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ORIGINAL PAPER

Wheat-alien metaphase I pairing of individual wheat genomes and D genome chromosomes in interspecific hybrids between *Triticum aestivum* L. and *Aegilops geniculata* Roth

Marta Cifuentes · Elena Benavente

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Abstract Homoeologous metaphase I (MI) pairing of *Triticum aestivum* × *Aegilops geniculata* hybrids ($2n = 5\times = 35$, ABDU^{g}M^{g}) has been examined by an in situ hybridization procedure permitting simultaneous discrimination of A, B, D and wild genomes. The seven D genome chromosomes (and their arms, except for 6D and 7D) plus some additional wheat chromosomes were also identified. Wheat-wild MI associations represented more than 60% of total, with an average ratio of 5:1:12 for those involving the A, B and D genomes, respectively. A remarkable between-chromosome variation for the level of wheat-wild genetic exchange is expected within each wheat genome. However, it can be concluded that 3DL and 5DL are the crop genome locations with the highest probability of being transferred to *Ae. geniculata*. Hybrids derived from the *ph2b* wheat mutant line showed increased MI pairing but identical pattern of homoeologous associations than those with active *Ph2*.

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M. Cifuentes · E. Benavente (✉)
Departamento de Biotecnología,
Escuela Técnica Superior de Ingenieros Agrónomos,
Universidad Politécnica de Madrid,
Ciudad Universitaria s/n, 28040 Madrid, Spain
e-mail: e.benavente@upm.es

Present Address:
M. Cifuentes
Station de Génétique et d'Amélioration des Plantes,
Institut Jean-Pierre Bourgin, INRA UR 254,
Route de Saint-Cyr, 78026 Versailles, France

Introduction

Wild species have traditionally been a matter of interest as genetic pools for introgression of agronomically beneficial traits into crops. The development of transgenic varieties has opened a new conceptual frame for research on those relatives that can hybridize with crops in nature because of the possible economic and/or ecological consequences of crop-to-wild gene flow (Ellstrand et al. 1999; Stewart et al. 2003). In the case of wheats, most of the attention from this recent perspective has been focused on *Aegilops cylindrica*, a weed for which introgression of herbicide resistance from bread wheat has been demonstrated (Seefeldt et al. 1998), but other related species should not be ignored.

The ovate goatgrass *Aegilops geniculata* Roth ($2n = 4\times = 28$, U^{g}U^{g}M^{g}M^{g}), one of the two wheat wild relatives most extended all over the world (Van Slageren 1994), meets the two scenarios noted above. So, based upon its ability to grow in varied extreme environmental conditions, this wild relative has been proposed to represent a helpful reservoir of genes involved in physiological adaptations to distinct abiotic stresses (Zaharieva et al. 2001a; Colmer et al. 2006). Resistances to several wheat pathogens have also been described (Gill et al. 1985; Zaharieva et al. 2001b), and some of them have indeed been successfully introgressed into crop wheat varieties (see Schneider et al. 2008). On the other hand, neither flowering time nor other prezygotic constraints constitute insurmountable barriers for hybridization between wheat and *Ae. geniculata* when they grow in sympatry (Zaharieva and Monneveux 2006). Accordingly, spontaneous hybrids in durum and bread wheat field margins are documented (Van Slageren 1994; Jacot et al. 2004). F_1 hybrids are mostly sterile, but viable seeds resulting in amphiploids with a higher fertility have been found (David et al. 2004; Loureiro et al. 2008). Thus

these hybrid-derived forms constitute an open route for unintended gene transfer from wheat to this wild relative. *Ae. geniculata* is not generally considered a weedy species, but its invasive behaviour could have risky consequences if wild populations acquired herbicide-resistance genes. Furthermore, the higher hybridization rates of ovate goatgrass with wheat compared to jointed goatgrass has led to predict that releasing of genetically modified wheat varieties will probably have a greater impact on *Ae. geniculata* than on *Ae. cylindrica* in areas, as Southern Europe, where the former shows a much wider geographical distribution (Schoenenberger 2005).

