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ORIGINAL PAPER

Complete characterization of wheat–alien metaphase I pairing in interspecific hybrids between durum wheat (*Triticum turgidum* L.) and jointed goatgrass (*Aegilops cylindrica* Host)

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Abstract The pattern of homoeologous metaphase I (MI) pairing has been fully characterized in durum wheat × *Aegilops cylindrica* hybrids ($2n = 4x = 28$, ABC^cD^c) by an in situ hybridization procedure that has permitted individual discrimination of every wheat and wild constituent genome. One of the three hybrid genotypes examined carried the *ph1c* mutation. In all cases, MI associations between chromosomes of both species represented around two-third of total. Main results from the analysis are as follows (a) the A genome chromosomes are involved in wheat–wild MI pairing more frequently than the B genome partners, irrespective of the alien genome considered; (b) both durum wheat genomes pair preferentially with the D^c genome of jointed goatgrass. These findings are discussed in relation to the potential of genetic transference between wheat crops and this weedy relative. It can also be highlighted that inactivation of *Ph1* provoked a relatively higher promotion of MI associations involving B genome.

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Introduction

Bread wheat (*Triticum aestivum* L.; $2n = 6x = 42$, AABBDD) and durum wheat (*T. turgidum* L.; $2n = 4x = 28$, AABB) can hybridize in nature with some closely related species when they grow in sympatry and have overlapping flowering periods (Jacot et al. 2004; Zaharieva and Monneveux 2006). Amongst their wild relatives, the jointed goatgrass (*Aegilops cylindrica* Host; $2n = 4x = 28$, $C^cC^cD^cD^c$) receives special attention; first, because of its weedy nature in wheat crop areas in North America, Asia and Europe, but mainly because transference of herbicide resistances from bread wheat into wild populations of *Ae. cylindrica* has been demonstrated (Seefeldt et al. 1998). Based on those evidences, wheat crops have been included in the group of crops with moderate risk of transgene escape, for which studies to minimize the possibility of unintended gene escapes are recommended (Stewart et al. 2003).

Homoeologous chromosome pairing is one of the critical steps for long-term successful incorporation of genetic sequences from a crop into a wild genome since only crop genome segments inherited as recombinant crop–wild chromosomes are stably transmitted to the next generations. Thus, the level and pattern of metaphase I (MI) pairing between wheat and wild homoeologues in the hybrids inform on which genome regions of the crop have a higher chance for introgression (Tomiuk et al. 2000; Wang et al. 2001).

MI pairing analysis conducted by conventional staining in bread wheat × *Ae. cylindrica* hybrids ($2n = 5x = 35$, $ABDC^cD^c$) led to designate A and B wheat genome as safe places for transgene integration in transformed wheat lines based upon the premise that all chromosome associations observed in these hybrids had to involve the shared D

genome (Zemetra et al. 1998; Wang et al. 2000). However, studies based on molecular markers support that wheat–wild recombination affecting A and B chromosomes can occur (Galaev et al. 2004; Schoenenberger et al. 2005). Actually, Wang et al. (2000) evidenced by genomic in situ hybridization (GISH) the transmission to hybrid-derived progenies of an intergenomic exchange involving a wheat chromosome that did not belong to the D genome, but their GISH procedure was unable to discriminate if the introgressed crop segment corresponded to the A or to the B genome. In a previous paper, we examined homoeologous MI pairing for individualized A and B wheat genomes in interspecific hybrids between durum wheat and *Ae. geniculata* ($2n = 4x = 28$, ABU^gM^g), which demonstrated that A genome chromosomes were more frequently involved in wheat–wild MI association than B genome chromosomes (Cifuentes et al. 2006). If applied to the analysis of hybrids involving *Ae. cylindrica*, a similar approach can provide valuable information to separately assess the potential of gene escape to jointed goatgrass from each of these wheat genomes.

