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Cytomolecular Identification of Individual Wheat-Wheat Chromosome Arm Associations in Wheat-Rye Hybrids

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Key Words
Chromosome pairing · FISH · GISH · Meiosis · Wheat

Abstract
Chromosome pairing in the meiotic metaphase I of wheat-rye hybrids has been characterized by sequential genomic and fluorescent in situ hybridization allowing not only the discrimination of wheat and rye chromosomes, but also the identification of the individual wheat and rye chromosome arms involved in the chromosome associations. The majority of associations (93.8%) were observed between the wheat chromosomes. The largest number of wheat-wheat chromosome associations (53%) was detected between the A and D genomes, while the frequency of B-D and A-B associations was significantly lower (32 and 8%, respectively). Among the A-D chromosome associations, pairing between the 3AL and 3DL arms was observed with the highest frequency, while the most frequent of all the chromosome associations (0.113/cell) was found to be the 3DS-3BS. Differences in the pairing frequency of the individual chromosome arms of wheat-rye hybrids have been discussed in relation to the homoeologous relationships between the constituent genomes of hexaploid wheat.
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Bread wheat (*Triticum aestivum* L.) is an allohexaploid (2n = 6× = 42) crop. As a result of 2 consecutive interspecific hybridization and chromosome doubling events, it has 3 related genomes (A, B and D). The first polyploidization, which took place 0.5–3 million years ago between *T. urartu* Tum. *ex* Gandil. (A^uA^u) and an unidentified species (BB) highly similar to *Aegilops speltoides* Tausch., resulted in the evolution of wild emmer wheat, *T. turgidum* ssp. *dicoccoides* Körn. (A^uA^uBB) [Sabot et al., 2005; Berkman et al., 2011]. After domestication, the second hybridization event between *T. turgidum* L.ssp. *dicoccum* (Schrank) Thell. and *Ae. tauschii* Coss. (DD) produced the allohexaploid genomic structure of *T. aestivum* (A^uA^uBBDD) approximately 8,000 years ago [Huang et al., 2002].

The homoeologous chromosomes of the A, B and D genomes are closely related genetically and capable of pairing, so the evolution of a strict regulation of chromosome pairing was necessary to ensure stable meiosis and the reproductive stability of polyploid wheat. In the tetraploid and hexaploid wheat, the principal regulator of meiotic chromosome pairing is the *Ph1* gene [Riley and Chapman, 1958; Sánchez-Morán et al., 2001]. *Ph1* suppresses pairing and recombination between homoeologous and less closely related chromosomes, resulting in

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diploid-like pairing between the homologous chromosome partners. The pairing between homologous chromosomes results in the gene order on the 3 members of a homoeologous group being relatively similar [Gale and Devos, 1998]. However, the macrosynteny between the homoeologous chromosomes is disturbed in some places due to the presence of intra- and intergenomic chromosome rearrangements [Qi et al., 2006; Badaeva et al., 2007]. Earlier studies revealed that a series of translocations had occurred between the chromosomes 4A, 5A and 7B [Naranjo et al., 1987; Devos et al., 1995; Hernandez et al., 2012]. It has also been proved that chromosome 4A underwent a pericentric inversion and a paracentric inversion [Devos et al., 1995; Hernandez et al., 2012]. A translocation has also been identified by genetic mapping between chromosomes 2B and 6B [Conley et al., 2004].

Chromosome pairing analysis is an excellent tool for the investigation of chromosome structure and for studying the homoeologous relationship of one genome relative to another [Fominaya and Jouve, 1985; Naranjo, 1992]. The meiotic behaviour of interspecific hybrids has been extensively studied in order to obtain valuable information about the homoeologous relationship between the genomes of wheat and rye [Miller et al., 1994; Cuadrado et al., 1997], wheat and *Aegilops* sp. [Fernandez-Kalvin and Orellana, 1992; Logojan and Molnár-Láng, 2000; Cifuentes at al., 2010], and wheat and barley [Molnár-Láng et al., 2000]. In interspecific hybrids, such as wheat-rye hybrids, the pairing between the rye and wheat chromosomes is strongly suppressed in the presence of the *Ph1* gene. Therefore, chromosome pairing analysis on wheat-rye hybrids in the presence of *Ph1* enables the homoeologous relationship between the constituent genomes of hexaploid wheat to be examined [Naranjo et al., 1987].