Either in nature or for breeding objectives, stable genetic exchange between wheat and a wild relative requires meiotic pairing and recombination between their corresponding genomes in the F_1 hybrids or in subsequent selfing or backcross progenies. Hence the pattern of wheat-wild chromosome associations at metaphase I (MI), when pairing and recombination have taken place, informs about which genome locations can be more easily transferred (see Benavente et al. 2008). In a former study, homoeologous MI pairing of interspecific hybrids between *Ae. geniculata* and *Triticum turgidum* L. ($2n = 4\times = 28$, AABB) was examined by a genomic in situ hybridization (GISH) procedure that simultaneously discriminate A and B wheat genomes from the wild homoeologues (Cifuentes et al. 2006). Then the potential of genetic transfer to *Ae. geniculata* from specific durum wheat genome regions could be established. The present study aimed to perform an equivalent analysis in hybrids of bread wheat, *T. aestivum* L. ($2n = 6\times = 42$, AABBDD). This is the wheat crop most common on a worldwide scale thus offering more chances than durum wheat for unintended hybridization. The presence of an additional set of homoeologues (D genome) increases the difficulty to individualize wheat genomes by GISH in these hybrids; even more when their simultaneous discrimination represents a technical goal in order to facilitate the scoring of large MI cell samples. To solve this problem we have recourse to pAs1, a D-genome-specific repeated DNA sequence isolated from *Ae. squarrosa* by Rayburn and Gill (1986). When used as a probe for in situ hybridization (ISH), pAs1 has proved to be suitable for labeling of D genome and for cytological identification of individual D genome chromosomes in bread wheat (e.g., Mukai et al. 1993; Hernandez et al. 2008) and other wild relatives (e.g., Rayburn and Gill 1987; Badaeva et al. 2002).

Quantitative MI pairing analyses of interspecific hybrids require that the number of observations for each type of MI association that can be discriminated is large enough to perform reliable statistics. Homoeologous pairing in wheat hybrids is largely prevented by two main loci, *Ph1* and *Ph2*, and some other minor loci that assure a diploid-like meiotic behaviour of polyploid wheats (Sears 1976). Wheat parental lines carrying *ph* mutations, like *ph1b* (Sears 1977) or *ph2b* (Wall et al. 1971; Sears 1984), can then significantly reduce the amount of pollen mother cells (PMCs) that need to be examined in a hybrid. A higher increment of MI pairing between homoeologues is achieved by using the *ph1b* mutant line (e.g., Naranjo and Maestra 1995; Benavente et al. 1998). However, Sanchez-Moran et al. (2001) demonstrated by GISH that plants with inactive *Ph1* genes, either by deficiency for 5BL or by mutation, can show aneuploid aberrant karyotypes and that intergenomic rearrangements are *de novo* generated at an averaged frequency of 0.95/plant/generation. The existence of chromosomal alterations in a *ph1b* plant, which cannot be totally ruled out even after detailed cytological examination, would have unpredictable consequences on the homoeologous pairing pattern of its hybrid progeny. For that reason, we decided to use the *ph2b* mutant line as wheat parent which behaves as karyotypically stable (Sanchez-Moran et al. 2001). In addition to, comparison with hybrids derived from the mother cultivar Chinese Spring could also serve to determine whether mutations at *Ph2* alter the pattern of homoeologous MI association, as evidenced for *Ph1* in hybrids between durum wheat and *Ae. cylindrica* (Cifuentes and Benavente 2009).

Materials and methods

Plant material

Interspecific hybrids between *T. aestivum* and *Ae. geniculata* ($2n = 5\times = 35$, $ABDU^gM^g$) were obtained by manual crossing. Spikes of the Moroccan ovate goatgrass accession INRA-103 (kindly provided by Dr. J. David; INRA, Montpellier) were emasculated and hand pollinated with bread wheat cultivar Chinese Spring (CS) and its *ph2b* mutant line (CS*ph2*). Five plants of each hybrid genotype were used in this study. Anthers of the emerging spikes containing PMCs at metaphase I were fixed in 1:3 (v/v) acetic acid:ethanol and stored at −20°C for a minimum of 2 weeks. Then anthers were squashed in 45% acetic acid and 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 *Ae. geniculata* and of the wheat diploid relatives *T. monococcum*, *Ae. speltoides* and *Ae. squarrosa* ($2n = 2\times = 14$; AA, SS and DD, respectively) following standard protocols. Diploid species genomic DNAs were labelled with digoxigenin-11-dUTP (A genome) or biotin-16-dUTP (S and D genomes) by random priming, and then mechanically sheared by autoclaving to 0.5–1.5 Kbp pieces. The

