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The synaptic behaviour of *Triticum turgidum* with variable doses of the *Ph1* locus

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Abstract Cultivated wheat *Triticum turgidum* is an allotetraploid (AABB) with diploid-like behaviour at metaphase I. This behaviour is mainly influenced by the action of the *Ph1* locus. To study the effect of *Ph1* on chromosome pairing in *T. turgidum* we have analysed the synaptic pattern in fully traced spread nuclei at mid- and late-zygotene and at pachytene of three different genotypes: a standard line, *ph1c* mutant and a duplication mutant, with zero, two and four doses of *Ph1*, respectively. The number of synaptonemal complex (SC) bivalents and of the different SC multivalent associations were determined in each nucleus. The mean number of lateral elements involved in SC multivalent associations (LEm) at mid-zygotene was relatively high in all lines and was similar in two and zero doses of *Ph1*. These means changed little with the progression of zygotene but decreased at pachytene because of the transformation of multivalents into bivalents. Multivalent correction was more efficient in the presence than in the absence of *Ph1*. The four doses of *Ph1* genotype showed a higher number of SC bivalents at mid-zygotene, and the frequency of multivalents decreased progressively throughout zygotene and pachytene. The results suggest that the main action of the *Ph1* locus on the diploidisation mechanism would be related to a process of checking for homology operating during prophase I.

Keywords *Ph1* gene · *Triticum turgidum* · Meiosis · Synaptonemal complex · Diploidisation

Introduction

Many allopolyploid species have developed a genetic mechanism that precludes heterogenetic chromosome pairing at metaphase I and ensures disomic inheritance. The best-studied example is common wheat *Triticum aestivum*, which is an allohexaploid species with the genome constitution AABBDD (2n=6x=42). *Triticum aestivum* arose from two hybridisation events involving three diploid species (reviewed by Dvorak 1998). Tetraploid wheat *T. turgidum* (genome constitution AABB) originated from hybridisation between *T. urartu* and most likely *Aegilops speltoides*, which contributed the A and B genomes, respectively. The second hybridisation event involved *T. turgidum* and *Ae. tauschii*. The latter species contributed the D genome. The three genomes of *T. aestivum* have evolved from a common ancestral genome and preserve a residual homology (Sears 1966). In spite of the potential for pairing among the homoeologous chromosomes, tetraploid and hexaploid wheats show a diploid-like behaviour with 14 and 21 bivalents at metaphase I, respectively. The diploid-like chromosome pairing of bread wheat is under the control of the *Ph1* locus on the long arm of chromosome 5B (Sears 1976).

In an earlier report, Feldman (1966) found that extra doses of the long arm of the 5B chromosome in bread wheat caused partial asynapsis of homologues, pairing of homoeologues and interlocking of bivalents at metaphase I. He proposed that the *Ph1* gene exerted its effect at the premeiotic stages and suggested that in the absence of *Ph1*, both homologous and homoeologous chromosomes are associated in premeiotic nuclei and therefore compete in pairing. In euploid *Ph1Ph1* plants, pre-synaptic alignment takes place only between homologous chromosomes, which undergo synapsis at zygotene. With six doses of *Ph1* all chromosomes are distributed randomly, which causes disturbed synapsis.

The use of spreading techniques for making whole mount preparations of synaptonemal complexes has enabled the study of the process of chromosome synapsis in hexaploid wheat and in hybrids of this species with some of its relatives (Gillies 1987; Holm and Wang 1988). The results of these studies indicated that homoeologous chromosomes may synapse at prophase I and that homoeologous associations persist until metaphase

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I only in the absence of *Ph1*. The disomic inheritance of hexaploid wheat was attributed to: (1) a restriction of synapsis to homologous chromosomes at early prophase; (2) elimination of the multivalents formed through a synaptic correction mechanism that operates at zygotene-pachytene; (3) occurrence of crossing-over only between homologues (Holm 1986). The *Ph1* gene was argued to affect both synapsis and crossing-over (Gillies 1987; Holm and Wang 1988; Luo et al. 1996).

Recent studies carried out using genomic *in situ* hybridisation have shown a premeiotic association of barley or rye homologues added to hexaploid wheat (Aragón-Alcaide et al. 1997; Schwarzacher 1997; Martinez-Perez et al. 1999). A similar approach demonstrated that the absence of *Ph1* affects both the premeiotic association and chromosome pairing of two rye telosomes in a wheat background (Mikhailova et al. 1998). Fluorescent *in situ* hybridisation (FISH) with DNA probes revealed the location of centromeres and telomeres of hexaploid wheat chromosomes in nuclei at premeiotic interphase and early prophase I (Martínez-Pérez et al. 1999). However, these studies provided no information about the behaviour of the remaining wheat chromatin.