Among the cytogenetic methods, Feulgen staining has been widely used to characterize the meiotic chromosome pairing behaviour of interspecific hybrids since the early seventies [Riley and Law, 1965; Fedák, 1977]. This technique allows the type of chromosome associations to be differentiated, but is not suitable for the discrimination of the parental genomes in interspecific hybrids or the identification of the individual chromosomes. The use of Giemsa C-banding allows individual mitotic chromosomes to be identified by visualizing the constitutive heterochromatic pattern and may provide information about homoeologous relationships at the chromosome arm level in the meiotic metaphase I [Ferrer et al., 1984]. Although the C-banding is a relatively nonexpensive technique, the identification of the individual chromosomes from complex banding patterns requires advanced experience in karyotyping [Naranjo and Fernández-Rueda 1996]. This may be why the arm level discrimination of meiotic chromosomes by C-banding is rare [Naranjo et al., 1987], and the use of this technique in meiotic chromosome pairing analysis is usually limited to the discrimination of the pairing partners at the genome level [Jauhar et al., 1991].

The advent of fluorescent in situ hybridization (FISH), allowing the visualization of different repetitive DNA classes, provided a new opportunity for the identification of individual chromosomes in wheat [Mukai et al., 1993; Molnár et al., 2009; Molnár-Láng et al., 2010] and related species [Badaeva et al., 1996; Molnár et al., 2011a, b]. FISH was also applied for the identification of the individual meiotic chromosomes involved in chromosome associations at metaphase I of meiosis [Cuadrado et al., 1997; Cifuentes et al., 2006; Cifuentes and Benavente, 2009]. Because of the differences in the chromatin structure of mitotic and meiotic chromosomes, the use of repetitive DNA probes made it possible to identify wheat chromosomes having specific cytogenetic landmarks (major nucleolus organizer regions) or specific FISH signals. Up till now, many of D and B chromosomes of wheat and some of the A chromosomes have been identified in the meiotic pairing studies on wheat-alien hybrids [Cuadrado et al., 1997; Cifuentes and Benavente, 2009; Cifuentes et al., 2006]. Genomic in situ hybridization (GISH), using total genomic DNA as a probe, is an excellent technique for discriminating the parental genomes of interspecific hybrids [Le et al., 1989; Schwarzacher et al., 1989] and identifying the intragenomic and intergenomic chromosome associations in meiotic metaphase I [Miller et al., 1994; Molnár-Láng et al., 2000; Cifuentes et al., 2010; Molnár and Molnár-Láng, 2010]. One disadvantage of the GISH technique is that individual chromosomes cannot be distinguished. However, the sequential use of FISH and GISH is able to maximize the amount of information that can be obtained from a cytological preparation. In the case of major cereals, sequential FISH and GISH has been widely applied on mitotic chromosomes for karyotypic analysis. By contrast, its potential has not been utilized for the meiotic pairing analysis of wheat-alien hybrids, and only one report has been found on their application in *Alstroemeria* hybrids [Kamstra et al., 2004].

The present study, involving wheat-rye hybrids, proved that the sequential use of GISH and FISH is able to identify all the wheat and rye chromosomes in meiotic metaphase I, making it a powerful method for the chromosome pairing analysis of major cereals. By determining

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the frequency of wheat-wheat chromosome associations in 2 wheat-rye F_1 hybrids, this research also provided additional information about the homoeologous relationships of the wheat genomes.

Materials and Methods

Plant Material

Two wheat-rye F_1 hybrids were used to investigate the frequency of individual chromosome associations. Two new wheat (*T. aestivum* L.) genotypes carrying the 1B.1R translocation and the *kr1* crossability gene were first produced by crossing the winter wheat cultivars 'Mv Béres' and 'Mv Magdaléna' with the winter wheat genotype Mv9kr$_1$ [Molnár-Láng et al., 2010]. These new recipient genotypes (Mv Béres kr1 and Mv Magdaléna kr1) were then pollinated with the rye (*Secale cereale* L.) cultivar 'Kriszta' and to produce wheat-rye F_1 hybrids (Mv Béres kr$_1$ × rye and Mv Magdaléna kr$_1$ × rye) [Molnár-Láng et al., 2010]. The plants were grown in a phytotron under controlled environmental conditions (Conviron, PGR-15 cabinet) [Tischner et al., 1997].