D-genome-specific pAs1 repeated DNA probe (Rayburn and Gill 1986) and the pTa71 ribosomal DNA probe (Gerlach and Bedbrook 1979) were labelled by nick translation. Labelling of probes was performed using standard kits from Roche following the manufacturer's instructions. The hybridization mix contained differentially labelled A and S genome probes (4 and 8 ng/µl, respectively), digoxigenin-labelled pAs1 probe (5 ng/µl) and the ribosomal DNA probe (2.5 ng/µl), either digoxigenin labelled, biotinylated or as mixture of digoxigenin- and biotin-labelled pTa71. In some experiments, the probe mix included also biotin-labelled D genomic DNA (4 ng/µl). Unlabelled genomic DNA from *Ae. geniculata* sheared to 0.3–0.7 Kbp by autoclaving was always added (400 ng/µl) to block shared DNA sequences. ISH protocol was essentially as described in Sanchez-Moran et al. (1999).

Immunological detection and visualisation

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 fluorescein and avidin 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

Combination of A and S genomic DNA probes and the D-genome-specific pAs1 repeated DNA probe in the hybridization mix has allowed simultaneous identification of the three wheat constituent genomes and their discrimination from the wild partners (U^g and M^g genomes) in MI cells of the interspecific hybrids examined. The inclusion of labelled *Ae. squarrosa* genomic DNA in the probe mix resulted in a distinct colouring of D genome chromatin but it was required neither to differentiate D genome nor for recognition of specific D chromosomes. Additionally, the ribosomal pTa71 probe has permitted to trace the major NOR bearing chromosomes 1B, 6B, $1U^g$ and $5U^g$. ISH analysis of both root tips and PMCs confirmed that all the hybrid plants examined had 35 somatic chromosomes and the expected $A^7 + B^7 + D^7 + U^gM^{g14}$ genome composition (Fig. 1a, b).

Table 1 shows the meiotic configurations observed in the hybrid plants examined. Almost all MI associations were distal and rod bivalent was the predominant configuration. As expected, the level of MI paring was higher in wheat × *Ae. geniculata* hybrids from the *ph2b* mutant line (g × CS*ph2*) than in those from wild-type cultivar Chinese Spring (g × CS), the difference between both hybrid genotypes being statistically significant ($t = 5.48$; $P < 0.001$; $df = 8$). Discrimination among A, B, D and wild chromosomes by ISH informed on which genomes were involved in each meiotic pairing configuration. Thus the following main classes of MI associations could be distinguished: intraspecific associations involving wheat genomes (A–B, A–D and B–D), intraspecific associations involving both wild genomes (U^g–M^g), and interspecific wheat-wild associations (A–wild, B–wild and D–wild) (Fig. 1b–g). The absolute frequencies of each type of MI association in the hybrids are given in Table 2. Wheat-wild MI associations represented more than 60% of total in the two $ABDU^gM^g$ genotypes, those involving D genome being the most frequent. B genome chromosomes showed the lowest level of MI pairing either with the wheat or wild homoeologues. The contingency χ^2 test performed to statistically compare the distribution of homoeologous MI associations in hybrids carrying and lacking the *ph2b* mutation demonstrated no significant difference between the MI pairing patterns in both bread wheat × *Ae. geniculata* genotypes ($\chi^2 = 6.94$; $P > 0.05$; $df = 6$).

