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M. Martinez · T. Naranjo · C. Cuadrado · C. Romero

Synaptic behaviour of the tetraploid wheat *Triticum timopheevii*

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Abstract *Triticum timopheevii* (2n=4x=A^tA^tGG) is an allotetraploid wheat which shows a diploid-like behaviour at metaphase-I. The synaptic process was analyzed in fully traced spread nuclei at mid-zygotene, late-zygotene and pachytene. The length and type of synaptonemal complexes, as well as the number of bivalent and multivalent associations, were determined in each nucleus. A high number of bivalents per nucleus was detected at all three stages. Nuclei at pachytene showed a lower frequency of multivalents than did zygotene nuclei, which suggests the existence of a pairing correction mechanism. At metaphase-I only homologous bivalents and, rarely, one pair of univalents were observed. Similarities between the diploidization mechanism of *T. timopheevii* and that of allohexaploid wheat, controlled by chromosome 5B, are discussed.

Key words *Triticum timopheevii* · Synaptonemal complex · Spreading · Diploidization mechanism

Introduction

Tetraploid wheats are divided into two groups: the emmer wheat group (*Triticum turgidum*), with the genome formula AABB, and the timpoheevii group (*T. timopheevii*), with the genome formula A^tA^tGG. It is generally accepted that both A and A^t genomes are derived from *T. urartu* (Dvorak et al. 1993; Takumi et al. 1993). It has also been proposed that both B and G genomes originated from either *Ae. speltoides* or an ancestral form closely related to it (Tsunewaki and Ogihara 1983; Ogihara and Tsunewaki 1988; Chen and Gill 1983; Jiang and Gill 1994). Both tetraploid wheats show a diploid-like behaviour at metaphase-I. The diploidization mechanism in *T. turgidum* is controlled by the *Ph1* gene, which is located on the long arm of chromosome 5B (Giorgi and Barbera 1981; Dvorak et al. 1984). Despite the scarcity of information concerning the diploidization of *T. timopheevii*, it is assumed that another gene homoeologous to *Ph1* controls the disomic inheritance in this species (Feldman 1966).

The application of the spreading technique for making whole mount preparations of synaptonemal complexes (SCs) showed that the mechanism of diploidization controlled by *Ph1* in allohexaploid wheat (*T. aestivum*) operates in the following way: (1) a high stringency for homologous synapsis occurs at early prophase; (2) correction of pairing reduces the number of multivalents from zygotene to later stages; and (3) crossing-over is restricted to homologous chromosomes (Holm and Wang 1988). Whether this mechanism of diploidization acts in the same way in allotetraploid wheats has not been confirmed.

In the present paper, the pairing process is studied in the allotetraploid wheat *T. timopheevii* in order to establish the mechanism of diploidization.

Materials and methods

Three plants of the allotetraploid wheat *Triticum timopheevii* Zhuk. (2n=28 A^tA^tGG), kindly supplied by Dr. J. Orellana (Genetics Department, ETSIA, Madrid, Spain), were analyzed in this work.

Single anthers of the emerging spikes were squashed in 2% acetic orcein to locate the stages of meiosis. Two sister anthers of the same floret at zygotene or pachytene were then prepared for SC isolation, as described by de Jong et al. (1991). Surface-spread preparations were silver stained using Loidl's method (1984).

For metaphase-I observations, the anthers were fixed in 1:3 acetic acid:ethanol and stored at 4°C. The fixed material was squashed and C-banded according to Giraldez et al. (1979).

Data from the three plants were pooled because they showed similar behaviour at prophase-I and metaphase-I. Thirty nine fully traced nuclei at prophase-I were analyzed and a total of 50 pollen mother cells (PMCs) at metaphase-I were scored. Identification of chromosomes belonging to the A^t and G genomes was carried out at metaphase-I according to Badaeva et al. (1991).

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M. Martinez · T. Naranjo · C. Cuadrado · C. Romero (✉)
Departamento de Genética, Facultad de Ciencias Biológicas, Universidad Complutense, Madrid, 28040, Spain

Results

General features of the SCs formed in the 39 fully traced nuclei are given in Table 1 where the axial-element length, the number of bivalent and multivalent configurations, and the degree of synapsis in each nucleus are shown. In addition to the percentage of the total axial-element length that showed synapsis, the percentage of synapsis in bivalent configurations and in the multivalent branches, as well as the extent of the asynaptic regions surrounding the pairing partner switch (PPS) points of multivalents, are indicated. Both, the total percentage of synapsis and the percentage of synapsis in SC bivalents were negatively correlated with the total length of axial elements.