Tetraploid wheat *T. turgidum* (AABB) is a very useful material for studying meiotic pairing since, in addition to having fewer genomes than hexaploid wheat, it also has mutants involving the *Ph1* locus. Two different mutants have been isolated, a deletion mutant designated *ph1c*, which lacks the *Ph1* locus (Giorgi and Cuozzo 1980), and another mutant with a duplicated segment that includes *Ph1*. This mutant was proposed to carry four doses of the gene (Dvořák et al. 1984). In contrast to hexaploid wheat, little information is available on chromosome synapsis in tetraploid wheat. Synaptonemal complex (SC) formation has been studied only in *T. timopheevii* (A^tA^tGG) (Martínez et al. 1996). In the experiments described here the effect of the *Ph1* gene on the synaptic process at prophase I and chromosome association at metaphase I in *T. turgidum* is analysed. Studies included plants with zero, two and four copies of *Ph1*.

Materials and methods

Three genotypes of durum wheat *Triticum turgidum* (AABB, 2n=4x=28) cv. Cappelli were used: a standard line, *ph1c* mutant and the *Ph1Ph1Ph1Ph1* mutant, with two, zero and four doses of *Ph1*, respectively. Ten plants of each genotype were sampled.

Single anthers of the emerging spikes were squashed in 2% acetic orcein to locate the zygotene or pachytene stages of meiosis. Two remaining anthers in the same floret were then prepared for SC isolation, as described by Holm (1986). Surface-spread preparations were silver-stained by Loidl's method (1984).

A total of more than 1000 nuclei at zygotene-pachytene were examined by electron microscopy (EM). EM images from 113 nuclei in the *Ph1Ph1* genotype, 64 nuclei in the *ph1cph1c* mutant and 42 nuclei in the *Ph1Ph1Ph1Ph1* mutant were captured. The complete image of each nucleus was mounted from four to six partially overlapping images, which were printed to a very high magnification to facilitate their study. Nuclei from different plants did not qualitatively differ in their synaptic pattern and, therefore, all reconstructed nuclei of each genotype at a similar prophase I stage were pooled.

For metaphase I observations, the anthers were fixed in 1:3 acetic-acid ethanol and stored at 40° C. The fixed material was squashed and C-banded using the Giemsa staining procedure of Giráldez et al. (1979). Chromosomes were identified according to Simeone et al. (1988).

Results

Synaptonemal complex formation at zygotene-pachytene

SCs could be completely traced and analysed in 48, 44 and 36 nuclei from plants with two, zero and four copies of *Ph1*, respectively. Nuclei not included in the study showed no apparent differences from the nuclei used. Their reconstruction was not accomplished because they had many multivalents and/or SCs which were partially destroyed, most likely in the partner exchange regions of some multivalents. The nuclei analysed at prophase I were classified into three substages, mid-zygotene, late-zygotene and pachytene, according to Martinez et al. (1996). Nuclei at mid-zygotene showed the bouquet structure and a degree of synapsis lower than 75%; nuclei at late-zygotene partially maintained the bouquet structure and the degree of synapsis was lower than 95%; nuclei at pachytene showed no bouquet arrangement and complete or almost complete synapsis.

General features of the SCs formed at mid-zygotene, late-zygotene and pachytene in the plants with two, zero and four doses of the *Ph1* gene are summarised in Table 1. Mean values for the total length of the axial elements, the total level of synapsis and the percentage of synapsis in SC bivalents per nucleus are given. Likewise, the mean number of bivalents, quadrivalents and other multivalent configurations per nucleus as well as the number of lateral elements involved in multivalents (LEm) per nucleus are indicated. Figure 1 shows representative drawings of nuclei in the three stages analysed. Each stage showed a different distribution of unmatched lateral elements in the SC bivalents and multivalents. At mid-zygotene, distal chromosome regions were synapsed. A number of intercalated synapsed segments scattered along all the SC bivalents and multivalents were also observed. The number of unmatched segments decreased at late-zygotene. At pachytene, unmatched regions were confined to a few bivalents, which were often interlocked, and to surrounding regions of the synaptic partner exchange (SPE) of multivalents. This synaptic pattern suggests that synapsis is initiated in distal regions and progresses with the formation of interstitial synapsis initiation sites in a similar way in the three genotypes.

