycles demonstrate that chromosome arms can be transferred from one translocation to another until the most suitable location is found. As a result of the manipulations described here, translocations of the same rye chromosome arm 1RS to all three group-1 homoeologues of wheat were produced. Effectively, both chromosome arms of the original translocation 1RS.1BL of ‘Kavkaz’ were replaced, one at a time, with the arms of the group-1 homoeologues of ‘Pavon’ (Figure 1). These new translocation stocks in a uniform genetic background of ‘Pavon’ should permit a detailed study of the possible position effect of the rye 1RS arm in wheat, and of its most desirable location from the point of view of agronomy and technological properties. Translocation 1RS.1AL originating from the ‘Amigo’ wheat has been shown to also increase yield (Villareal et al. 1996) but is less detrimental to quality than the 1RS.1BL translocation (Graybosch et al. 1993).

At present it appears that the chromosomes 1R$_i$ and 1B$_i$ recovered in the original study (Lukaszewski, 1993) resulted from non-centric breakage and fusion rather than homoeologous recombination between wheat arm 1BS and rye arm 1RS. Chromosome 1R$_i$ had lower male transmission than chromosomes 1R$_e$ and 1RS$_v$.1RL$_e$(data not shown), and substitution 1R$_i$(1B) had somewhat reduced vigor and fertility relative to the 1R$_e$(1B) and 1RS$_v$.1RL$_e$(1B) substitutions. This suggests that chromosome 1R$_i$ may carry a deletion. Translocations of small segments of chromosomes produced by induced homoeologous recombination between 1BS and 1RS have normal male transmission rates and no reductions in vigor or fertiltiy are apparent (Lukaszewski, unpublished). If chromosome 1R$_i$ carries a deletion then chromosome 1B$_i$ very likely carries a corresponding homoeologous duplication in the vicinity of the translocation breakpoint. No adverse effects of 1B$_i$ on male transmission rate, fertility or plant vigor were observed.

Following the manipulations described in this article the following stocks of rye chromosome 1R in ‘Pavon’ wheat are available:

- substitutions 1R$_e$(1A), 1R$_e$(1B), and 1R$_e$(1D)

- substitutions $1RS_v.1RL_e$(1A), $1RS_{v.1RLe}$(1B), $1RS_v.1RL_e$(1D)
- centric translocations $1RS_e.1AL_p$, $1RS_e.1BL_p$, $1RS_e.1DL_p$, $1AS_p.1RL_e$, $1BS_p.1RL_e$, $1DS_p.1RL_e$
- centric translocations $1RS_v.1AL_p$, $1RS_v.1BL_p$, $1RS_v.1DL_p$
- substitution of $1BL_v$ into ‘Pavon’ (20" + $1BS_p.1BL_v$")
- translocation $1RS_e.1BL_v$
- translocation $1B_i$ and $1RS_v.1BS_p.1BL_p$
- substitutions$1R_i$(1B) and $1BS_p.1RS_v.1RL_e$(1B)

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