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ORIGINAL PAPER

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A cytomolecular approach to assess the potential of gene transfer from a crop (*Triticum turgidum* L.) to a wild relative (*Aegilops geniculata* Roth.)

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Abstract When a crop hybridizes with a wild relative, the potential for stable transmission to the wild of any crop gene is directly related to the frequency of crop–wild homoeologous pairing for the chromosomal region where it is located within the crop genome. Pairing pattern at metaphase I (MI) has been examined in durum wheat × *Aegilops geniculata* interspecific hybrids ($2n = 4x = ABU^gM^g$) by means of a genomic in-situ hybridization procedure that resulted in simultaneous discrimination of A, B and wild genomes. The level of MI pairing in the hybrids varied greatly depending on the crop genotype. However, their pattern of homoeologous association was very similar, with a frequency of wheat–wild association close to 60% in all genotype combinations. A–wild represented 80–85% of wheat–wild associations which supports that, on average, A genome sequences are much more likely to be transferred to this wild relative following interspecific hybridization and backcrossing. Combination of genomic DNA probes and the ribosomal pTa71 probe has allowed to determine the MI pairing behaviour of the major NOR-bearing chromosomes in these hybrids (1B, 6B, $1U^g$ and $5U^g$), in addition to wheat chromosome 4A which could be identified with the sole use of genomic probes. The MI pairing pattern of the wild chromosome arms individually examined has confirmed a higher chance of gene escape from the wheat A genome. However, a wide variation regarding the amount of wheat–wild MI pairing among the specific wheat chromosome regions under analysis suggests that the study should be extended to other homoeologous groups.

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Introduction

Spontaneous hybridization with wild-related species has been reported for most of the main crops in the world (Ellstrand et al. 1999). This explains a serious objection to the introduction of new GM varieties for crop breeding that refers to the possibility that transgenes could become incorporated into the genetic pool of wild species by vertical transmission following unintended interspecific hybridization. The ecological or economical consequences potentially derived, if any, are difficult to predict and assess (Dale et al. 2002), but many advisory institutions are encouraging research that allows to design new strategies in order to minimize the risk of gene escape from GMOs (FAO 2002; ESF 2004).

The stable transference of genes from a crop to a wild relative requires that their chromosomes pair with each other and subsequently recombine during the meiosis of the interspecific hybrid. Otherwise, aneuploid crop chromosomes will likely be lost early during the karyotypic evolution that follows backcrossing of the hybrid to the related species. The risk of effective introgression into the wild for any gene (or transgene) is then directly related to the probability of intergenomic pairing and recombination for the chromosomal region where that particular DNA sequence is located within the crop genome (Tomiuk et al. 2000). According to that, the homoeologous recombination pattern in interspecific hybrids could serve to predict safe genome sites for transgene integration in a crop. Subsequently, the location of the insert could be used as an additional criterion for the selection of transformed lines.

Punctual markers (either molecular or genetic) provide reliable information to determine the actual recombination rate of specific DNA sequences, and have been successful to demonstrate gene transfer from crop

species to wild relatives (e.g. Guadagnuolo et al. 2001), but they prove inadequate for recombination analyses at a broad genome level in interspecific hybrids. So, a set of markers that physically covers the entire chromosome complement and a full characterization of the wild parental species marker profiles are both required, which is hardly feasible if different genotypic combinations of both the crop and the wild species need to be examined. Nevertheless, the unsolvable impediment for estimation of crop–wild recombination parameters is the high sterility of most of these hybrids, whose offspring sizes are usually too small for reliability in the statistics to be performed.

The use of total genomic DNA probes for genomic in-situ hybridization (GISH) has proven to be very useful for discrimination of parental genomes in interspecific hybrids and derivatives, and it is being routinely used in many polyploid complexes. Within the Triticeae, GISH karyotype analysis of wheat × alien hybrid progenies allows characterization of genome composition and intergenomic exchanges eventually transmitted to the offspring (Wang et al. 2000; Benavente et al. 2001), although some limitations have been recently pointed out (Lukaszewski et al. 2005). When used for the analysis of meiotic metaphase I (MI) associations in interspecific hybrids, GISH reveals the outcome of synapsis and crossing-over between the constituent genomes (e.g. Cai and Jones 1997; Benavente et al. 1998; Chen et al. 2001; Jauhar et al. 2004) thereby allowing to estimate the level and pattern of intergenomic recombination. Contrary to most approaches based upon punctual markers, this cytomolecular technique is insensitive to intraspecific molecular polymorphisms.