Introgression in opposite direction as considered above (i.e. from a wild into a crop genome) is the goal in breeding programs aimed to incorporate alien desirable traits into cultivated plants. Here, again, the amount and pattern of MI chromosome association in the hybrids are critical when homoeologous recombination-based protocols are followed. In the case of wheat crops, the main impediment is imposed by the *Ph1* locus (Sears 1976) which restricts MI pairing to homologous partners by preventing crossing over (and, therefore, recombination) between homoeologues even if perfectly synapsed (Gillies 1987). Thus, the use of mutants for *Ph1* has become a very effective strategy to minimize efforts in order to produce wheat–alien transfers (see Ceoloni and Jauhar 2006; Qi et al. 2007). These mutants are *ph1b* (Sears 1977) and *ph1c* (Giorgi 1978) in bread and durum wheat, respectively.

The final objective of this work was to determine the pattern of wheat–wild MI association in hybrids between durum wheat and jointed goatgrass. As a previous requirement, we had to develop a GISH procedure to identify individual genomes in these hybrids.

Materials and methods

Plant material

Manual crosses were made between *T. turgidum* ssp. *durum* and *Ae. cylindrica*. The wild accession used as female parent is maintained by selfing since the 1980s in the seed bank of Escuela Técnica Superior de Ingenieros Agrónomos (UPM, Madrid), but its geographical origin is unknown. Three different wheat genotypes were used as male parents: cultivars Langdon (L) and Cappelli (Cp), and the mutant line *ph1c* obtained in Cappelli (Cp*ph*) (Giorgi 1978). Hybrid plantlets ($2n = 4x = 28$, ABC^cD^c) were grown in a green-house until flowering. Anthers of the emerging spikes containing pollen mother cells (PMCs) at MI were fixed in 1:3 (v/v) acetic acid:ethanol and stored at −20°C for a minimum of 2 weeks. Then anthers were stained in 1% aceto-carmine for 20–30 min and squashed in 45% acetic acid. The slides were stored at 4°C prior to in situ hybridization.

DNA extraction, probe labelling and ISH

Total genomic DNAs were isolated from young leaves of the diploids *T. monococcum* and *Ae. speltoides* ($2n = 14$; AA and SS, respectively) following standard protocols. DNAs were labelled with digoxigenin-11-dUTP (A genome) or biotin-16-dUTP (S genome) by random priming, and then mechanically sheared by autoclaving to 0.5–1.5 kbp pieces. The pTa71 ribosomal DNA probe (Gerlach and Bedbrook 1979) and the pAs1 repeated DNA probe (Rayburn and Gill 1986) were labelled by nick translation. Labelling of probes was performed using standard kits from Roche following the manufacturer's instructions. The standard hybridization mix contained differentially labelled A and S genome probes (4 and 8 ng/µl, respectively), the ribosomal DNA probe (2.5 ng/µl, either digoxigenin or biotin labelled) and unlabelled genomic DNA from *Ae. cylindrica* sheared to 0.3–0.7 kbp by autoclaving (400 ng/µl) as blocking. Digoxigenin-labelled pAs1 probe (5 ng/µl) was included in the mix on the majority of the meiotic slides. In situ hybridization protocol was as described in Sánchez-Morán et al. (1999).

Immunological detection and visualization

Digoxigenin-labelled probes were revealed with 5 ng/µl goat antidigoxigenin antibody conjugated with fluorescein isothiocyanate (FITC, Roche), whereas biotinylated probes were detected with 5 ng/µl avidin conjugated with Cy3 dye (Roche). Slides were screened using an Axiophot epifluorescent microscope (Zeiss) equipped with a double filter for green and red fluorescence. Images were captured with a CoolSnap digital camera and processed with Adobe Photoshop v8.0 for brightness and contrast when required.

Statistical analysis

Statistical analyses have been performed with Statistix v8.0.

Results

The standard GISH hybridization mix used in this study allowed simultaneous identification of A and B genome chromosomes and their discrimination from the wild homoeologues in the wheat × *Ae. cylindrica* hybrids examined (Fig. 1). Preliminary observation of MI cells served to confirm that all of them had the expected $A^7 + B^7 + C^cD^{c14}$ karyotypic composition. Table 1 shows the numbers of meiotic configurations and the frequency of MI associations in each hybrid genotype. The level of MI paring was similar in those derived from durum wheat cultivars Langdon and Cappelli (c × L and c × Cp, respectively), but significantly lower than in the hybrid genotype carrying the *ph1c* mutation (c × Cp*ph*).