The meiotic pAs1 FISH patterns of D genome chromosomes were not identical as described in mitotic cells (e.g., Mukai et al. 1993), and some critical fluorescent signals showed different relative positions and sizes depending on whether the affected chromosome arm was paired or unpaired (Fig. 1). Nevertheless, all seven D chromosomes (and their arms, except in the case of 6D and 7D) were undoubtedly identified in a large sample of PMCs, where their homoeologous partner could then be scored. Taking into account that the three types of MI associations involving D genome (A–D, B–D and D–wild) showed a similar distribution in g × CS and g × CS*ph2* hybrids ($\chi^2 = 4.97$; $P > 0.05$; $df = 2$), data from the two $ABDU^gM^g$ genotypes have been pooled in Table 3. Chromosome 3D showed the highest level of pairing (15.8% of PMCs) whereas 4D was the less frequently involved in MI association (6.6% of PMCs). Differences among chromosome arms were remarkable, with less than 1% of MI pairing for 4DS while around 9% for 1DL, 3DL and 5DL. Regarding wheat pairing partners, A genome was always more frequent than B genome except for 5DL. However, a clear preferential MI association with the wild homoeologues has generally been found, 2DS, 2DL and 7D being the only exceptions. The presence of major NORs on *Ae. geniculata* chromosomes $1U^g$ and $5U^g$ was used as a criterion for a more detailed