Only a few transgenic wheat varieties have been commercialized up to date, but a lot of research is currently focused on wheat transformation for breeding purposes (Sahrawat et al. 2003; Jones 2005). The relative potential of stable transference for individual wheat genomes or specific chromosomal regions then represents a useful piece of information in order to develop new wheat varieties that reduce the chance of gene flow to wild relatives. Keeping that in mind, this study was aimed to compare the probability of genetic transfer from specific genomic regions of durum wheat according to the pattern of meiotic chromosome pairing revealed by GISH in interspecific hybrids. *Aegilops geniculata* Roth. (synonymous of *Ae. ovata* L.), the wild species used for the analysis, is the relative most extended around the Mediterranean basin (van Slageren 1994). The main obstacle for interspecific hybridization between *Ae. geniculata* and both durum and bread wheat is their autogamy (Dr. J. David, personal communication). However, hybrid forms have actually been reported in nature since the nineteenth century (van Slageren 1994). It has also been shown that either spontaneous or artificial durum wheat × *Ae. geniculata* hybrids can produce viable and fertile offspring by way of unreduced gametes (David et al. 2004).

Materials and methods

Plant material

Twelve interspecific hybrids between durum wheat (*Triticum turgidum* ssp. *durum*; $2n = 4x = 28$, genome composition AABB) and *Ae. geniculata* ($2n = 4x = 28$, genome composition $U^gU^gM^gM^g$) were examined in this study. Their parental genotypes are noted in Table 1. In all crosses, the wild species was used as the female parent. For most presumed hybrids, root tips were collected and fixed to confirm their expected hybrid genome composition ($2n = 4x = ABU^gM^g$). Hybrid g21w×A was collected as a kernel in a population of *Ae. geniculata* from which accessions INRA-211 (early-flowering line) and INRA-212 (late flowering line) were originated. Cultivar Ardente, the durum wheat variety growing in the crop field surrounding that wild population, has been confirmed as the wheat parent of that spontaneous hybrid by means of molecular marker analysis (Dr. J. David, personal communication).

Anthers of the emerging spikes containing pollen mother cells 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 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 durum wheat, *Ae. geniculata* and their ancestral diploids *Triticum monococcum*, *Ae. speltoides*, *Ae. umbellulata* and *Ae. comosa* ($2n = 14$; genome constitution AA, SS, UU and MM, respectively), following standard protocols. Different labelling methods and probe mix compositions were checked for GISH, with remarkable differences in the degree of genome discrimination achieved (see Fig. 1). Best results were obtained by the procedures and GISH mixture compositions described here. Diploid species genomic DNAs were labelled with digoxigenin-11-dUTP (A- and U-genome probes) or biotin-16-dUTP (S- and M-genome probes) by random priming, and then mechanically sheared by autoclaving to 0.5–1.5 kbp pieces. The ribosomal probe pTa71 (Gerlach and Bedbrook 1979) was labelled with digoxigenin-11-dUTP and biotin-16-dUTP by Nick translation. Labelling of probes was performed using standard kits from Roche following the manufacturer's instructions. For most meiotic slides examined, hybridization mixtures contained differentially labelled A and S genomic probes, to a final concentration of 4 and 8 ng/μl, respectively. Unlabelled *Ae. geniculata* genomic DNA

Table 1 Parental lines of the interspecific durum wheat × *Ae. geniculata* hybrids examined ($2n = 4x = 28$; ABU^gM^g)

Hybrid	*Ae. geniculata* (♀)	*T. turgidum* ssp. *durum* (♂)	No. of individuals
g003×L	ETSIA-3 (unknown)	cv. Langdon	4
g103×L	INRA-103 (Morocco)	cv. Langdon	3
g211×A	INRA-211 (Baillargues, France)	cv. Ardente	1
g212×A	INRA-212 (Baillargues, France)	cv. Ardente	2
g21w×A	Wild (Baillargues, France)[a]	cv. Ardente	1
g17×C*ph*	INRA-17 (unknown)	cv. Creso (*ph* mutant line)	1