Discrimination amongst A, B and alien chromatin by GISH allowed to identify the following types of MI associations: intraspecific associations involving both wheat genomes (A–B), intraspecific associations involving both wild genomes (C^c–D^c), wheat–*Ae. cylindrica* associations

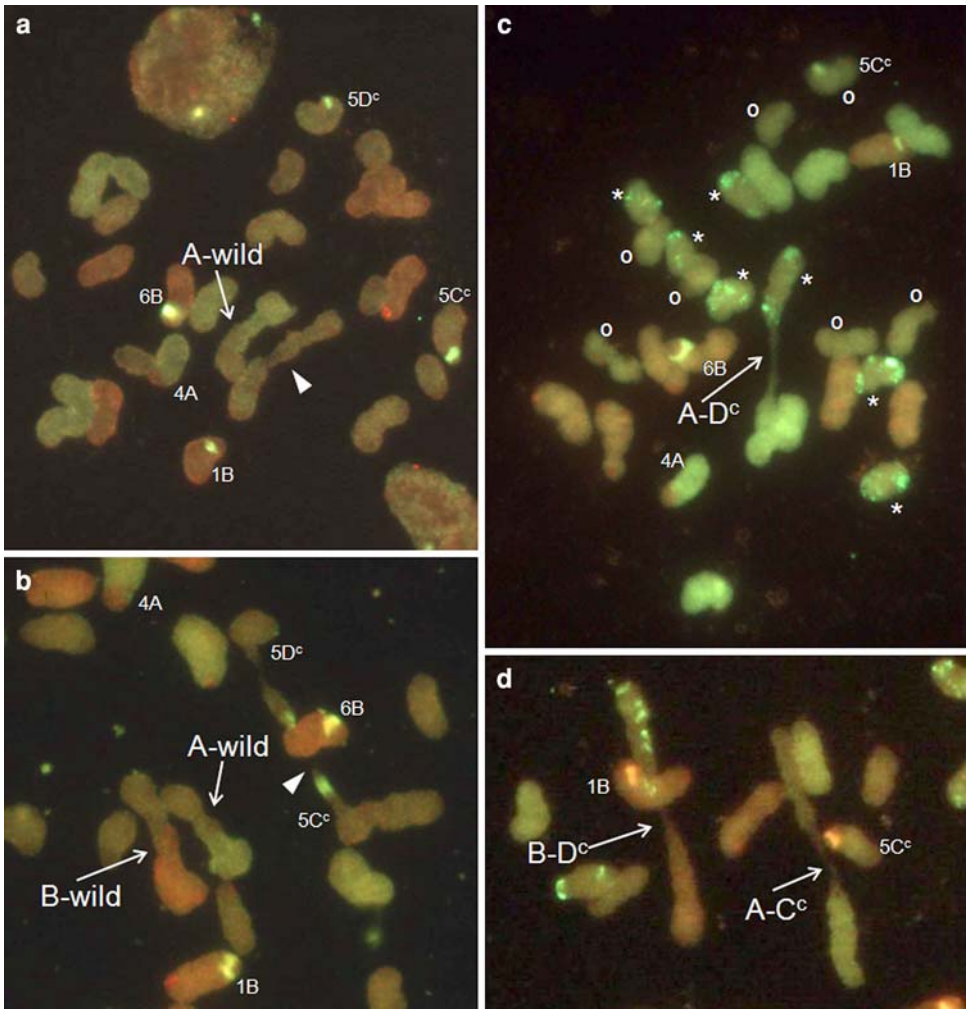

Fig. 1 Micrographies from MI cells of durum wheat × *Ae. cylindrica* interspecific hybrids ($2n = 4x = 28$, ABC^cD^c) after ISH combining differentially labelled A and S genomic DNA probes, and the ribosomal pTa71 probe. Wheat constituent genomes are homogeneously coloured in *green* (A chromatin) and *red* (B chromatin) whilst the blocked wild genome chromosomes are in *brown*. In **c** and **d**, the hybridization mixture included also the pAs1 probe, which produced distinctive fluorescent *green* signals on D^c genome chromosomes. Specific chromosomes that can be individually identified have been indicated. The pTa71 probe was digoxigenin labelled in **a**–**c** and DNA ribosomal sites are visualized as *yellow* or *green* signals depending on whether they correspond to a B genome (1B, 6B) or to a blocked wild genome ($5C^c$, $5D^c$) chromosome. In **d**, where pTa71 was used as biotin-labelled probe, ribosomal sites are recognized by *bright red* fluorescence. Complete (**a**) and partial (**b**) PMCs with MI association between the A or the B wheat genome and a wild genome (*arrows*). *Arrowheads* point out MI pairing involving both jointed goatgrass genomes, which in **b** involves the short arms of $5C^c$ and $5D^c$. **c** PMC with discrimination between the two wild genomes based upon the presence (D^c chromosomes; *asterisks*) or absence (C^c chromosomes; *circles*) of pAs1 FISH signals. **d** Wheat–wild associations of the same types as shown in **b** where the wild genome has been identified according to its pAs1 FISH pattern

Table 1 Meiotic configurations at metaphase I in durum wheat × *Ae. cylindrica* hybrids

Hybrid	Cells	MI pairing configuration					MI associations	
		I	Rod II	Ring II	III	IV	Total	Mean/cell
c × L	276	7,349	188	0	1	0	190	0.69
c × Cp	800	21,878	261	0	0	0	261	0.33
c × Cp*ph*	83	1,497	327	25	41	0	459	5.53

I univalent, *II* bivalent, *III* trivalent, *IV* quadrivalent

Table 2 MI associations in durum wheat × *Ae. cylindrica* hybrids

Hybrid	Intraspecific MI associations		Wheat–wild MI associations		Others[a]
	A–B	C^c–D^c	A–wild	B–wild	