SC bivalent and quadrivalent configurations were observed in nuclei of the three types of plants at all the prophase I stages analysed (Figs. 2 and 3). All nuclei at mid- and late-zygotene of both the standard line and the *ph1c* mutant showed SC multivalents while in the *Ph1Ph1Ph1Ph1* mutant, 40% of zygotene nuclei had only bivalents. Configurations higher than quadrivalents were also observed in plants with zero or two doses of

Table 1 Summary of the analysis of synaptonemal complexes (SC) of fully traced nuclei at mid-zygotene (MZ), late-zygotene (LZ) and pachytene (P) of tetraploid wheat *T. turgidum* with different *Ph1* doses. The mean number of the different SC associations per nucleus as well as the mean number of the lateral elements involved in multivalent associations (LEm) per nucleus are indicated

Ph1 doses	Stage	Number of nuclei	Axial element length (μm)	Total synapsis (%)	Bivalent synapsis (%)	SC configurations per nucleus						LEm
						II	III	IV	VI	VIII	X	XII	
2	MZ	7	3285±93	62.2±2.8	68.7±2.9	8.57±0.53		1.43±0.48	0.29±0.18	0.43±0.20			10.86±1.06
	LZ[a]	12	3310±154	79.7±1.9	86.4±2.1	8.17±0.60	0.25±0.18	1.42±0.23	0.33±0.14	0.33±0.14			11.67±1.20
	P[b]	29	2225±102	97.7±0.5	99.3±0.2	12.41±0.37		0.48±0.12	0.10±0.08	0.07±0.05			3.10±0.75
0	MZ	6	3218±194	50.8±3.5	55.2±3.5	8.17±0.75		2.67±0.49	0.17±0.17				11.67±1.50
	LZ	8	3228±174	78.5±2.0	86.7±2.3	7.87±0.61		1.75±0.37	0.50±0.33	0.12±0.12	0.12±0.12		12.25±1.22
	P	30	2573±119	93.6±0.9	98.7±0.3	10.33±0.40		1.17±0.17	0.23±0.08	0.07±0.05	0.03±0.03	0.03±0.03	7.33±0.79
4	MZ	5	3950±463	61.3±2.8	63.2±2.7	12.40±0.75		0.80±0.37					3.20±1.50
	LZ	5	3451±332	79.0±3.4	81.7±2.6	12.80±0.49		0.60±0.24					2.40±0.98
	P	26	2392±81	97.6±0.5	98.2±0.4	13.54±0.20		0.23±0.10					0.92±0.40

[a] One nucleus had one heptavalent
[b] One nucleus had two univalents

Fig. 1 Representative idiograms of mid-zygotene, late-zygotene and pachytene nuclei. The SC bivalents are placed in order of size from the highest to the lowest, then the synaptonemal complexes involved in multivalent associations are represented. Identification of the different bivalents was not possible by this technique

Ph1. These originate from synapsis between non-homoeologous chromosomes (chromosomes belonging to different homoeologous groups). A maximum of 12 and 8 lateral elements in the SC multivalents were observed in plants with zero and two doses of *Ph1*, respectively. The frequency of multivalents per nucleus measured as the mean number of LEm showed similar and relatively high values at mid-zygotene in the plants with zero and two doses of *Ph1* (χ^2=0.31; 0.7>*P*>0.5). These values barely changed with the progression of zygotene in such genotypes. At pachytene the number of LEm decreased, while the number of bivalents increased. However, the increase in the number of bivalents was higher in plants with two doses of *Ph1* than in plants with zero doses. The genotype with four copies of *Ph1* showed a different be-