Table 1 Summary of the analysis of synaptonemal complexes of fully traced nuclei at mid-zygotene (MZ), late-zygotene (LZ) and pachytene (P) of tetraploid wheat *T. timopheevii*

Nucleus	Axial element length (μm)	Bivalents	Quadrivalents	SC pairing (%)			
				Total	In bivalents	In multivalent branches	Asynapsis in the PPS regions
MZ1	2990	8	3	45.7	53.4	54.6, 43.0, 90.0	27.6, 20.1, 64.6
MZ2	3017	10	2	54.3	53.8	80.0, 74.8	34.8, 21.8
MZ3	2689	10	2	54.8	58.2	67.4, 52.7	35.8, 7.4
MZ4	3070	10	2	57.3	60.7	67.7, 53.0	21.9, 12.4
MZ5	2930	10	2	57.8	60.0	71.4, 67.6	40.7, 21.2
MZ6	2792	12	1	59.5	61.4	60.7	18.5
MZ7	2124	8	3	60.5	67.0	80.2, 75.6, 76.7	23.9, 39.3, 39.7
Mean	2842±124	9.7±0.5	2.1±0.4	55.7±1.9	59.2±1.8	67.6±10.9	28.6±13.4
LZ1	1994	12	1	75.4	80.1	70.5	23.5
LZ2	1950	12	1	86.3	86.4	95.9	10.6
LZ3	2497	12	1	86.4	88.3	88.4	15.2
LZ4	3051	12	1	87.0	89.5	79.9	8.4
LZ5	2653	14	0	87.0	87.0	–	–
LZ6	2050	10	2	87.8	90.6	76.5, 97.6	6.6, 14.6
LZ7	2837	12	1	90.1	92.1	99.2	20.1
Mean	2685±184	12.0±0.4	1.0±0.2	85.7±1.8	87.9±1.5	86.9±18.6	14.1±5.3
P1	2221	10	2	93.2	97.8	100, 95.4	12.4, 16.7
P2	1620	12	1	93.7	94.2	97.2	6.5
P3	1878	10	2	95.6	100	100, 98.6	8.4, 19.8
P4	1958	12	1	95.8	99.4	88.7	26.9
P5	1315	12	1	96.1	99.7	100	38.0
P6	2017	12	1	96.1	99.1	100	24.4
P7	1691	12	1	96.2	99.3	100	11.9
P8	1762	14	0	98.9	100	–	–
P9	1472	11	1VI	98.9	100	100	6.1
P10	1255	12	1	99.2	99.7	100	3.8
P11	1703	12	1	99.2	99.8	100	5.3
P12	2119	12	1	99.3	100	100	5.7
P13	1621	14	0	99.3	100	–	–
P14	1993	14	0	99.5	100	–	–
P15	1317	14	0	99.5	100	–	–
P16	1746	14	0	99.6	100	–	–
P17	1338	14	0	99.7	100	–	–
P18	1590	14	0	99.8	100	–	–
P19	1317	14	0	100	100	–	–
P20	1579	14	0	100	100	–	–
P21	1990	14	0	100	100	–	–
P22	1630	14	0	100	100	–	–
P23	1632	14	0	100	100	–	–
P24	1938	14	0	100	100	–	–
P25	1466	14	0	100	100	–	–
Mean	1687±54	12.9±0.3	0.5±0.1	98.4±0.4	99.3±0.2	98.4±0.8	14.3±8.3
Total	2073±98	12.2±0.28	0.9±0.14	88.4±3.10 r^a=−0.80	90.1±3.10 r^a=−0.81	82.9±8.3	14.3±8.3