c × L	13 (0.05)	44 (0.16)	120 (0.43)	13 (0.05)	0 (0.00)
c × Cp	18 (0.02)	73 (0.09)	148 (0.19)	19 (0.02)	3 (0.004)
c × Cp*ph*	64 (0.77)	96 (1.16)	212 (2.55)	83 (1.00)	4 (0.05)

Mean values per cell are in parentheses

[a] Includes non-homologous associations and multiple (non-two-by-two) chromosome arm associations

involving the A wheat genome (A–wild) and wheat–*Ae. cylindrica* associations involving the B wheat genome (B–wild) (Fig. 1a, b). Their frequencies are given in Table 2. A–B association accounted for 7% of total in hybrids from Langdon and Cappelli, but increased up to 14% when from the *ph1c* mutant parent. The level of MI pairing between the wild homoeologues (C^c–D^c associations) slightly varied amongst genotypes (21–28%). Wheat–wild MI associations represented about two-third of total associations in all cases, A genome being always more frequently associated with the wild homoeologues than B genome. So, A–wild association represented 90% of wheat–wild MI pairing in hybrids c × L and c × Cp, and 72% in the hybrid c × Cp*ph*.

In order to determine whether those apparent between-genotype differences had any statistical significance, the hybrids were two-by-two when compared by means of contingency χ^2 tests (Table 3). This demonstrated identical MI pairing patterns in c × L and c × Cp. The hybrid genotype carrying the *ph1c* mutation did not statistically differ from the others regarding the ratio of intraspecific:wheat–wild MI paring, but it showed a higher frequency of intraspecific MI associations between the two wheat genomes as well as a highly significant increase of wheat–wild associations involving the B wheat genome.

The four genomes present in the hybrids could be simultaneously discriminated in MI cells where the D-genome-specific repeated DNA probe pAs1 was added to the A and S genomic DNA probes in the hybridization mix. This was the case in 71, 88 and 100% of the PMCs scored in c × L, c × Cp and c × Cp*ph* genotypes, respectively. This technical procedure permitted to recognize whether the wild

Table 3 Contingency χ^2 tests for comparison of MI associations between durum wheat × *Ae. cylindrica* hybrid genotypes

Hybrid genotypes compared	MI associations compared, χ^2		
	Intraspecific vs. wheat–wild	A–B vs. C^c–D^c	A–wild vs. B–wild
c × L–c × Cp	1.37^{ns}	0.19^{ns}	0.20^{ns}
c × L–c × Cp*ph*	1.60^{ns}	5.43*	17.76***
c × Cp–c × Cp*ph*	0.00^{ns}	10.78**	17.41***