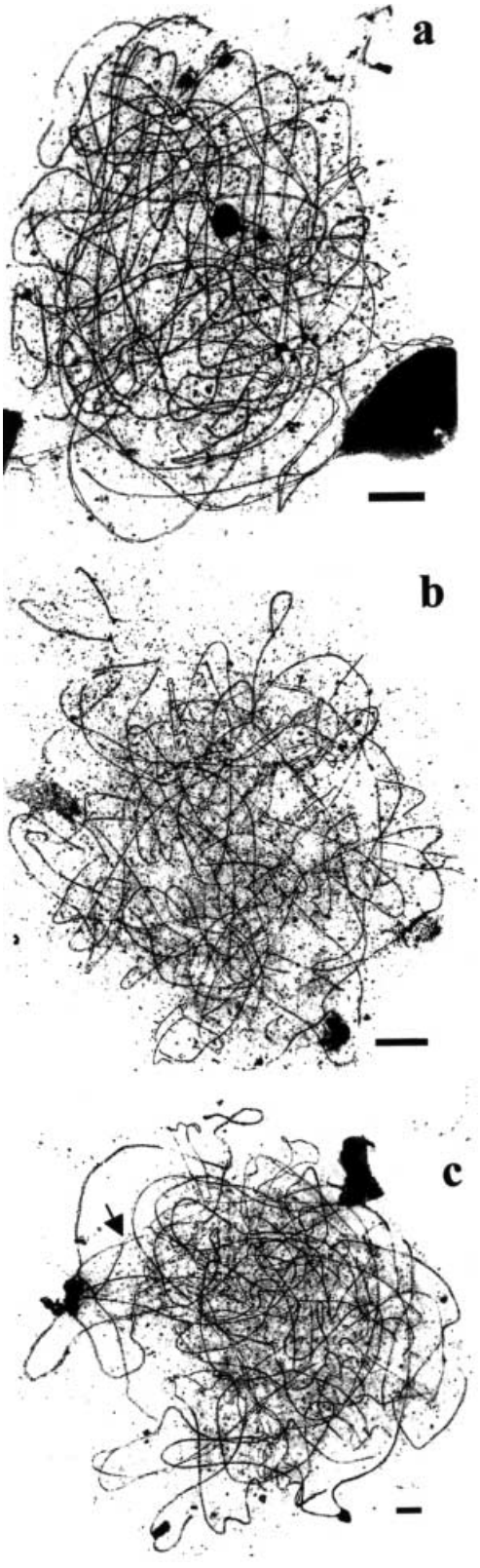

Fig. 2 Electron micrographs of entire nucleus at late-zygotene of: **a** *T. turgidum Ph1Ph1*, **b** *T. turgidum ph1cph1c*, **c** *T. turgidum Ph1Ph1Ph1Ph1* (*arrow* indicates an interlocking). Bar: 5 µm

Table 2 Summary of the mean number of interlockings (ILs) per nucleus at the mid-zygotene (MZ), late-zygotene (LZ) and pachytene (P) substages of tetraploid wheat *T. turgidum* with different *Ph1* doses

Ph1 doses	Substage	Total IL	ILs associated with synaptic partner exchange points
2	MZ	4.0±0.84	2.0±0.72
	LZ	4.5±1.03	3.08±0.79
	P	0.5±0.16	0.41±0.14
0	MZ	4.5±0.81	2.33±0.61
	LZ	4.5±0.38	3.5±0.42
	P	1.73±0.35	1.23±0.32
4	MZ	4.4±0.75	0.8±0.37
	LZ	3.2±1.16	0.8±0.58
	P	0.54±0.14	0.11±0.06

haviour from the earlier stages. Most chromosomes were synapsed in SC bivalents, and a significantly lower number of LEm was observed relative to the *Ph1Ph1* line (χ^2=30.70; P<0.001). The few multivalents observed in the *Ph1Ph1Ph1Ph1* mutant were mostly converted into bivalents throughout zygotene and pachytene.

The majority of quadrivalents showed one SPE. Only four quadrivalents in the *ph1c* genotype and one quadrivalent in the four-doses mutant had two SPEs. A study of the distribution of the SPE regions in the quadrivalents with one SPE was carried out following the procedures of Santos et al. (1995) and Martínez et al. (1996). Each chromosome was divided in two halves; one of which contained the SPE site. This half was divided into five segments of equal relative length which were numbered from 1 to 5 in the direction from the telomere to the chromosome centre.Consequently, the number of the segment containing the SPE site defines its position relative to the chromosome centre. Whether a given SPE site was located in the short or in the long arm could not be determined because the technique does not preserve the centromeric region. Therefore, the SPEs located a similar length from either of the chromosome ends were combined. A total of 61, 41, and 12 quadrivalents in the plants with zero, two and four copies of *Ph1*, respectively, were analysed. Most SPEs were centrally located at mid-zygotene (Fig. 4), which is consistent with a synaptic process that starts at the chromosome ends and progresses towards the central region. At late zygotene and pachytene, SPEs were more scattered in the *ph1c* mutant than in the *Ph1Ph1* standard line. In the *ph1c* mutant at pachytene a considerable proportion of the quadrivalents showed a distal SPE site.