[a] Correlation coefficient between axial element length and pairing percentage

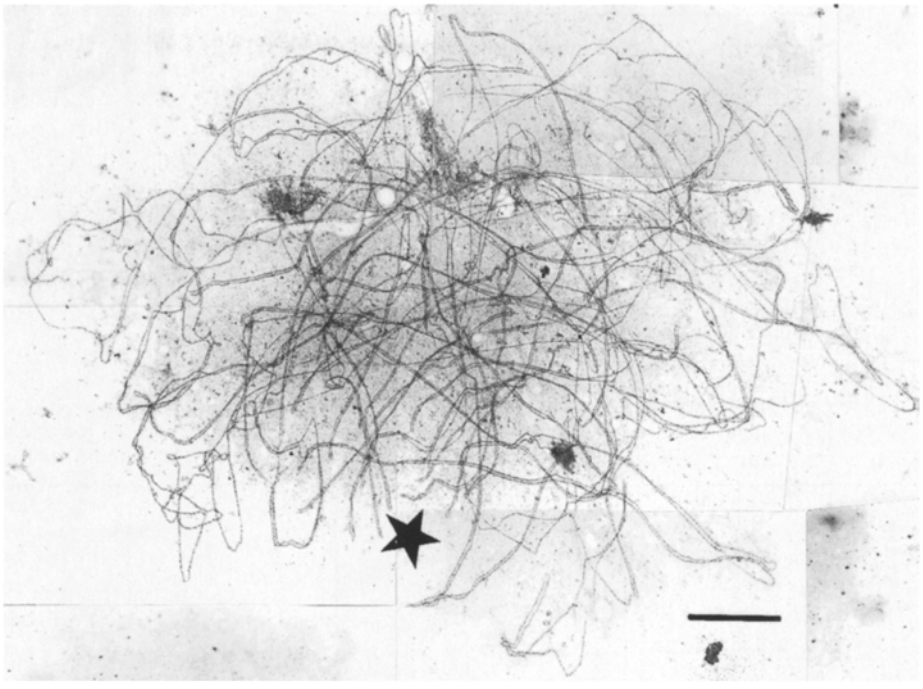

Fig. 1 Electron micrograph of an entire nucleus at mid-zygotene. The bouquet is marked with a *star*. Scale bar=5 μm

Nuclei analyzed were classified into three substages, mid-zygotene, late-zygotene and pachytene, according to the degree of synapsis, the distribution of unpaired segments, and the presence or absence of the bouquet arrangement of telomeres. In nuclei at mid-zygotene the presence of the bouquet was apparent (Fig. 1). Synapsis involved distal segments with variable length as well as a high number of intercalary segments scattered along all the chromosomes. The level of synapsis was lower than 60% in most nuclei at mid-zygotene. The bouquet chromosome arrangement was partially disrupted in the nuclei at late-zygotene. The majority of bivalents and multivalents showed incomplete synapsis with a mean pairing value of 87.9% in bivalents. Nuclei at pachytene showed complete or almost complete synapsis (Fig. 2). Asynapsis at this stage was confined to a few bivalents which were often interlocked, and to the PPS surrounding region of multivalents.

All the nuclei showed a high number of SC bivalents, which indicated that the chromosome involved in such configurations were homologous. Individual bivalents could not be identified because their length was very similar and centromeric regions were not preserved. In the 14 nuclei at pachytene that showed 14 bivalents, the relative length of the bivalents ranged from 5.71% to 8.74%.

With the exception of one hexavalent found in nucleus P9, all the other SC multivalent configurations observed were quadrivalents. No univalents were found. Taking into account the high number of bivalents per nucleus, quadrivalents were most likely formed by two homoeologous chromosome pairs. Each quadrivalent showed only one PPS point which was generally located in a relatively long asynaptic region. On average, the degree and distribution of SC pairing in the branches of the quadrivalents was not very different from that of the bivalents (Table 1).

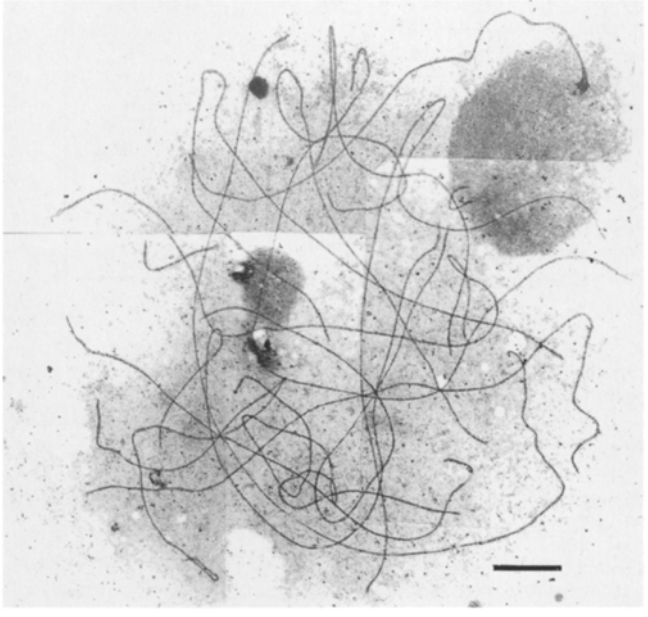

Fig. 2 Electron micrograph of an entire nucleus at pachytene showing 14 bivalents. Scale bar=5 μm