In all tests performed the number of degrees of freedom is equal to 1

ns not significant ($P > 0.05$), *$P > 0.01$, **$P > 0.001$, ***$P < 0.001$

pairing partner belonged to the C^c or to the D^c genome in A–wild and B–wild associations (Fig. 1c, d). Full characterization of MI homoeologous pairing in the ABC^{c}D^c hybrids was then possible. It evidenced that both durum wheat genomes paired preferentially with the D^c alien genome in any of the genotypes under study (Table 4). Taking into account that no significant difference had been detected between the MI pairing patterns of hybrids c × L and c × Cp (Table 3), these genotypes were pooled and renamed as c × [L + Cp] to perform further statistical comparisons. The relative amount of MI associations with the B genome resulted greater in c × Cp*ph* than in c × [L + Cp] for any of both wild genomes, the differences being statistically significant (A–C^c vs. B–C^c: $\chi^2 = 14.65$, $P < 0.001$, 1 degree of freedom; A–D^c vs. B–D^c: $\chi^2 = 11.50$, $P < 0.001$, 1 degree of freedom). This was in full agreement with the observed when the frequencies of A–wild and B–wild MI associations had been earlier compared between hybrids lacking or having *ph1c* (Table 3). However, no significant

Table 4 Types of wheat–wild MI associations in durum wheat × *Ae. cylindrica* hybrids after individual discrimination of the four constituent genomes

Hybrid	Cells	Wheat–wild MI association			
		A–C^c	A– D^c	B–C^c	B–D^c
c × L	196	23	56	1	8
c × Cp	706	34	97	4	12
c × [L + Cp]		57 (24.3)	153 (65.1)	5 (2.1)	20 (8.5)
c × Cp*ph*	83	55 (18.6)	157 (53.2)	30 (10.2)	53 (18.0)

Their relative proportions expressed as percentages of total wheat–wild MI pairing are in parentheses

differences were obtained when the proportions of B–C^c versus B–D^c associations in the two genotypic classes were contrasted ($\chi^2 = 2.29$, $P > 0.05$, 1 degree of freedom). This evidenced that the increase of B–wild homoeologous association demonstrated under the effect of *ph1c* mutation affected to the same extent the two constituent genomes of *Ae. cylindrica*. Statistical differences were not detected when the test included A–B associations (20 and 64, respectively, in the MI cell samples of c × [L + Cp] and c × Cp*ph* hybridized with pAs1), and a 3 × 2 contingency table compared the three types of MI pairing involving the B genome in hybrids with active and inactive *Ph1* (A–B vs. B–C^c vs. B–D^c: $\chi^2 = 2.28$, $P > 0.05$, 2 degrees of freedom).

As illustrated in Fig. 1, some specific chromosomes were recognized although their MI pairing has not been individually analysed. Identification was based upon their distinctive GISH pattern (i.e., 4A, which carries an intergenomic A/B translocation in its long arm) or FISH signals for pTa71 (i.e., 1B and 6B). Distinction between the alien chromosomes bearing major nucleolus organizer regions (5C^c and 5D^c) was also possible, even in PMCs where the C^c and D^c wild genomes were not discriminated, because of the different size of their ribosomal DNA sites (Badaeva et al. 2002) (Fig. 1a, b).

Discussion

All former MI pairing analyses on hybrids between wheat and *Ae. cylindrica* have been conducted by conventional staining procedures (Giorgi and Barbera 1981; Bai et al. 1995; Zemetra et al. 1998). These techniques are unable to differentiate individual genomes if, as in the Triticeae, homoeologous partners show similar chromosomal morphologies. This prevents from reliably determining the level and pattern of wheat–wild MI associations in the hybrids, since the goal requires cytological discrimination between parental genomes. Thus, GISH is currently the most extended analytical tool not only in the case of wheats and related species but also for most other important crops (see Benavente et al. 2008). Only two previous studies have reported the use of GISH to visualize specific genomes in wheat × jointed goatgrass hybrids or derived progenies. Using genomic DNA of *Ae. caudata* L. ($2n = 2x = 14$, CC) as a probe, Wang et al. (2000) achieved discrimination of C^c genome chromosomes in hybrids between *T. aestivum* and *Ae. cylindrica* and in their backcross progenies. Later, Wang et al. (2002) demonstrated that such procedure is a useful tool to determine whether a BC_1 individual derives from a hybrid backcrossed to bread wheat or to jointed goatgrass. Wang et al. (2000) also evidenced the retention and involvement in intergenomic translocations of wheat chromosomes that did not belong to the D genome in the second selfed progeny from a hybrid backcrossed to jointed goatgrass twice (BC_2S_2 individuals) when durum wheat genomic DNA was used as the labelled probe. But they could not discern whether the introgressed wheat chromosomes or segments belonged to the A or to the B genome.