Synaptic abnormalities, such as interlockings (ILs) were observed in nuclei of all the genotypes and prophase I stages analysed (Table 2). Identification of these interlocks was based on the tensioned configuration adopted by the lateral elements involved, as shown in Fig. 2. Two-dimensional analysis of nuclei possibly underestimates the actual interlocking frequency. However,

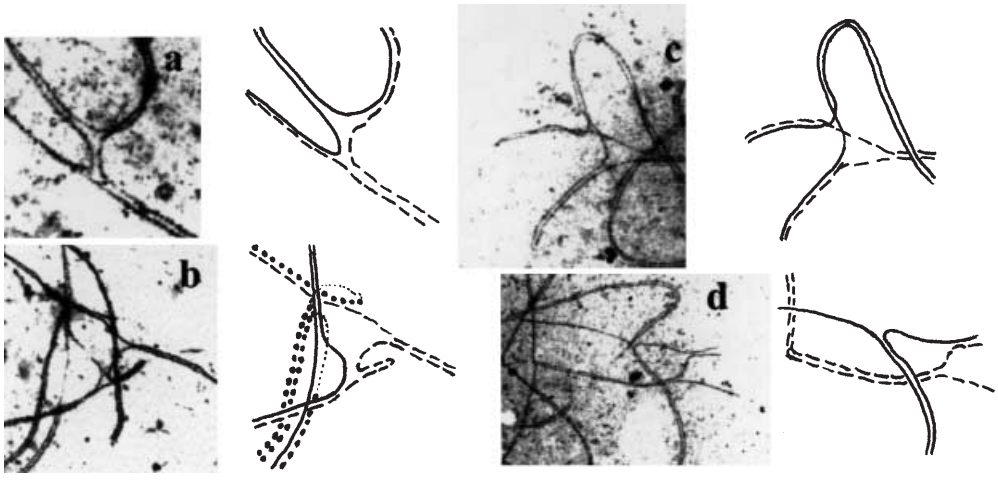

Fig. 3 Higher magnification electron micrographs of synaptic partner exchanges in synaptonemal complexes of *T. turgidum Ph1Ph1* (**a, b**) and *T. turgidum ph1cph1c* (**c, d**) and their correspondent schematic drawings. **a**, **c** and **d** show a quadrivalent, and **b** shows an hexavalent where four of six lateral elements involved in the association can be continuously traced, and the remaining two are broken (represented by *thin dotted lines* in the drawing)

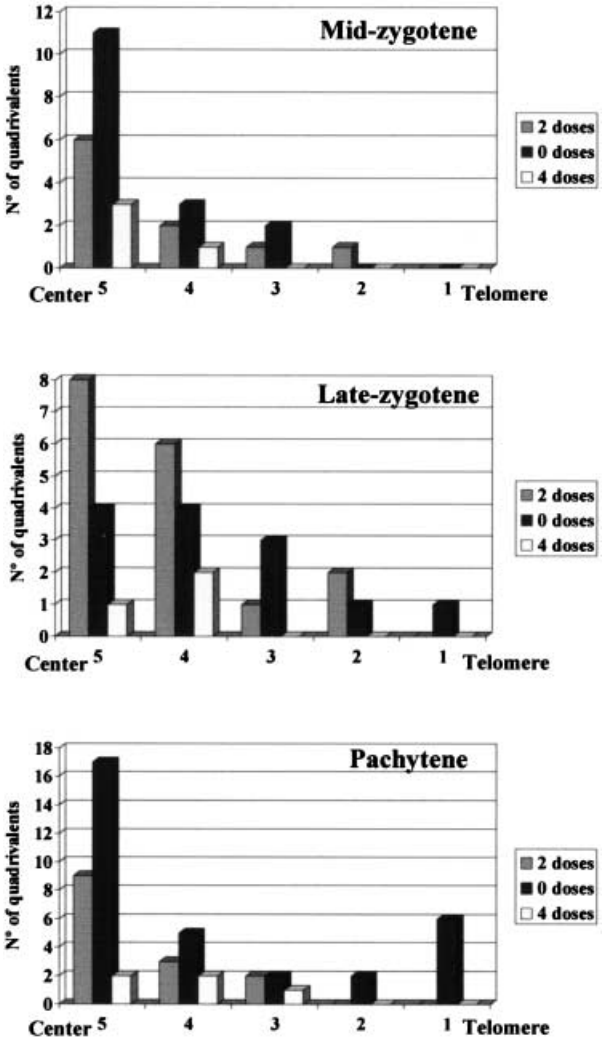

Fig. 4 Location of the synaptic partner exchange point at mid-zygotene, late-zygotene and pachytene

at mid-zygotene the estimated values of ILs were similar in all three genotypes (F=0.113; P=0.89). The number of ILs decreased at pachytene, but the reduction was lower in the absence of *Ph1*. In plants with zero and two doses of *Ph1*, which formed more multivalents than plants with four doses, a considerable number of interlocks were associated with SPE regions.