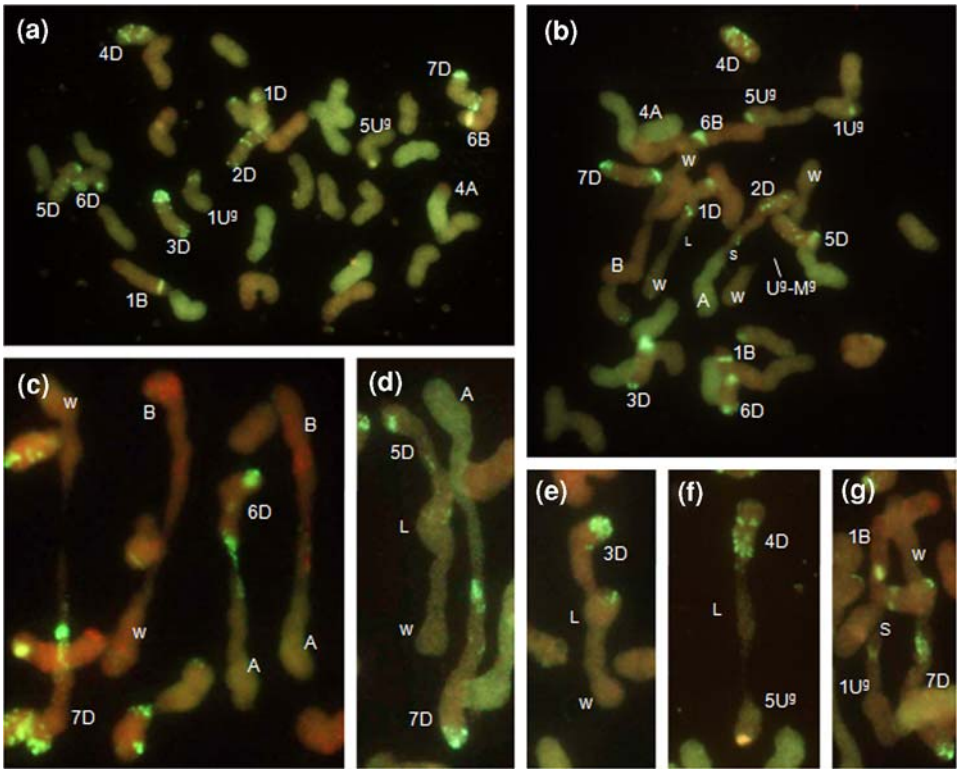

Fig. 1 Micrographies from PMCs of bread wheat × *Ae. geniculata* interspecific hybrids ($2n = 5\times = 35$, ABDU^{g}M^g) after ISH combining differentially labelled A and S genomic DNA probes, the D-genome-specific pAs1 probe and the ribosomal pTa71 probe. A and B wheat genomes are homogeneously coloured in *green* and *red*, respectively, whilst the blocked wild genome chromosomes are *brown*. The pAs1 FISH sites appear as *bright green* signals on D chromosomes, which are coloured in *red* when the probe mix included labelled *Ae. squarrosa* genomic DNA. Ribosomal sites on major NOR chromosomes (1B, 6B, 1U^g and 5U^g) are visualized in *green*, *yellow* or *red* according to the varied ratios of digoxigenin-:biotin-labelled pTa71 used in the experiments (see text). All chromosomes that have been individually recognized are indicated. **a** MI cell of a g × CS hybrid showing the whole chromosome complement as univalents. **b** MI cell of a g × CS*ph2* hybrid with four rod bivalents; one of them, that involves two *Ae. geniculata* partners, is indicated as 'U–M'. **c**–**g** Selected meiotic pairing configurations. In **b**–**g**, '*S*' and '*L*' indicate if MI associations occurred for the short or long chromosome arms; unidentified chromosomes of the A, B and *Ae. geniculata* genomes are noted by '*A*', '*B*' and '*w*', respectively

Table 1 Meiotic configurations at metaphase I in bread wheat × *Ae. geniculata* hybrids

Hybrid	PMCs	MI pairing configuration[a]				MI associations	
		I	Rod II	Ring II	III	Total	Mean/cell
g × CS-1	70	2,291	78	0	1	80	1.14
g × CS-2	34	1,085	51	0	1	52	1.53
g × CS-3	71	1,833	76	0	1	78	1.10
g × CS-4	149	4,127	144	0	3	150	1.01
g × CS-5	114	3,169	107	0	4	115	1.01
Total g × CS	438	12,505	456	0	10	475	1.08
g × CS*ph2*-1	54	1,341	84	0	1	86	1.59
g × CS*ph2*-2	31	741	59	0	2	65	2.10
g × CS*ph2*-3	71	1,911	134	0	4	142	2.00
g × CS*ph2*-4	26	790	57	0	2	61	2.35
g × CS*ph2*-5	57	1,503	109	1	8	115	2.02
Total g × CS*ph2*	239	6,286	443	1	17	469	1.96

[a] I, univalent; II, bivalent; III, trivalent

characterization of D-wild associations involving some D chromosome arms. For instance, Fig. 1b shows one MI association between 1DL and a wild homoeologue lacking a conspicuous pTa71 FISH signal (1 M^g) while 1U^g is present as a univalent. It must be noted that the U genome of *Ae. umbellulata* possesses the 4L/5L translocation present

Table 2 Frequency of MI homoeologous associations in bread wheat × *Ae. geniculata* hybrids (ABDU^{g}M^{g})

Hybrid	Intraspecific associations				Wheat–wild associations			Others[a]
	A–B	A–D	B–D	U^{g}–M^{g}	A–wild	B–wild	D–wild	
g × CS-1	2	12	2	16	14	5	29	0
g × CS-2	2	10	1	9	9	4	17	0
g × CS-3	4	15	3	10	14	2	30	0
g × CS-4	6	20	7	22	26	1	68	0
g × CS-5	5	13	2	17	22	4	52	0
Total g × CS	19	70	15	74	85	16	196	0
g × CS*ph2*-1	0	15	0	15	17	3	36	0
g × CS*ph2*-2	2	9	3	5	15	3	28	0
g × CS*ph2*-3	8	27	1	19	26	2	59	0
g × CS*ph2*-4	3	10	1	7	13	3	24	0
g × CS*ph2*-5	4	28	2	13	13	5	49	1
Total g × CS*ph2*	17	89	7	59	84	16	196	1

[a] non-homoeologous associations

in several Triticeae genomes, but absent in the D genome (King et al. 1994). As then expected, 5U^{g} was found to be paired with 5D when for its short arm but with 4D when for its long arm (Fig. 1f). For chromosome arms 1DS, 1DL, 5DS and 4DL, the numbers of MI associations with the U^{g} (marked by pTa71 signals) and the M^{g} (unmarked) homoeologues have been included in Table 3.

Wheat-wild associations were also scored for three additional wheat chromosomes that could be identified on the basis of their distinctive GISH pattern (4A) or ribosomal DNA probe signals (1B and 6B) (Fig. 1a, b, g). No pairing with *Ae. geniculata* chromosomes was found for 4A (S or L) nor for 6BL in the 677 MI cells examined in the hybrids, while 3, 3 and 2 MI associations with the wild homoeologues were recorded for 1BS, 1BL and 6BS, respectively.