In an earlier study, A and B genomes were individually recognized in *T. turgidum* × *Ae. geniculata* interspecific hybrids using differentially labelled genomic DNA from their diploid donors as probes (Cifuentes et al. 2006). We have successfully adapted that protocol to hybrids between durum wheat and jointed goatgrass by simply replacing the wild species genomic DNA used as blocking DNA (Fig. 1a, b). Furthermore, the inclusion of the D-genome-specific pAs1 repeated DNA probe in the hybridization mix has permitted a full characterization of homoeologous MI pairing pattern in the hybrids (Fig. 1c, d). Linc et al. (1999) had used this probe to discriminate C^c from D^c genome chromosomes in *Ae. cylindrica* accessions. We have now proved its usefulness to discern the genome origin of jointed goatgrass chromosomes when in a durum wheat background. Besides, our results evidence that combination of two differentially labelled genomic probes with an appropriate genome-specific repeated DNA probe permits easy and simultaneous visualization of four distinct, though closely related, genomes, which obviously may increase the potential uses of in situ hybridization procedures for the analysis of multigenomic materials.

As expected, inactivation of *Ph1* locus resulted in a significant increase of MI associations (Table 1). However, the overall levels of MI pairing found here are much lower than those reported by Giorgi and Barbera (1981) in durum wheat × *Ae. cylindrica* hybrids derived from the mother cultivar Cappelli and its *ph1c* mutant (1.86 and 9.58, respectively). Such striking discrepancies could be attributable to the presumably distinct wild parental accessions used in the two studies, as has been evidenced in wheat × *Ae. geniculata* hybrids (Farooq et al. 1996). Nevertheless, the influence of non-genotypic factors known to affect the pairing and chiasma formation processes

(e.g., Bayliss and Riley 1972; Stern 1986) should not be ruled out. It can be noted that the frequencies of A–B associations per cell in the hybrids examined in the present study (see Table 2) are also lower, although maintaining the ratio, than those observed in haploids from *Ph1* and *ph1c* durum wheats (0.23 and 3.00, respectively) by Jauhar et al. (1999).

Despite the different levels of MI pairing in the durum wheat × *Ae. cylindrica* hybrids examined here, their homoeologous MI pairing pattern and its derived consequences can be generalized as follows. Wheat–wild associations are significantly more abundant than intraspecific MI associations, remarkably those involving the A genome chromosomes which represent around 60% of total in hybrids c × L and c × Cp (Table 2). Although these are hybrids with limited MI pairing, A–*Ae. cylindrica* recombinant chromosomes can be generated in almost half of their PMCs. On their turn, B genome chromosomes associate with the wild homoeologues in 2–5% of meiocytes. The potential of gene flow from any of both wheat genomes will obviously increase as the overall level of MI pairing does. This is well exemplified in the c × Cp*ph* genotype where 3.5 wheat–wild associations per cell have been observed. Results in Table 4 further support that, irrespective of their location within the crop genome, stable incorporation of durum wheat genes will more likely occur into D^c genome chromosomes. Conversely, from a breeding perspective, any agronomically favourable trait present in jointed goatgrass will be more difficult to introgress into durum wheat by recombination-mediated strategies if the responsible gene(s) is allocated on the C^c genome. It agrees with the exclusive finding of wheat–*Ae. cylindrica* addition lines within the resistant progeny of advanced backcross generations for leaf and stem rust resistance genes that had been assigned to C^c genome chromosomes (Bai et al. 1995).