In situ Hybridization

Chromosome Preparations. The meiotic behaviour of wheat-rye hybrids was analysed in pollen mother cells at metaphase I of meiosis. Anthers containing pollen mother cells were fixed in 1:3 (v/v) acetic acid/ethanol and squashed in 45% acetic acid, according to Molnár and Molnár-Láng [2010].

DNA Probes and Labelling. Total rye genomic DNA was isolated according to Sharp et al. [1988] and labelled with biotin-16-dUTP (Roche, Mannheim, Germany) by nick translation. The repetitive DNA sequences pSc119.2 [Bedbrook et al., 1980] and Afa family [Nagaki et al., 1995] were amplified and labelled with biotin-16-dUTP (Roche) and digoxigenin-11-dUTP (Roche), respectively, using PCR [Nagaki et al., 1995; Contento et al., 2005]. The 18S-5.8S-26S rDNA clone pTa71 [Gerlach and Bedbrook, 1979] was labelled with 50% biotin-16-dUTP and 50% digoxigenin-11-dUTP. Digoxigenin and biotin were detected using anti-digoxigenin-rhodamine Fab fragments (Roche) and streptavidin-FITC (Roche), respectively.

Sequential GISH and FISH. GISH was carried out on meiotic chromosome spreads of the wheat × rye hybrids using a rye genomic probe according to Molnár-Láng et al. [2010]. After the documentation of the GISH sites, the slides were washed and rehybridized. FISH was carried out using repetitive DNA probes (pSc119.2, Afa family, pTa71) according to Molnár et al. [2009]. Images were captured with a Zeiss Axioskop-2 fluorescence microscope using a Plan Neofluar oil objective (Zeiss, Oberkochen, Germany) equipped with filter sets appropriate for DAPI, FITC and Rhodamin (Zeiss filter set 24) with a Spot CCD camera (Diagnostic Instruments, Sterling Heights, Mich., USA). The images were compiled with Image Pro Plus software (Media Cybernetics, Silver Spring, Md., USA).

Pairing Analysis. The calculated pairing frequency represents the percentage of cells in which a given configuration was observed. The number of chromosome associations was evaluated on the basis of their meiotic configuration: one association per rod bivalent, 2 associations per ring bivalent and chain trivalent, and 3 per frying pan trivalent [Cuadrado et al., 1997]. Chi-square was calculated for the comparison of the expected and observed pairing frequencies of the wheat chromosome arms within and between the genotypes.

Results

Investigation of Chromosome Pairing by GISH

GISH resulted in strong fluorescent signals on the rye chromosomes, which confirmed the presence of 7 rye chromosomes and the short arm of the 1R rye chromosome involved in the 1BL.1RS translocation among the 28 meiotic chromosomes of the wheat-rye F_1 hybrids (fig. 1A).

A total of 330 chromosome associations were observed in the 274 pollen mother cells examined. GISH was able to discriminate between intraspecific and interspecific chromosome associations. Meiotic configurations and the frequency of total MI associations per PMC for the hybrids are shown in table 1. The relatively high level of MI associations per cell observed in the 2 hybrids (1.06 and 1.25) could be attributed to the high frequency of pairing between the 1RS arms of rye and the 1BL.1RS translocation chromosome. A total of 133 chromosome associations were observed between the 1RS chromosome arms. The homologous pairing between the short arms of the 1R chromosomes was not the subject of the present study, so this data has been omitted from further comparisons. The number of chromosome associations without 1BL.1RS-1RS associations was 194 (table 1), most of which (93.8%) were detected between the wheat chromosomes (table 2), while wheat-rye and rye-rye associations were observed with significantly less frequency (5.2 and 1.0%). Pairing between the chromosomes was limited almost exclusively to the formation of rod bivalents. Multivalent formations were extremely rare; only a few trivalents were observed.