Geographical origin of the *Ae. geniculata* accessions is indicated in brackets
[a]The hybrid seed was found on an *Ae. geniculata* plant growing in the wild population from which accessions INRA-211 and INRA-212 were developed (see text)

sheared to 0.3–0.7 kbp by autoclaving was also added in excess (60-fold the A genome-labelled probe concentration) to block shared DNA sequences. However, on a sample of MI cells from one of the hybrids (g212×A-1), the GISH mixture contained differentially labelled U and M genomic probes (4 ng/μl each) and unlabelled durum wheat blocking DNA (400 ng/μl). When added to the mix of genomic probes, the final concentration of the ribosomal probe was 2.5 ng/μl. It was used either biotinylated (Fig. 1h) or as a mixture of digoxigenin- and biotin-labelled pTa71 in a ratio of 2:3 (Fig. 1 except h). ISH protocol was as described in Sanchez-Moran 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 fluorescein and avidin fluorescence. Images were captured with a CoolSnap digital camera. No further image processing but adjustment of brightness has been done for any of the micrographies in Fig. 1.

Results

GISH analysis of both root tips and MI cells confirmed that all the hybrids had 28 somatic chromosomes and the expected $A^7 + B^7 + U^gM^{g14}$ genome composition (Fig. 1a).

Analysis for individualized genomes

Table 2 shows the numbers of meiotic configurations and the frequency of MI associations in all individuals examined. Hybrids from durum wheat cv. Langdon (later grouped as g×L) showed the lowest level of MI pairing, whereas the highest value was reached in the hybrid from cv. Creso *ph* mutant (also noted as g×C*ph*). The spontaneous hybrid g21w×A behaved as individuals obtained from crosses with cv. Ardente (grouped as g×A), thus showing an intermediate MI pairing level. Two-sample Student t tests have demonstrated very significant differences in the mean number of chromosome associations per cell between hybrids from distinct wheat parental lines (g×L–g×A: $t = 35.61$, $P < 0.001$; g×L–g×C*ph*: $t = 23.56$, $P < 0.001$; g×A–g×C*ph*: $t = 9.79$, $P < 0.001$).

Discrimination among A, B and wild (U^g or M^g) chromatin by GISH allowed to identify the homoeologous genomes involved in each meiotic pairing configuration. The following types of MI associations could be distinguished: intraspecific associations involving both wheat genomes (A–B), intraspecific associations involving both *Ae. geniculata* genomes (U^g–M^g), wheat–wild associations involving the A wheat genome and one *Ae. geniculata* genome (A–wild) and wheat–wild associations involving the B wheat genome and one *Ae. geniculata* genome (B–wild) (see Fig. 1). It is worthy of noting that associations were predominantly distal. Table 3 shows the distribution of these types of MI associations for the durum wheat × *Ae. geniculata* genotypic combinations analysed. Data from individuals within each combination have been pooled because all statistical comparisons that were conducted failed to detect significant differences for hybrids from the same parental lines.

Metaphase I associations between wheat and wild homoeologues represented around 60% of total associations in all hybrids examined (Table 3), despite the differences in their level of MI pairing described above. Accordingly, contingency χ^2 tests did not detect any significant difference in the ratio of intraspecific and wheat–wild associations, either between hybrid combinations sharing the same durum wheat parent or between hybrids from distinct wheat genotypes (Table 4). The two types of intraspecific association (A–B and U^g–M^g) showed a certain variation between hybrids but in all cases the frequency of MI pairing involving both wild genomes exceeded that involving A and B wheat genomes (Table 3). Regarding wheat–wild