A study of the positional distribution of the PPS points in the quadrivalents was carried out in the following way. Chromosomes involved in quadrivalents were divided into ten intervals of equal relative length. Bearing in mind that

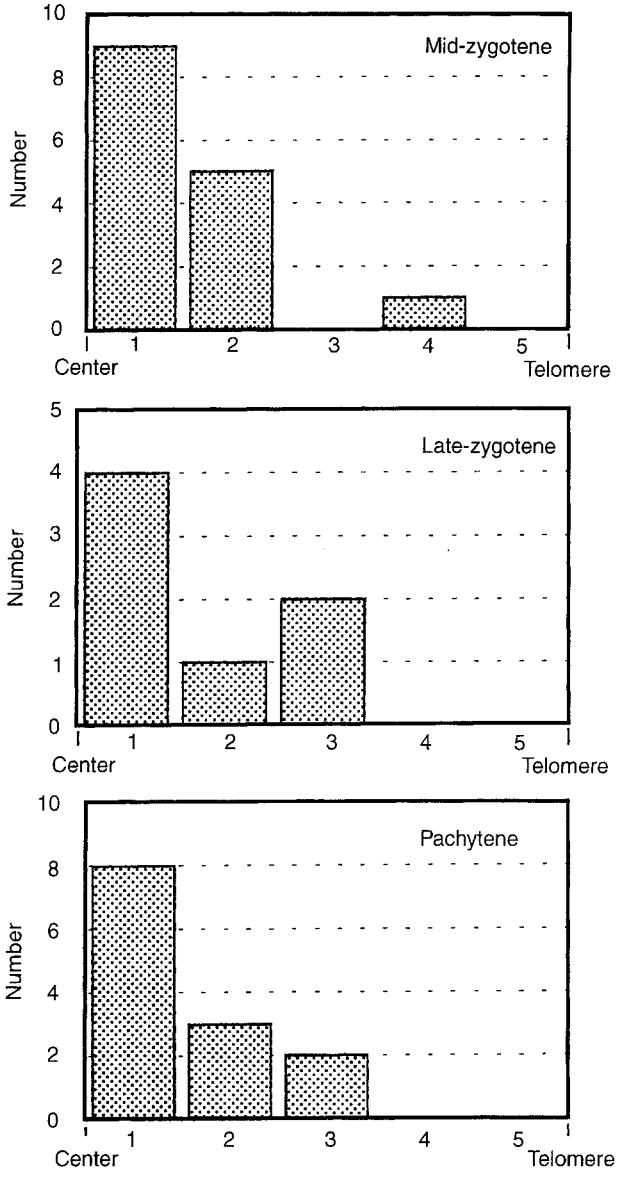

Fig. 3 Location of the PPS point at mid-zygotene, late-zygotene and pachytene

the orientation of a given chromosome with respect to the PPS point could not be ascertained, because centromeric regions were not preserved, only the five intervals of a chromosome-half from the telomere to the center of the chromosome were considered. Because the length of the asynaptic region associated with the pairing partner exchange varied between quadrivalents, the switch point was considered to be located in the middle of the asynaptic region in all cases. Under this assumption the PPS point was mainly centrally located in the multivalents at all analyzed stages (Fig. 3), which is consistent with a pairing end-initiation model.