Identification of Chromosome Arm Associations by FISH

In order to identify the individual chromosome arm associations, the GISH signals were washed off, and the slides were reprobed for FISH, using the Afa family, pSc119.2 and pTa71 probes (fig. 1B). After the FISH experiments, there were no detectable structural changes in the chromatine structure of the meiocytes, indicating the feasibility of performing sequential GISH and FISH on meiotic chromosome preparations. Apart from the fact that identification was complicated by the stretching of the meiotic chromosomes, their FISH pattern was very

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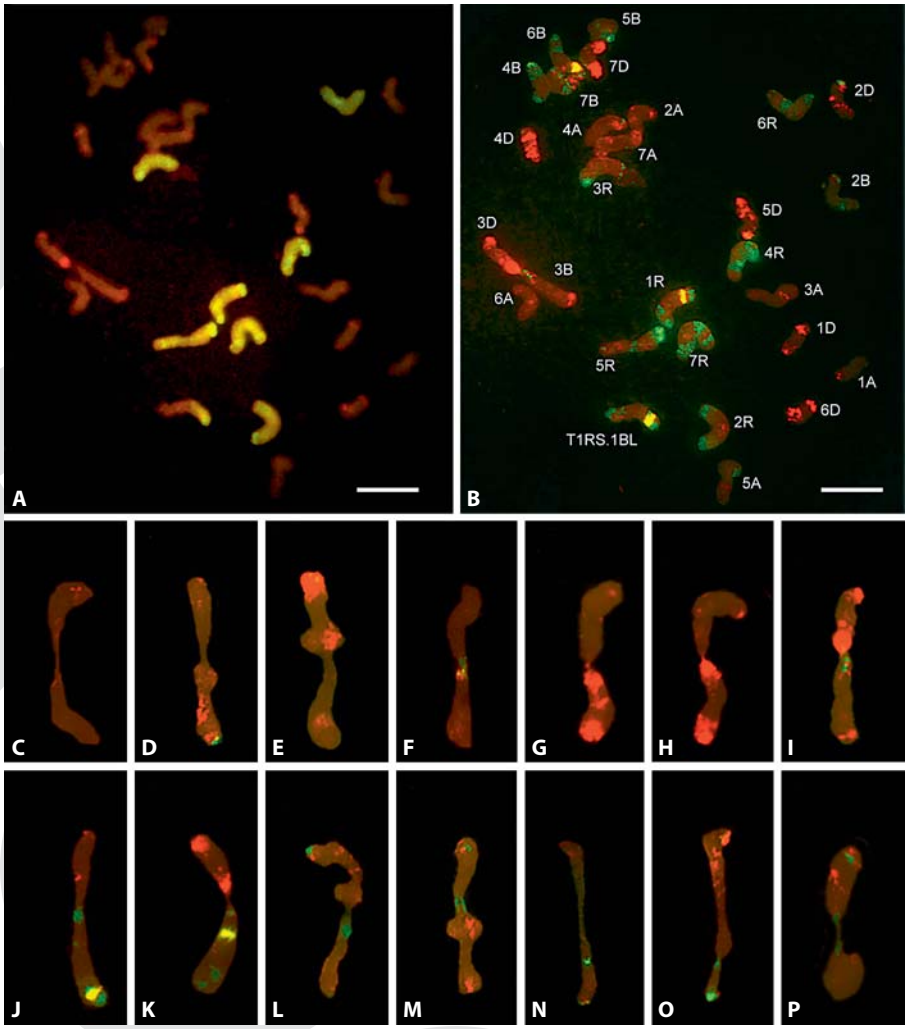

Fig. 1. In situ hybridization on meiotic chromosomes of wheat-rye hybrids: **A** GISH on chromosomes in the meiotic metaphase I of pollen mother cell. The rye chromosomes and the 1RS arm of the 1BL.1RS translocation are yellowish-green, while the wheat chromosomes are unlabelled. **B** FISH with repetitive DNA probes pSc119.2 (green), Afa family (red) and pTa71 (yellow) on the same pollen mother cell. **C–H** Homoeologous chromosome associations between the A and D genomes of wheat: **C** 1DS-1AS, **D** 2AL-2DL, **E** 3DL-3AL, **F** 5AS-5DS, **G** 6AL-6DL, **H** 7AL-7DL. **I–L** Homoeologous chromosome associations between the B and D genomes of wheat: **I** 3DS-3BS, **J** 1DL-1BL, **K** 6DS-6BS, **L** 5DL-5BL. **M** Non-homoeologous association between the long arms of the 1D and 7B chromosomes (1DL-7BL). **N** Homoeologous association between the long arms of the 2A and 2B chromosomes (2AL-2BL). **O**, **P** unidentified wheat-wheat chromosome associations. Scale bar = 10 μm.