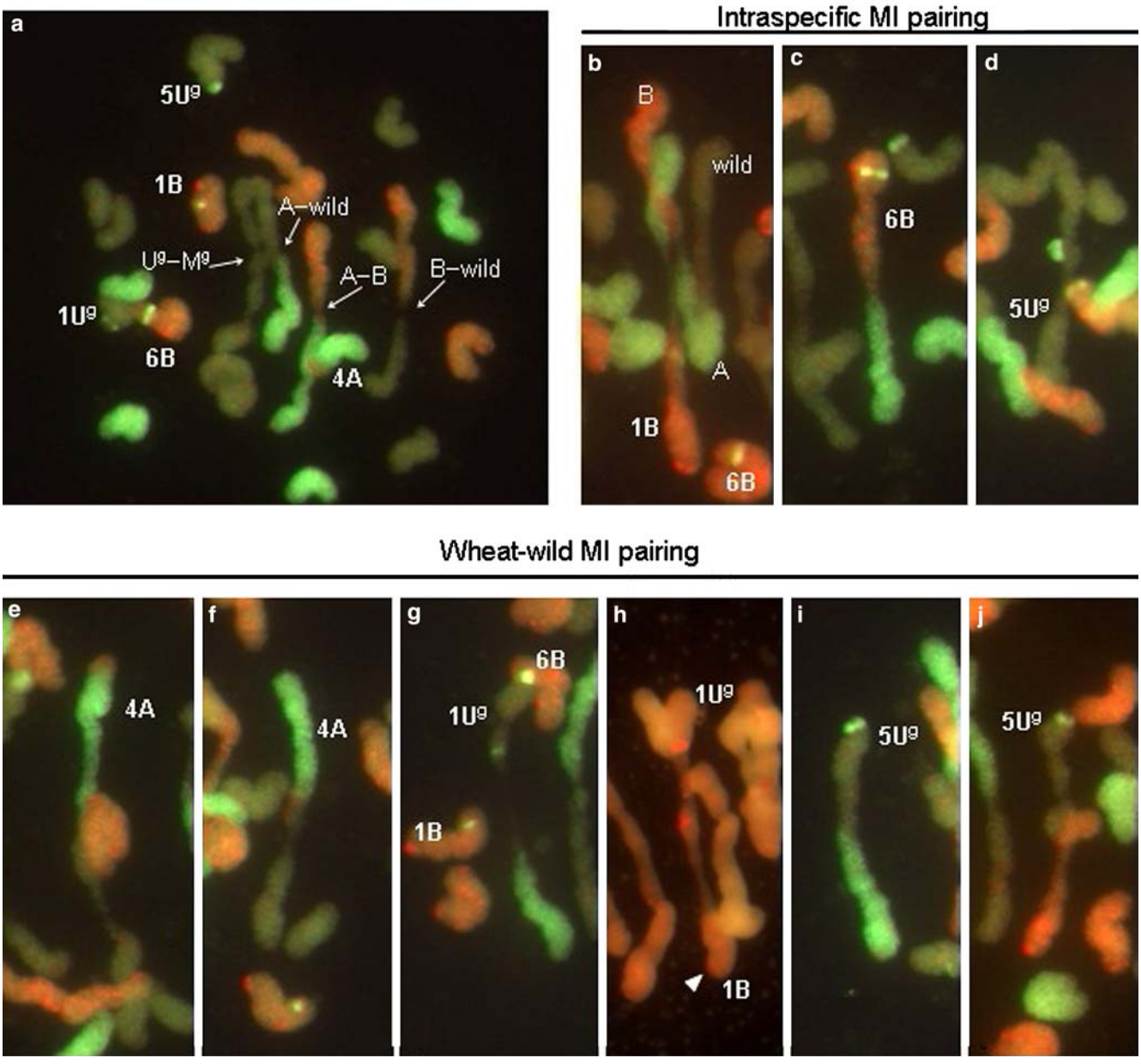

Fig. 1 Micrographies from MI cells of durum wheat × *Ae. geniculata* interspecific hybrids ($2n = 4x = ABU^gM^g$) after ISH combining differentially labelled A and S genomic DNA probes, and the ribosomal pTa71 probe. In all but **h**, the GISH mixture was as described in the Materials and methods section; wheat constituent genomes are *green* (A chromatin) and *red* (B chromatin), the blocked wild genome is *brown* and ribosomal sites are revealed by *bright yellow-green* signals. In **h**, a different ratio of A-genome probe:S-genome probe:blocking DNA was used and the pTa71 probe was biotinylated. This resulted in lower discrimination between A (*yellow*), B (*red-brown*) and wild (*brown*) chromatin and red signals for ribosomal sites. **a** MI cell with four rod bivalents showing the different types of homoeologous MI pairing in these hybrids. All five specific chromosomes individually analysed appear as univalents. **b–j** Selected bivalents with intraspecific (**b–d**) or wheat–wild interspecific (**e–j**) MI association. **b** A–B association for 1BS chromosome arm. The genomes involved in a trivalent pairing configuration also included have been noted. **c** A–B association for 6BL. **d** U^g–M^g association for $5U^gS$. **e** A–wild association for 4AS. **f** Wheat–wild association for the distal segment of 4AL, thus scored as B–wild. **g** A–wild association for $1U^gS$. The *bright fluorescent dot* at the distal region of $1U^gL$ corresponds to the NOR site of 6B. **h** B–wild association involving 1BS and $1U^gS$ chromosome arms. The GISH signal that differentiates 1BL from 6BL is indicated by an *arrowhead*. **i** A–wild association for $5U^gL$. **j** B–wild association for $5U^gL$