Synaptic abnormalities, such as interlockings and foldback loops, were observed in the nuclei at the three prophase-I stages analyzed. The frequencies of interlocking in mid-zygotene, late-zygotene and pachytene were 5.14,

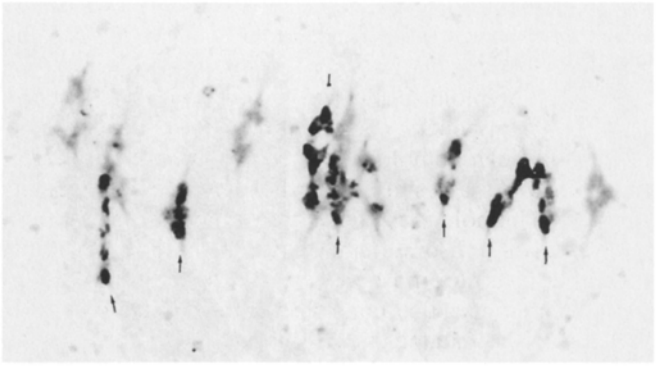

Fig. 4 Micrograph of a metaphase-I cell showing seven ring bivalents of the A^t genome and six ring bivalents and one open bivalent of the G genome (*arrows*)

2.86 and 0.92 per nucleus, respectively. A considerable number of this type of abnormality was associated with the PPS point in multivalents (2.00, 1.43 and 0.40 in each substage respectively). The number of interlockings was negatively correlated with the degree of synapsis (r=–0.80) and decreased from mid-zygotene to pachytene. Foldback loops showed a very low frequency at the three stages, being at their minimum value in pachytene nuclei (0.28).

The chromosome configurations observed at metaphase-I were ring bivalents ($\bar{x}$=12.44), rod bivalents ($\bar{x}$=1.52), and one pair of univalents which was found in only two PMCs ($\bar{x}$=0.04). The C-banding pattern indicated that the chromosomes involved in each bivalent were homologous (Fig. 4). Therefore, no homoeologous A^t-G bivalents were present. This result implies that, although a reduced level of homoeologous pairing persisted in the pachytene multivalents, chiasma formation was restricted to homologous chromosomes. Chromosomes of the A^t and G genomes did not differ in their ability to form chiasmata (with 6.34 as the mean of ring bivalents in the A^t compared with 6.10 in the G genome). The mean number of ring bivalents per cell at metaphase-I (12.44) was quite similar to that of SC bivalents at pachytene (12.9). This result confirms the homologous nature of SC bivalent pairing and demonstrates that such SC bivalents were able to form chiasmata in both chromosome arms. Open bivalents observed at metaphase-I might be derived from chromosome pairs involved in multivalents or from SC bivalents that were interlocked.

When the number of chromosomes involved in bivalent and multivalent configurations at the three prophase-I stages and at metaphase-I were compared, using a contingency chi-square test, significant differences were detected between mid- and late-zygotene (χ^2=15.0, P<0.001), between late-zygotene and pachytene (χ^2=7.82, P<0.001), and between pachytene and metaphase-I (χ^2=110.85, P<0.001). The frequency of quadrivalents showed a reduction of 53.3% from mid- to late-zygotene, of 48% from late-zygotene to pachytene, and of 100% from pachytene to metaphase-I, while the frequency of bivalents progressively increased along this progression.

Discussion

Many allopolyploid species have developed a mechanism, which is under genetic control, for exclusive chiasma formation between homologous chromosomes at prophase-I of meiosis. As a result of this behaviour only bivalents formed by homologous chromosomes are present at metaphase-I, and a disomic inheritance is ensured. However, homoeologous chromosomes are able to pair at prophase-I and, when genes that control the diploid-like behaviour are absent or mutated, homoeologous associations may persist until metaphase-I (Holm and Wang 1988; Gillies 1989).

The results obtained in *T. timopheevii* clearly show the presence of both bivalent and multivalent configurations at prophase-I while only homologous associations persist at metaphase-I. The SC bivalents found at prophase-I were homologous, as deduced from the correspondence between the number of bivalents at pachytene and the number of ring bivalents at metaphase-I. SC multivalents were mainly quadrivalents, which suggests that non-homologous pairing occurred between homoeologous chromosomes.