Chromosome associations at metaphase I

Plants with two and four copies of *Ph1* behaved in a similar way (Table 3). Homologous chromosomes formed mainly ring bivalents, less frequently open bivalents, and rarely univalents (Fig. 5a). No homoeologous A-B association was detected in these two genotypes, which indicates that chiasmata were formed only between homologous chromosomes. In the plants lacking the *Ph1* gene, some multivalents, trivalents and quadrivalents involving chromosomes from the A and B genomes were observed at metaphase I (Table 3 and Fig. 5b). Such configurations demonstrated the occurrence of homoeologous crossing-over at prophase I. In addition, these plants showed a higher frequency of open bivalents and univalent pairs than plants carrying *Ph1*.

Discussion

Synapsis development

An average of 8.17, 8.57 and 12.40 SC bivalents at mid-zygotene in plants with zero, two and four copies of *Ph1*, respectively, indicates that synapsis occurs preferentially between homologous chromosomes. Also apparent is the higher degree of stringency of synapsis initiation in the mutant with four doses of *Ph1* than in the zero- and two-dose genotypes. Extensive multivalent formation observed in the *ph1c* mutant and in the standard tetraploid wheat may be attributed mainly to homoeologous synapsis, although additional heterologous synapsis was in-

Table 3 Mean number per cell of chromosome associations at metaphase I in tetraploid wheat *T. turgidum* (AABB) with different *Ph1* doses

Ph1 doses	Number of cells	Genome	Ring bivalents	Open bivalents	Univalent pairs	Trivalents plus univalent	Quadrivalents
2	50	A	6.90±0.04	0.10±0.04			
		B	6.74±0.04	0.24±0.08	0.02±0.02		
		Total	13.64±0.09	0.34±0.09	0.02±0.02		
0	100	A	4.72±0.12	1.82±0.11	0.27±0.05		
		B	4.74±0.12	1.87±0.12	0.20±0.04		
		Total	9.46±0.18	3.69±0.17	0.47±0.06	0.02±0.01	0.17±0.04
4	50	A	6.86±0.06	0.12±0.05	0.02±0.02		
		B	6.58±0.09	0.38±0.08	0.04±0.04		
		Total	13.44±0.09	0.50±0.09	0.06±0.04		

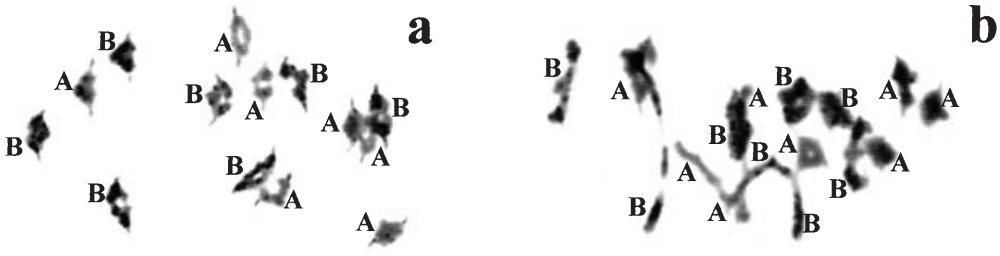

Fig. 5 **a** Micrograph of a metaphase I nucleus of *T. turgidum Ph1Ph1* showing 7_A-ring II, 7_B-ring II. **b** Micrograph of a metaphase I nucleus of *T. turgidum ph1cph1c* showing 5_A+3_B-ring II, 1_A+3_B-rodII and $1_{A\text{-}B}$-quadrivalent

ferred from the presence of multivalent configurations above quadrivalents. That the number of SC multivalents was lower in the *Ph1Ph1Ph1Ph1* genotype than in the *Ph1Ph1* and *ph1cph1c* genotypes indicates that multivalents were not misinterpreted because of the two-dimension analysis of spread nuclei. Furthermore, two-dimension analysis provides unambiguous evidence of synaptic partner exchange in the multivalents (Fig. 3).