MI pairing, associations involving the A wheat genome were always much more frequent than those involving the B wheat genome, with A–wild:B–wild ratios ranging from ≈8:1 in g103×L to ≈4:1 in g17×C*ph* (Table 3). No significant differences have been found between hybrid combinations with the same wheat parent. However, hybrids from cv. Langdon showed a slightly greater proportion of A–wild MI associations than hybrids from cv. Ardente or cv. Creso (Table 4).

Table 2 Meiotic configurations at metaphase I in ABU^{g}M^g interspecific hybrids

Hybrid	Cells	MI pairing configuration[a]					MI associations	
		I	Rod II	Ring II	III	IV	Total	Mean/cell
g003×L-1	132	3,524	86	0	0	0	86	0.65 ± 0.07
g003×L-2	62	1,672	32	0	0	0	32	0.52 ± 0.09
g003×L-3	80	2,160	40	0	0	0	40	0.50 ± 0.08
g003×L-4	111	2,953	76	0	1	0	78	0.70 ± 0.08
g103×L-1	86	2,384	12	0	0	0	12	0.14 ± 0.04
g103×L-2	101	2,806	11	0	0	0	11	0.11 ± 0.03
g103×L-3	311	8,318	188	1	4	0	198	0.64 ± 0.04
g211×A-1	114	2,289	394	12	29	1	479	4.20 ± 0.17
g212×A-1	69	1,456	215	6	10	1	250	3.62 ± 0.18
g212×A-1[b]	49	991	169	4	9	2	201	4.10 ± 0.26
g212×A-2	71	1,397	251	5	25	1	314	4.42 ± 0.19
g21w×A-1	78	1,668	236	4	12	0	268	3.44 ± 0.18
g17×C*ph*-1	83	1,319	395	15	55	5	551	6.64 ± 0.26

[a] *I* univalent, *II* bivalent, *III* trivalent, *IV* quadrivalent
[b] Cell sample where U^g and M^g wild genomes were individualized by GISH

The types of associations distinguished on the sample of MI cells which was hybridized with differentially labelled U and M genomic probes in hybrid g212×A-1 as well as their frequencies are included in Table 3. It must be noted that the relative frequencies of A–B, U^g–M^g and wheat–wild associations (U^g–wheat + M^g–wheat) were not significantly different to the values found for this individual when the standard GISH probe mix was used ($\chi^2 = 0.65$; $df = 2$; $P > 0.05$). Similar proportions for U^g–wheat and M^g–wheat homoeologous pairing indicated that none of the wild species genomes was preferentially involved in MI association with their wheat homoeologues.

Analysis for specific chromosome arms

The intergenomic translocation 4AS·4AL/7BS present in both durum and bread wheats (Naranjo et al. 1987; Naranjo 1990) has provided a suitable cytomolecular marker for the identification of the MI pairing pattern of chromosome 4A by means of GISH in the interspecific hybrids examined (Fig. 1a, e, f). In addition to 4A, four major NOR-bearing chromosomes (1B, 6B, 1U^g and 5U^g) were undoubtedly discriminated in MI cells from those slides where the ribosomal probe pTa71 was added to the mix of genomic probes (Fig. 1a–d, g–j). Additional minor NOR signals have been described in both wheat and *Ae. geniculata* FISH karyotypes (Mukai et al. 1991; Badaeva et al. 2004), but their identification on meiotic chromosomes was sometimes lacking or doubtful and therefore these have not been considered for our analysis. The two wheat NOR chromosomes showed a similar morphology and NOR location at the MI meiotic stage, although their FISH signals were slightly different in size and intensity. In addition, they differed by the presence/absence of a strong GISH signal corresponding to B genome repeated DNA sequences on the distal long arm (Fig. 1a, b, g). Such a distinctive signal could be assigned to 1B when the marked chromosome