similar to that of the mitotic chromosomes of wheat and rye [Sepsi et al., 2008; Szakács and Molnár-Láng, 2008]. Strong Afa family signals were observed on the D genome chromosomes, while diagnostic pSc119.2 signals were detected on the rye and B genome chromosomes. The strong signals of the pTa71 probe were observed on the nucleolus organizer region of the satellited rye 1R and wheat 6B chromosomes, while weak signals were detected on the short arms of the 3D and 5D chromosomes. A strong pTa71 signal has also been reported on the short arm of the satellited 1B chromosome, but this chromosome arm was not found in the genetic material used here due to the 1BL.1RS translocation. The A genome chromosomes could also be discriminated on the basis of their weak Afa signals and the diagnostic pSc119.2 signals on the 4A and 5A chromosomes.

Two wheat-wheat associations could not be identified from their FISH signals (fig. 1O, P), but the other chromosome associations were identified at the genomic level (table 2), and 129 wheat-wheat associations were identified at the chromosome arm level (table 3).

The FISH discrimination of the constituent genomes showed that intergenomic associations were more frequent than intragenomic associations in the F_1 hybrids. Most of the wheat-wheat associations were found between the A and D genomes, with similar pairing frequency in both hybrid combinations (0.342 and 0.354) (table 2 and fig. 1C, H). There were significantly fewer B-D chromosome associations (0.190 and 0.226) than A-D, while the least pairing frequency was detected between the A and B genome chromosomes (pairing frequency: 0.038 and 0.056). More frequent pairing was ob-

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Table 1. Meiotic configuration at metaphase I of meiosis in the wheat-rye hybrids

Combination	No. of PMCs	Total No. of configurations				Chromosome associations			
						with 1 BL.1RS-1RS		without 1 BL.1RS-1RS	
		I	rod II	ring II	III	total	mean/cell	total	mean/cell
Mv Magdaléna kr1 × rye	79	2,052	75	2	2*	84	1.06	58	0.71
Mv Béres kr1 × rye	195	4,976	232	1	6**	246	1.25	136	0.70

PMC = Pollen mother cell; I = univalent; II = bivalent; III = trivalent.
* One frying pan and one chain trivalent. ** Six chain trivalents.

Table 2. Frequency of intergenomic and intragenomic chromosome associations per PMC at metaphase I of meiosis in the wheat-rye hybrids

Combination	No. of PMCs	Wheat-wheat							Wheat-rye				Rye-rye[a]
		A-A	A-B	A-D	B-B	B-D	D-D	total	A-R	B-R	D-R	total	R-R
Mv Magdaléna kr1 × rye	79	0.101	0.038	0.342	0.000	0.190	0.013	0.684	0.000	0.013	0.025	0.038	0.013
Mv Béres kr1 × rye	195	0.005	0.056	0.354	0.005	0.226	0.010	0.656	0.010	0.015	0.010	0.036	0.005
Sum	274	0.033	0.051	0.350	0.004	0.215	0.011	0.664 (93.8%)	0.007	0.015	0.015	0.036 (5.2%)	0.007 (1.00%)

[a] The frequency of associations between rye chromosomes does not include the 1BL.1RS-1RS chromosome arm association.
PMC = Pollen mother cell.

served between the homoeologous wheat chromosome arms, while intragenomic A-A, B-B and D-D associations were extremely rare.

Type and Frequency of Wheat-Wheat Chromosome Arm Associations

Most of the chromosome arms involved in the wheat-wheat chromosome associations were identified according to the standard karyotype of hexaploid wheat [Mukai et al., 1993] (fig. 1C, N). Table 3 shows the wheat chromosome arm associations identified. The different associations were compared on the basis of pairing frequency (number of chromosome arm associations/cell). In the case of A-D associations, preferential pairing between the long arms was observed (table 3), where the significantly highest pairing frequency (0.048) was observed between the 3AL-3DL arms. Associations between the short arms of the A and D chromosomes were rare.

A very interesting type of pairing affinity was observed between the B and D chromosomes (fig. 1I, L). The overall pairing frequency was low (0.004–0.018) as compared to the A and D chromosomes, except for one chromosome arm association (3BS-3DS), where the pairing frequency was significantly higher (0.113) in both hybrid combinations than for any other chromosome arm association. It should be noted that non-homoeologous pairing was also observed mainly between the B and D genomes but at very low frequency (fig. 1M).