Figure 2 shows the number of wheat-wild associations relative to 1,000 PMCs for all the wheat chromosomes and chromosome arms individualized in the study. It also includes the averaged frequency per chromosome for each wheat genome estimated from data of A–wild, B–wild and D–wild MI associations in Table 2.

Table 3 Frequency of homoeologous MI associations for individual D genome chromosomes and chromosome arms in the bread wheat × *Ae. geniculata* hybrids examined

Chromosome arm [Chromosome]	A–D	B–D	D–wild (U^{g} + M^{g})[a]	Total
1DS	2	0	26 (13 + 13)	28
1DL	9	1	27 (1 + 26)	37
[1D]	11	1	53	65
2DS	12	3	9	24
2DL	8	4	7	19
[2D]	20	7	16	43
3DS	2	0	26	28
3DL	9	1	31	41
[3D]	11	1	57	69
4DS	2	0	1	3
4DL	2	0	24 (19 + 5)	26
[4D]	4	0	25	29
5DS	3	0	11 (1 + 10)	14
5DL	2	2	33	37
[5D]	5	2	44	51
[6D]	18	3	35	56
[7D]	25	1	22	48

Data correspond to 438 PMCs (298 and 140 in g × CS and g × CS*ph2*, respectively)

[a] Numbers of MI associations involving the U^{g} and the M^{g} homoeologues for the D chromosome arms whose wild partner could be identified by distinctive pTa71 FISH signals (see text)

Discussion

In a previous report (Cifuentes et al. 2006), we examined by GISH the homoeologous MI pairing pattern of individual wheat genomes in interspecific hybrids between durum wheat and *Ae. geniculata*. Addition of the pAs1 repeated DNA probe to the standard hybridization mix used there has served to perform the analysis in bread wheat hybrids with a minimum technical modification. It can be noted that such a combination of labelled probes allows also identification of the four constituent genomes in durum wheat × *Ae. cylindrica* hybrids ($2n = 4\times = 28$, ABC^{c}D^{c}), where the pAs1 permits distinguishing between the two wild genomes (Cifuentes and Benavente 2009). Although the inclusion of labelled *Ae. squarrosa* genomic DNA in the probe mix was

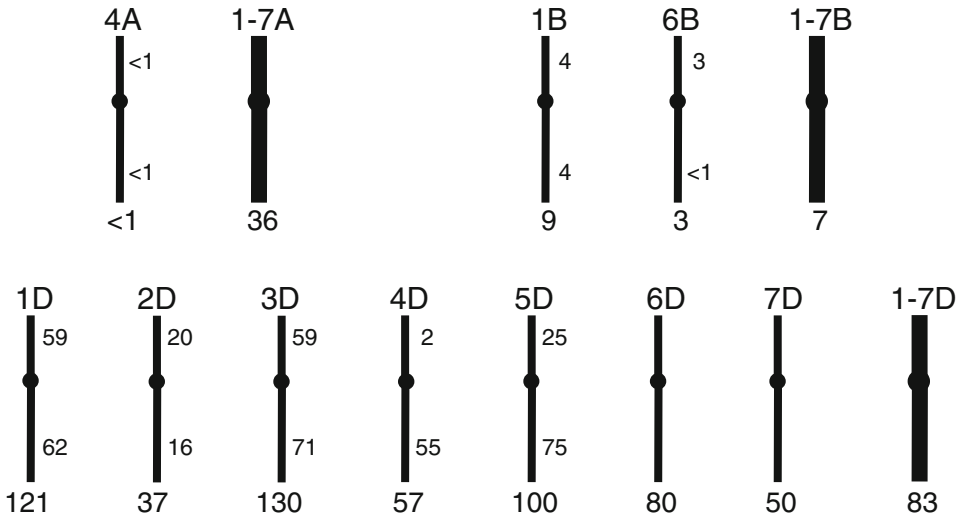

Fig. 2 Wheat-wild MI associations expected in 1,000 PMCs for the wheat chromosomes individually discriminated in bread wheat × *Ae. geniculata* hybrids. *Upper* and *lower values* in small lettering correspond to the short and long arms, respectively. The mean frequencies per A, B and D genome chromosome, estimated from data in Table 2, are indicated under the '1–7' headings

not necessary for identifying individual D genome chromosomes in MI pairing configurations of $ABDU^gM^g$ hybrids, it could facilitate physical characterization of alien segments introgressed into this wheat genome.