In the standard *Ph1Ph1* line of hexaploid wheat, *T. aestivum*, Holm and Wang (1988) reported a much lower frequency of multivalent associations than that observed here. The presence of the D genome in hexaploid wheat, which also carries pairing regulator genes (Sears 1976), may account for the differences in synapsis initiation between tetraploid and hexaploid wheats.

The number of SC bivalents increased at pachytene in the three genotypes as a result of the correction of multivalent associations. This has also been reported in *T. aestivum* (Holm 1986; Holm and Wang 1986), *Lotus corniculatus* (Davies et al. 1990) and *T. timopheevii* (Martínez et al. 1996). The different numbers of bivalents at pachytene when zero or two copies of *Ph1* were present suggest that the synaptic correction mechanism was more effective when the *Ph1* gene was present. The distribution of SPEs in the quadrivalents supports this conclusion. In plants with two and four doses of the *Ph1* locus, the SPEs were proximally located in most quadrivalents at all substages of prophase I. This distribution suggests a rapid dissolution of branches from the SC multivalents involving non-homologous chromosomes and the subsequent completion of synapsis between the two homologous axial elements (Martínez et al. 1996). In plants lacking the *Ph1* locus, the frequency of quadrivalents with the distally located SPE increased during the course of prophase I, suggesting a shift of SPEs from central positions towards the chromosome ends. The detection of SPE movement might be derived from a synaptic correction mechanism that operates more slowly in the absence of *Ph1* than when two or four copies of this locus are present.

All three genotypes of *T. turgidum* used in this work were able to complete synapsis. In studies on hexaploid wheat, *T. aestivum*, Holm and Wang (1988) analysed plants with zero, one, two, four and six doses of the 5BL arm, where the *Ph1* locus is located. Plants with zero, four and six copies of 5BL lacked chromosome arm 5BS. These plants did not complete synapsis, and the interlocking of bivalents persisted until metaphase I. Holm and Wang (1988) attributed these defects to the absence of the 5BS chromosome arm, which carries a pairing promoter gene (Riley and Chapman 1967; Cuadrado et al. 1990). Our results support such an explanation and illustrate the convenience of using mutants of *Ph1* instead of 5B-deficient plants in these studies.

Chiasma formation

The use of C-banding confirmed that in all three genotypes all bivalents at metaphase I were formed by homologous chromosomes. Homoeologous associations at metaphase I were found only in trivalents and quadrivalents in plants lacking the *Ph1* locus.

The number of lateral elements involved in the SC bivalent associations at pachytene in plants with four doses of *Ph1* corresponds to the number of chromosomes involved in ring bivalents at metaphase I (χ^2=0.66; 0.5>*P*>0.3). This

confirms the homologous nature of the SC bivalents and demonstrates that such SC bivalents were able to form chiasmata in both chromosome arms. In plants with two copies of *Ph1*, the number of ring bivalents at metaphase I was significantly higher than the number of SC bivalents at pachytene (χ^2=72.31; P<0.001). This means that a considerable number of metaphase I ring bivalents were derived from the SC multivalents and that some crossing-overs had to be formed after the transformation of multivalents into bivalents at pachytene. This is consistent with other reports suggesting that synapsis is not always dependent on a previous recombination event in higher eukaryotes (Dernburg et al. 1998; McKim et al. 1998; Santos 1999). Such a sequence seems to be different from that of yeast where concomitant with intermediate and late stages of recombination, synaptonemal complexes are formed (Padmore et al. 1991; Schwacha and Kleckner 1994, 1995; Roeder 1997; Zickler and Kleckner 1998). On the other hand, if some SC multivalents did persist in the plants with two and four *Ph1* doses when crossing-over was formed, the absence of multivalent associations at metaphase I indicates that homoeologous synapsis does not imply the occurrence of chiasmata, hence, the crossing-over between homoeologous chromosomes. *Ph1*-mediated restriction of crossing-over in *T. aestivum* was postulated by Gillies (1987) and Holm and Wang (1988), on the basis of the degree of synapsis and the number of chiasmata in hybrids of wheat with rye or *Ae. kotschyi* and in wheat haploids. They found similar levels of synapsis both in the presence and in the absence of *Ph1*, while chiasmata were much more frequent in genotypes without the functional allele. A similar conclusion was also reported by Luo et al. (1996) from estimates of the frequency of recombination across chromosome pairs composed of homologous and homoeologous chromosome segments.