Table 3 Pattern of MI association in the durum wheat × *Ae. geniculata* hybrid combinations examined

Hybrid	Intraspecific MI associations			Wheat–wild MI associations			Others[a]
	Total	%A–B	%U^g–M^g	Total	%A–wild	%B–wild	
g003×L [1–4]	86	24.4	75.6	149	87.2	12.8	1
g103×L [1–3]	89	25.8	74.2	131	88.5	11.5	1
g211×A [1]	190	31.6	68.4	282	82.6	17.4	7
g212×A [1–2]	234	31.6	68.4	321	80.7	19.3	9
g21w×A [1]	114	25.4	74.6	154	83.8	16.2	0
g17×Cph [1]	204	39.2	60.8	335	79.4	20.6	12
g×L	175 (38.5)	25.1	74.9	280 (61.5)	87.9	12.1	2
g×A	538 (41.5)	30.3	69.7	757 (58.5)	82.0	18.0	16
g×Cph	204 (37.8)	39.2	60.8	335 (62.2)	79.4	20.6	12
	Total	%A–B	%U^g–M^g	Total	%U^g–wheat	%M^g–wheat	Others
g212×A [1][b]	77	28.6	71.4	121	50.4	49.6	3

The relative proportions (%) of intraspecific and wheat–wild associations for hybrids sharing the wheat parent are in brackets
[a] Includes non-homologous associations and multiple (non-two-by-two) chromosome arm associations
[b] Cell sample where U^g and M^g wild genomes were discriminated by GISH

Table 4 Contingency χ^2 tests for comparisons of MI-pairing pattern between ABU^{g}M^g hybrid combinations

Hybrid combinations compared	MI associations	
	Intraspecific vs wheat–wild	A–wild vs B–wild
g003×L–g103×L	$\chi^2 = 0.71$*	$\chi^2 = 0.11$*
g211×A–g212×A	$\chi^2 = 0.38$*	$\chi^2 = 0.38$*
g211×A–g21w×A	$\chi^2 = 0.37$*	$\chi^2 = 0.09$*
g212×A–g21w×A	$\chi^2 = 0.01$*	$\chi^2 = 0.66$*
g×L–g×A	$\chi^2 = 1.33$*	$\chi^2 = 5.06$**
g×L–g×C*ph*	$\chi^2 = 0.04$*	$\chi^2 = 7.82$***
g×A–g×C*ph*	$\chi^2 = 2.16$*	$\chi^2 = 1.05$*

In all tests performed the number of degrees of freedom is equal to 1
*$P > 0.05$; **$0.05 > P > 0.01$; ***$0.01 > P > 0.001$; ****$0.001 > P$

was found to behave as pairing partner of chromosome 1U^g (Fig. 1h).

The level and pattern of homoeologous MI association for the long and short arms of all chromosomes analysed is given in Table 5. The results described for NOR chromosomes pool observations on 664, 291 and 83 MI cells from g×L, g×A and g×C*ph* hybrids, respectively. A considerable variation in the amount of MI pairing has been found for the specific chromosomes under analysis, ranging from the minimum value of 0.03 associations per cell for 4A (38 associations in 1,298 cells) to the maximum value of 0.15 for 5U^g (162 associations in 1,038 cells). For all chromosomes but 1U^g, the short arms showed a lower level of pairing than their long counterparts. The pattern of association was also remarkably variable, even between the long and short arms of a given chromosome. So, all associations for chromosome arm 6BS involved the wheat A genome partner, whereas an intraspecific:interspecific (wheat–wild) ratio close to 1:1 was found for 4AL, 1BL, 1U^{g}S and 1U^{g}L, and a preferential MI pairing with the wild homoeologue was observed for 4AS, 1BS and 6BL. Between-arm differences resulted striking for 5U^g, where the percentages of wheat–wild associations were 19.3 (11 out of 57 associations) and 83.8 (88 out of 105 associations) for 5U^{g}S and 5U^{g}L, respectively.

It can be finally noted that the relative frequencies of MI association with the wheat A and B genomes for all the four *Ae. geniculata* chromosome arms individually examined (1U^{g}S, 1U^{g}L, 5U^{g}S and 5U^{g}L) are in agreement with the proportions of A–wild and B–wild reported in Table 3 (80–88 and 12–20%, respectively).