The mean number of SC bivalents found in autotriploid (2.66) and autotetraploid (4.87) rye (Santos et al. 1995) fits the expected value assuming random chromosome pairing, initiation at both ends, and lack of interstitial autonomous pairing sites. Nuclei at any of the three stages analyzed in *T. timopheevii* showed a number of SC bivalents (9.7–12.9, Table 1) much higher than that expected (4.87) for random pairing between homologous and homoeologous chromosomes in each homoeologous group. Therefore, in this allotetraploid species, there is a severe restriction of pairing initiation to homologous chromosomes, comparable to that found in *T. aestivum* (Holm and Wang 1988). In most allopolyploid species reported in the literature, synapsis is largely confined to homologous chromosomes and SC bivalents are mainly formed at prophase-I. Nevertheless, there are differences between species in the degree to which restriction of pairing initiation to homologous chromosomes is exerted. While a certain number of multivalents are present at prophase-I in *Festuca*, *Scilla*, *Lotus*, *Aegilops* and *Triticum* (Holm 1986; Jenkins et al. 1988; Davies et al. 1990; Thomas and Thomas 1993; Cuñado et al. 1996) only bivalents were observed in *Avena* (Jones et al. 1989).

In the nuclei at mid-zygotene all distal chromosome regions were paired and SC segments of variable length extended along all of the chromosomes. At this stage 30% of the chromosome complement, on average, was involved in quadrivalents. The number of multivalents per nucleus decreased during the course of zygotene and at pachytene only 7% of the chromosome complement formed quadrivalents (Table 1). No nucleus with 14 bivalents was found at mid-zygotene and only one at late-zygotene. Therefore, retardation or arresting of nuclei carrying multivalents was not the reason for the low number of multivalents at pachytene. Quadrivalents were transformed into pairs of bivalents from zygotene to pachytene and no trivalents or univalents were generated in this transition.

Multivalent elimination at prophase-I has been described in *T. aestivum* (Holm 1986) and *Lotus corniculatus* (Davies et al. 1990). The correction mechanism suggested in these cases was the progressive dissolution of non-homologous (homoeologous) SC segments of multivalents and their immediate reconstitution in the branches formed by homologous chromosomes, which would result in a displacement of the PPS sites towards the chromosome end.

Only one PPS per quadrivalent was observed in *T. timopheevii* and its position did not depend on the prophase-I stage. All quadrivalents showed a proximal location of the PPS point (Fig. 3). Therefore, a movement of PPS sites towards the chromosome ends during the course of zygotene and pachytene was not apparent. Segments of SC multivalents involving homoeologous chromosomes probably suffered a rapid disolution and each axial element immediately started synapsis with its homologous partner. The large asynaptic region associated with the PPS point in many quadrivalents at mid- and late-zygotene, and even in some pachytene multivalents (Table 1), could facilitate their quick elimination at these stages. Quadrivalents with a short asynaptic region might be more stable and persist throughout the pachytene stage.

A low number of PPS points per quadrivalent has also been reported in autotetraploids of related species such as *T. monococcum* (Gillies 1989) and *S. cereale* (Jenkins and Chaterjee 1994; Santos et al. 1995). However, quadrivalents with two PPSs, in addition to quadrivalents with one PPS, as well as a more variable location of PPS sites, were found in these materials. This difference might be influenced by the homologous nature of such quadrivalents.

Because almost all metaphase-I cells showed 14 bivalents, and no multivalent was found at this stage, SC multivalents that were maintained until the end of pachytene were also transformed into two bivalents. Whether this transition took place before or after chiasma formation could not be determined. It is likely that multivalents, and therefore SC segments formed by homoeologous chromosomes, persisted when crossing over occurred, but crossing over was restricted to homologous SC pairing. This was suggested to occur in *T. aestivum* (Holm and Wang 1988) and *Lolium* (Jenkins 1988).

Consequently, disomic inheritance in *T. timopheevii* is the result of a high pairing stringency of homologous chromosomes at zygotene, which is followed by a pairing correction mechanism that transforms quadrivalents into pairs of bivalents during zygotene and pachytene and, ultimately, by the suppression of crossing over in any homoeologous SC segments that might persist at this stage. This diploidization mechanism seems to be very similar to that proposed for *T. aestivum* (Holm and Wang 1988) which is under the control of the *Ph1* gene located on chromosome 5B (Sears 1976). Chromosomes 5B and 5G are homoeologous as deduced from the positive results of compensation-substitution tests developed by Badaeva et al. (1991). In crosses of mono-5B *T. aestivum* with the amphidiploid *T. timopheevii-Ae. squarrosa* Feldman (1966) found a similar chiasma frequency in hybrids carrying chromosomes

5B and 5G and hybrids with only 5G. This meiotic behaviour and the homoeologous relationships suggest that chromosome 5G carries a pairing suppressor gene homoeologous to *Ph1* which is responsible for the diploidization mechanism in *T. timopheevii*.

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