In the absence of *Ph1*, the number of SC bivalents at pachytene was significantly higher than the number of ring bivalents at metaphase I (χ^2=11.75; P<0.001). Thus, chiasma formation failed to some degree in the SC bivalents of this genotype. Chiasma formation must also have failed in the multivalent associations since there was a sixfold reduction in quadrivalent frequency between pachytene and metaphase I. Such SC quadrivalents probably formed chiasmata in the two homologous branches and in one of the two homoeologous branches. The remaining pachytene multivalents most likely originated open bivalents and/or univalents pairs. Because most chiasmata are distally located in wheat and related species, the absence of crossing-over in some multivalent segments has been attributed to interference between chiasma formation and the shift of the SPE site towards distal chromosome regions (Gillies et al 1987; Sybenga et al. 1994; Santos et al. 1995). It is also possible that some SC quadrivalents were formed by two chromosome pairs from different homoeologous groups. Such quadrivalents would disappear with the dissolution of the SCs, since some degree of homology is required for recombination. In fact, multivalent associations above quadrivalents, which were present at pachytene, were not observed at metaphase I.

The effect of the *Ph1* locus

Different studies in plants, including hexaploid wheat, have indicated that chromosome pairing is preceded by premeiotic association of homologous chromosomes (Stack and Brown 1969; Loidl 1990; Aragón-Alcaide et al. 1997a; Schwarzacher 1997; Mikhailova et al. 1998; Martínez-Pérez et al. 1999). Schwarzacher (1997) suggested that this association is loosened at leptotene and early-zygotene where homologous chromosomes appear in separate domains. However, Mikhailova et al. (1998) propose that the premeiotic association may be maintained at leptotene in telomeric or subtelomeric regions which provide the initiation points of synapsis, and Martínez-Pérez et al. (1999) suggest that premeiotic association starts in centromeric regions and is maintained at the onset of leptotene. These suggestions agree, to some extent, with the hypothesis that the *Ph1* gene exerts its action by controlling the spatial arrangement of chromosomes at premeiotic interphase (Feldman 1966). However, the similar high frequencies of multivalent associations at mid-zygotene in tetraploid wheat with zero and two doses of the *Ph1* locus observed here are difficult to reconcile with the action of *Ph1* before the onset of meiosis. The frequency of the multivalent associations was significantly lower in the *Ph1Ph1Ph1Ph1* genotype. Two different explanations of this result are possible. First, extra doses of the *Ph1* gene enhance the efficiency of the mechanism responsible for the search of the homologous partner. Second, four copies of *Ph1* change the presynaptic arrangement of homologues and homoeologues relative to that produced by two or zero doses. If the number of copies of *Ph1* affects premeiotic chromosome arrangement, plants with four copies of *Ph1* would be expected to have lower frequencies of ILs than plants with two and zero doses. The similar IL frequencies at mid-zygotene in the three genotypes analysed seems to be in agreement with the first possibility.

Several studies suggest that the *Ph1* locus controls the initiation of synapsis and the occurrence of crossing-over between homologous chromosomes (Gillies 1987; Holm and Wang 1988; Luo et al. 1996). Our results agree with an effect of *Ph1* related to a mechanism checking for homology operating at the onset of synapsis, during the synaptic process in the correction of non-homologous pairing and, probably, impeding crossing-over in non-homologous SCs stretches that escape the pairing correction mechanism. With four doses of this locus, the mechanism acting at the early stage of synapsis is very efficient, and homologous bivalents are formed. When two doses of *Ph1* are present, the mechanism checking for homology completes its action later, resulting in the correction of multivalents into bivalents. In the absence of *Ph1*, the mechanism checking for homology is less efficient, and crossing-over may occur between homoeologous chromosomes involved in multivalent associations.

Mikhailova et al. (1998) studied the effect of the absence of the *Ph1* locus on the behaviour and morphology of a pair of rye telosomes in wheat. The study showed

that the organisation of the chromatin was different when *Ph1* was absent. It has been suggested that the *Ph1* locus is involved in chromosome condensation and/or scaffold organisation. The different organisation of chromatin in the absence of *Ph1* might hinder the movement of the chromosome during the multivalent correction process, thereby reducing its efficiency. The possibility exists that this different chromatin organisation is related to the mechanism checking for homology proposed as the main action of this gene.

In summary, the similar high frequencies of LEm observed at mid-zygotene in plants with zero and two doses of *Ph1* suggest that the effect of this locus on the diploidisation mechanism of tetraploid wheat occurs during meiotic prophase I. The *Ph1* locus would be mainly involved in the correction of homoeologous synapsis and in the suppression of crossing-over between homoeologous regions.

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