Discussion

Identification at meiosis of individual wheat genomes by means of GISH has been reported earlier in durum wheat haploids (Jauhar et al. 1999) and durum and bread wheat lines (Sanchez-Moran et al. 2001), but has never been used before for the meiotic analysis of interspecific hybrids between wheat and related species (see for instance Cai and Jones 1997; Chen et al. 2001; Jauhar et al. 2004). The GISH method described here has resulted in clear and easy discrimination between the A, B and wild genomes. In addition, our study demonstrates that combination of genomic and repeated DNA probes represents a significant improvement in order to increase the potentials of ISH procedures for chromosome analysis. It can be noted that the enormous difficulty and huge training which require the unequivocal identification of all wheat chromosomes by the other methods available (C-banding and FISH with multiple repeated DNA probes) explain their use for quantitative analyses of meiotic pairing has been very scarce.

The level of MI pairing in the hybrid genotypes examined seems to depend mainly on their durum wheat parent (Table 2). The highest level of chromosome association reached in the hybrid from cv. Creso (g×C*ph*) is attributable to the effect of *Ph1*$^-$ mutation, which promotes homoeologous pairing. However, significant differences have also been found between hybrids from cv. Langdon (g×L) and cv. Ardente (g×A), both with a functional *Ph1* gene. Ozkan and Feldman (2001) demonstrated genotypic variation in tetraploid wild wheats affecting homoeologous pairing in interspecific hybrids, which they attributed to the existence of allelic variants for *Ph1* activity. To date, only the null allele *Ph1*$^-$ has been considered in both durum and bread wheat, but a recent report of Martinez et al. (2005) in haploids from different cultivars of *T. aestivum* and our results support that weak active alleles might be present in some crop wheats as cv. Ardente.

The relative ratios of the two types of intraspecific associations in ABU^{g}M^g hybrids (Table 3) suggest a closer phylogenetic relationships between U^g and M^g wild genomes than between A and B wheat genomes, which is in agreement with the results reported by Lucas and Jahier (1988) for interspecific hybrids between their diploid donors.

In all hybrids analysed here the frequency of wheat–wild associations (58–62%) was significantly higher than the frequencies of associations involving the two genomes of each parental species, A–B and U^g–M^g representing 10–15 and 23–29% of total associations, respectively (see Table 3). Fernandez-Calvin and Orellana (1992) examined the MI pairing pattern of bread wheat × *Ae. ovata* (syn. *Ae. geniculata*) interspecific hybrids ($2n = 35$; genome composition ABDU^{g}M^g) by means of C-banding. These authors obtained a lower frequency of MI pairing involving chromosomes of both species (48% of wheat–wild associations), though these were also more abundant than wheat–wheat (38%) and wild–wild (14%) associations. Obviously, the presence of the D genome, showing a well-documented high pairing affinity with the A genome (e.g. Jauhar et al. 1991), greatly determines the outcome of homoeologous chromosome pairing in hybrids from bread wheat, and thus can account for most of the MI-pairing pattern differences when compared with hybrids between durum wheat and the same wild species. Much lower values of

Table 5 Frequency and pattern of homoeologous associations for individual chromosome arms

Chromosome	Short arm				Long arm			
	Total	MI pairing partner			Total	MI pairing partner		
		A	B	Wild		A	B	Wild
4A	9		2	7	29	15		14
1B	15	4		11	33	16		17
6B	8	8		0	53	18		35
$1U^g$	33	14	3	16	14	5	1	8
$5U^g$	57	9	2	46	105	76	12	17

Data for chromosome 4A correspond to 1,298 MI cells; data for the NOR-bearing chromosomes correspond to 1,038 MI cells

interspecific MI pairing have been reported in bread wheat × rye hybrids ($2n = 4x = 28$; ABDR), even under the promoting effect of *ph* mutations (Naranjo and Fernandez-Rueda 1996; Cuadrado et al. 1997; Benavente et al. 1998), and in trigeneric hybrids ($2n = 4x = 28$; genome composition ABJE) synthesized by crossing lines of durum wheat carrying and lacking *Ph1*, and fertile amphiploids developed from hybrids between *Thinopyrum bessarabicum* ($2n = 2x = 14$; JJ) and *Lophopyrum elongatum* ($2n = 2x = 14$; EE) (Jauhar et al. 2004). Nevertheless, ABDR and ABJE wheat × alien combinations are both very unlikely to occur in nature which calls into question their relevancy in the scope of the present study.