Preferential A–D MI association has been reported in haploids of bread wheat ($2n = 3x = 21$, ABD) (Jauhar et al. 1991) and in hybrids between hexaploid wheat and related species (e.g., Naranjo et al. 1987). It has been attributed to a higher MI pairing affinity between the A and D wheat genomes compared to other pairwise combinations. The pattern of homoeologous association in ABC^cD^c hybrids demonstrates that A genome is also closer related, at least in terms of pairing relationship, with the D^c genome of *Ae. cylindrica*. This agrees with previous evidences on the high homology between the D genomes of wheat and *Ae. cylindrica* (Kimber and Zhao 1983; Rayburn and Gill 1987; Badaeva et al. 2002).

Wang et al. (2001) assumed that all MI pairing in *T. aestivum* × *Ae. cylindrica* hybrids involved the common D genome and then concluded that the A and B genomes were safe sites for transgene integration. However, the observation of MI cells containing more than seven bivalents (Bai et al. 1995; see also Fig. 1B in Zemetra et al. 1998) and different molecular marker analyses (e.g., Galaev et al. 2004) support that A and/or B bread wheat chromosomes can actually pair with jointed goatgrass partners. Unless individual genomes are discriminated it is not possible to characterize the homoeologous MI pairing pattern of $ABDC^cD^c$ hybrids, but some picture can be attempted based upon the results reported in Table 4. It is expected that the majority of wheat–wild MI associations, other than $D–D^c$, involves A genome chromosomes. If so, A genome markers will be more frequently transmitted as wheat–wild exchanges to the derived generations than B genome markers. The latter, mostly inherited as retained wheat chromosomes, will be easily lost in the hybrid lineage. Schoenenberger et al. (2005) reported introgression of A and B genome molecular markers in BC_1 progenies from bread wheat × *Ae. cylindrica* hybrids backcrossed to the wild parent. In agreement with that inferred from our observations in durum wheat hybrids, the B genome marker was never found in the BC_1S_1 generation (first selfed progeny from BC_1 plants) whilst retention of the A genome marker was evidenced in some individuals having 28 chromosomes. The presence of a common D genome will surely modify the pattern of meiotic pairing in hybrids between bread wheat and *Ae. cylindrica*. Nevertheless, it would be interesting to check whether, and then how much, B genome chromosomes associate there with the wild homoeologues in order to reliably assess the potential of stable gene transfer to jointed goatgrass from this a priori safest wheat genome.

A final consideration must be made on the finding that not only the level but also the pattern of homoeologous association is altered under the effect of *ph1c* mutation. Intraspecific associations of type A–B and wheat–wild associations of type B–wild were significantly more frequent in the hybrid c × Cp*ph* than in hybrids c × L and c × Cp (Table 3). Similar results were obtained after comparison between durum wheat × *Ae. geniculata* hybrids derived from Langdon and from the Creso *ph1c* mutant (Cifuentes et al. 2006). Jauhar and Peterson (2006) examined A–wild and B–wild MI pairing by GISH in hybrids between durum wheat and *Thynopirum bessarabicum* ($2n = 2x = 14$, JJ). These authors reported also a greater increase of B–J than A–J MI pairing in ABJ hybrids carrying a 5D(5B) substitution (therefore, lacking *Ph1* activity) compared to the observed in euploid hybrids. All these evidences support that inactivation of *Ph1* provokes a particularly remarkable induction of homoeologous MI pairing on B genome chromosomes. Comparative analysis of genotypes c × [L + Cp] and c × Cp*ph* has further demonstrated that such a greater promoting effect similarly affects all types of homoeologous associations involving the B genome, irrespective of its pairing partner (see "Results"). The primary mode of action of locus *Ph1* to suppress crossing

over between homoeologues is still under debate (e.g., Dvorak and Lukaszewski 2000; Prieto et al. 2005; Corredor et al. 2007) whilst disclosing its molecular structure progresses (e.g., Gill and Gill 1991; Segal et al. 1997; Griffiths et al. 2006; Sidhu et al. 2008). Both questions need to be answered if a controlled use of *Ph1* alteration for alien gene introgression into cultivated wheats is intended (Able and Langridge 2006). Studies revealing side effects of *Ph1* mutations on homoeologous MI pairing, as the reported here, should then be taken into account for a definite functional characterization of this locus, mainly responsible of the meiotic stability and fertility of cultivated wheats.

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