Once interspecific hybridization has occurred, long-lasting incorporation of crop genes into a wild species requires meiotic pairing and recombination between their corresponding genomes. Otherwise, crop markers eventually transmitted as complete chromosomes to the earlier hybrid progenies will be lost in the subsequent backcross generations. The degree of hom(oe)ology between parental genomes will then largely determine the probability of stable genetic transference. Weissmann et al. (2005) have reported spontaneous introgression of wheat B-genome-specific DNA into wild populations of *Ae. peregrina* ($2n = 4\times = 28$, SSUU), a species having one constituent genome (S) very closely related to the B genome. Introgression of wheat D genome markers into *Ae. cylindrica* ($2n = 4\times = 28$, $C^cC^cD^cD^c$) has also been demonstrated (Kroiss et al. 2004; Schoenenberger et al. 2006). The likelihood of unintended gene escape to wild relatives that do not have common genomes with wheat, though lower than to the formerly mentioned species, could be not negligible at a population scale. The case of *Ae. geniculata* may be specially relevant in Europe, where this is the most widespread wheat relative and spontaneous hybrids are documented since the XIX century (Van Slageren 1994; Zaharieva and Monneveux 2006).

The possibility that herbicide resistances can be easily transferred by homologous recombination to *Ae. cylindrica* has led to advise against the production of transgenic wheat varieties where the insert is localized on D genome chromosomes (Zemetra et al. 1998). Our study demonstrates that wheat genetic sequences on D genome are also the most likely to be stably incorporated into *Ae. geniculata* (Table 2). The preferential pairing observed in bread wheat haploids and interspecific hybrids between the A and D wheat genomes (e.g., Naranjo et al. 1987; Jauhar et al. 1991) has been explained by their closer relationship compared to other homoeologous combinations. Taken into account that D–wild (that is to say, $D–U^g$ plus $D–M^g$) MI associations are more than twice as frequent as A–D associations in $ABDU^gM^g$ hybrids, MI pairing affinity of D genome must be even higher with any of the two *Ae. geniculata* constituent genomes.

Nevertheless, it is also clearly evidenced that the pattern of MI pairing obtained for a given genome cannot be directly extrapolated to all the chromosomes belonging to that particular genome. So, as an average, 2/3 of MI pairing involving D genome chromosomes (A–D, B–D and D–wild associations in Table 2) occurs with their wild homoeologues, but the frequency of D–wild association is close to 90% for 1DS, 3DS, 4DL and 5DL while around 40% for 2DS, 2DL and 7D (Table 3). The negative influence of structural differentiation on homoeologous pairing has been adduced to explain the low level of MI association of chromosome arms 4DL and 5DL with the 4L/5L-rearranged A genome partners in bread wheat hybrids (e.g., Naranjo et al. 1987; Naranjo and Maestra 1995), a feature also observed in the present study. The remarkable variation in the number of D–wild associations between chromosomes and, in some instances, between their arms found in the hybrids (Table 3) could then be ascribed to extensive chromosome modifications known to have occurred during the evolution of *Ae. geniculata* and its diploid donors (Zhang et al. 1998; Badaeva et al. 2004). However, the finding of quite abundant MI association between 4DL and $5U^gL$ put into question structural dissimilarity as the sole explanation and confirms that other undisclosed differences must be governing the level of MI pairing between two homoeologous partners.

Figure 2 incorporates results on wheat–wild MI association for specific A and B genome chromosome arms and mean values per chromosome for the three wheat genomes.