Our analysis has clearly evidenced that A-genome chromosomes are much more prone to pair and therefore recombine with the wild homoeologues of *Ae. geniculata* than B-genome chromosomes (Table 3). This trend is confirmed by the results described for the long and short arms of both $1U^g$ and $5U^g$ wild chromosomes, whatever their relative MI pairing frequency with a wheat homoeologue (close to 50% in $1U^gS$ and $1U^gL$, 19.3% in $5U^gS$ and 83.8% in $5U^gL$) (Table 5). Even more, their ratios of A:B MI pairing partner (4–6:1) fit the range found for the wild genome as a whole. All that agrees with the different levels of MI associations reported in hybrids between *Ae. umbellulata* (U^g genome donor) and the diploid donors of durum wheat genomes (Lucas and Jahier 1988). Fernandez-Calvin and Orellana (1992) could not distinguish A from D genome in AB-DU^gM^g hybrids, which makes it impossible to perform a direct comparison of the ratio A–wild:B–wild between durum and bread wheat hybrids. However, these authors found that *Ae. geniculata* chromosomes paired much more frequently with wheat AD than with wheat B partners.

The consistency for most results obtained in the different hybrids (Table 4) supports that the relative proportions of intergenomic associations observed in our study could be generalized to any durum wheat × *Ae. geniculata* hybrid, whatever its origin (artificial or spontaneous), parental genotype combination or overall level of MI pairing. So, it is expected that around 60% of meiotic recombination events in any ABU^gM^g hybrid will occur between chromosomes of the crop and the wild species, the A wheat genome being much more frequently involved in genetic exchange with the wild homoeologues than the B genome. The MI-pairing pattern in the sample of cells where U^g and M^g could be discriminated (see last row in Table 3) suggests no differences between both *Ae. geniculata* constituent genomes regarding their ability to pair with a wheat partner.

The potential of transference to the wild of any wheat genetic sequence must be directly related to the frequency of wheat–wild MI pairing for the chromosome region where it is located. Despite the general trends depicted above, wide variation regarding not only the level but also the pattern of homoeologous pairing has been found for the chromosome arms individually analysed here (Table 5). Great between-arm differences have also been reported in bread wheat × alien hybrids when most of the 21 wheat chromosomes were identified at meiosis by C-banding (Naranjo and Fernandez-Rueda 1996; Maestra and Naranjo 2000) or by simultaneous fluorescent in-situ hybridization (FISH) of multiple repeated DNA probes (Cuadrado et al. 1997).

The lower MI association frequency observed for most short arms when compared with their long counterparts is in agreement with the well-established relationship between chromosome arm size and homologous pairing frequency. In interspecific hybrids, homoeologous pairing frequencies can also be determined by the degree of structural differentiation between their constituent genomes, which is known to be variable for the distinct groups of homoeology (e.g. Maestra and Naranjo 2000). This source of variation could be relevant in our hybrids; firstly, as the structural changes relative to wheat that affect to a different extent all seven U genome chromosomes have been reported in *Ae. umbellulata* (Zhang et al. 1998) and, secondly, because speciation of *Ae. geniculata* has been accompanied by modifications of parental U and M genomes, including chromosomal rearrangements (Badaeva et al. 2004). Differences in the pattern of homoeologous association as found here even for the short and long arm of a given chromosome (i.e. 6B, $5U^g$) could then be also related to structural divergence among the four genomes involved that might have resulted in differential pairing affinities for distinct chromosome segments. Whatever the reason

for these differences, our analysis evidences that wheat genetic sequences located on certain genome regions such as the distal portions of both 5AL (the A genome partner of $5U^gL$) and 6BL have a greater chance to be transmitted to *Ae. geniculata* when interspecific hybrids act as a bridge for transference, compared to other wheat regions that behave as much safer to avoid unintended wheat gene transmission.

Further studies will allow to extend the analysis to other chromosome regions to draw a final picture of the potential of gene escape for all wheat groups of homoeology. It will be also of interest to determine to what extent the trends described here are extensive to bread wheat hybrid combinations as well as to interspecific hybrids involving other wild or weedy species capable of hybridizing in nature with crop wheats.

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