This provides some clues for a more complete assessment on the relative chance for genetic transfer from distinct bread wheat genome regions. On average, the probability of transference for genes allocated on the A, B and D wheat genomes is intermediate, low and high, respectively, with a relative ratio of 5:1:12 (Table 2; Fig. 2). It can also be concluded that 3DL and 5DL are the wheat genome locations most prone for unintended gene flow to *Ae. geniculata*. However, our results further predict that some A genome regions can be stably introgressed into this wild relative more frequently that those on certain D genome chromosomes (i.e., 2D), whereas others like chromosome arms 4AS and 4AL are even safer to avoid gene escapes than the majority of B genome chromosomes. This evidences the need to perform wheat–wild homoeologous MI pairing analyses at a chromosome-specific level.

Farooq et al. (1996) obtained a great variation in the frequency of MI association in interspecific hybrids involving three bread wheat varieties and three distinct *Ae. ovata* (synonymous of *Ae. geniculata*) accessions, with values ranging from 1.00 to 10.00 chiasmata per PMC. For each of the wild parental genotypes, hybrids derived from cultivar Chinese Spring showed the lowest MI pairing frequencies. This could explain the reduced level of MI pairing observed in the g × CS genotype examined here (Table 1), just slightly higher than the reported by Fernandez-Calvin and Orellana (1992) in hybrids between this wheat cultivar and a presumably distinct *Ae. geniculata* genotype (0.88 associations/cell). It is then very likely that MI associations are more abundant in hybrids eventually produced in nature from other wheat varieties, which will obviously increase the chance of wheat-to-wild gene transfers. The absence of significant differences between g × CS and g × CS*ph2* genotypes (Table 2) allows to expect that the pattern of wheat-wild MI pairing in hybrids with a higher level of MI association fits also the described above.

Homoeologous pairing of interspecific hybrids can also be explored from a breeding perspective, which considers the reverse (wild-to-crop) gene transfer direction. Introgression of alien traits by recombination-mediated strategies (i.e., interspecific crossing followed by repeated backcrossing) will require less efforts if responsible genes are located on genome regions showing higher levels of wheat-wild MI pairing. According to the results illustrated in Fig. 2, agronomically useful genes localized on *Ae. geniculata* chromosome arms 3L and 5L (or 4L, if on the U^g genome) are then good candidates for successful incorporation into wheat whereas a more troublesome improvement is expected for traits controlled by genetic sequences on the short arm of homoeologous group 4. Interestingly, the long arm of homoeologous group 5 possesses the highest concentration of major genes and QTLs related to the most important plant adaptations for abiotic stress (especially, salt and cold tolerance) in the Triticeae genomes (Cattivelli et al. 2002). The pattern of MI association in the hybrids examined predicts also that incorporation of favourable alleles from this wild relative will more frequently occur into D genome chromosomes than into A or B genome partners. This has indeed been demonstrated for the three *Ae. geniculata* resistance genes transferred to bread wheat successfully mapped to date (Zeller et al. 2002; Kuraparthy et al. 2007).

MI associations recorded in the hybrids were mostly distal. Therefore the results presented here refer to the potential for escape or introgression of genetic sequences located close to the chromosome ends. A MI pairing-based prediction on the likelihood of genetic exchange for genes at more internal locations would require interstitial cytological markers, but it can be generalized that their stable transference either from wheat to *Ae. geniculata* or in the opposite way is very unlike. In the frame of the gene flow risk assessment, this is not a very reassuring assertion since transgenes are predominantly inserted at telomeric or distal chromosome locations in transformed cereals (Pedersen et al. 1997; Jackson et al. 2001). When for breeding purposes, the following inference is that radiation-induced strategies can be then a more effective alternative than homoeologous-recombination based strategies for successful wild trait integration.

A final consideration refers to the similar MI pairing patterns observed in $ABDU^gM^g$ hybrids lacking and carrying the *ph2b* mutation (Table 2). It is acknowledged that *Ph2* regulates the diploid-like meiotic behaviour of wheat by a distinct mechanism than *Ph1* (Sears 1982; Martinez et al. 2001), but the varied approaches followed so far for its molecular identification have resulted inconclusive (Ji and Langridge 1994; Sutton et al. 2003; Lloyd et al. 2007). Irrespective of its actual mode of action, our results demonstrate that mutation at this locus does affect the level but not the pattern of MI pairing in interspecific hybrids thus supporting that it is not involved in the recognition of homoeology itself.

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