Pairing between the A and B chromosomes was only found very rarely, the highest pairing affinity being detected between 2AL and 2BL (pairing frequency: 0.011, fig. 1N).

Only 4 wheat chromosome arms (4AS, 5AL, 6BL, and 4DS) did not pair with another chromosome arm in the cells examined.

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Table 3. Frequency (as association per PMC) of wheat-wheat chromosome arm associations identified by FISH (with the probes pTa71, pSc119.2 and Afa family)

	Chromosome associations	Mv Magdaléna kr1 × rye	Mv Béres kr1 × rye	Total
Homoeologous chromosome associations	1AS-1DS	0.000	0.005	0.004
	1AL-1DL	0.013	0.031	0.026
	1DL-1BL	0.000	0.026	0.018
	2AS-2DS	0.013	0.005	0.007
	2AL-2DL	0.025	0.031	0.029
	2AS-2BS	0.013	0.000	0.004
	2AL-2BL	0.013	0.010	0.011
	2DS-2BS	0.013	0.000	0.004
	2DL-2BL	0.013	0.021	0.018
	3AS-3DS	0.025	0.000	0.007
	3AL-3DL	0.063	0.041	0.048*
	3DS-3BS	0.089	0.123	0.113*
	3DL-3BL	0.013	0.005	0.007
	4AL-4DL	0.000	0.005	0.004
	4DL-4BL	0.000	0.010	0.007
	5AS-5DS	0.013	0.010	0.011
	5DL-5BL	0.013	0.005	0.007
	6AS-6DS	0.013	0.015	0.015
	6AL-6DL	0.051	0.026	0.033
	6AS-6BS	0.013	0.005	0.007
	6DS-6BS	0.000	0.005	0.004
	7AL-7DL	0.025	0.015	0.018
	7AL-7BL	0.000	0.010	0.007
	7DS-7BS	0.013	0.000	0.004
	7DL-7BL	0.000	0.005	0.004
Non-homoeologous chromosome associations	3AS-6DS	0.025	0.000	0.007
	3AL-4AL	0.000	0.005	0.004
	4AL-5DL	0.013	0.000	0.004
	1DS-4DL	0.000	0.005	0.004
	1DL-7BL	0.000	0.005	0.004
	2DS-6BS	0.013	0.000	0.004
	2DL-5BS	0.013	0.000	0.004
	3DS-6BS	0.000	0.005	0.004
	3DL-4BL	0.000	0.005	0.004
	4DL-6BS	0.000	0.005	0.004
	4BS-7BS	0.000	0.005	0.004

* The observed frequency of chromosome arm associations differs significantly from the expected pairing frequency at the p = 0.001 significance level. PMC = Pollen mother cell.

Chi square was calculated to evaluate the chromosome arm associations statistically. The results confirmed the more frequent pairing between chromosome arms in homoeologous group 3. The significantly highest pairing frequency was detected between the long arms of 3A and 3D and the short arms of the 3D and 3B chromosomes.

Discussion

The present study confirmed that sequential GISH and FISH caused no structural modification in the chromatin structure and allowed the discrimination of the constituent genomes and the identification of all the wheat and rye chromosomes. Cifuentes et al. [2006] and Cifuentes and Benavente [2009] has also applied genomic and repetitive DNA probes in a single step in situ hy-

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bridization for the meiotic chromosome pairing analysis of wheat-alien hybrids, but the probe applied (pTa71) only allowed the identification of 3 wheat chromosomes (4A, 1B and 6B) having visualized cytological landmarks. By reprobing the slides of wheat-rye hybrids, Cuadrado et al. [1997] visualized 5 repetitive_DNA probes (pSc74, pSc119.2, pAs1, pTa71, and pTa794), allowing the identification of most of the wheat chromosomes. By combining of GISH and FISH in sequential experiments, the present study was able to maximize the available information (genome discrimination and identification of individual chromosomes) for the systematic analysis of meiotic chromosome pairing in major cereals.

GISH proved that the frequency of wheat-wheat chromosome associations was much higher than that of wheat-rye and rye-rye associations in the 2 wheat-rye F_1 hybrid combinations in the presence of *Ph1*. The similar pairing frequency of the 2 wheat-rye hybrids could be due to the close genetic relationship between the parental lines [Molnár-Láng et al., 2010]. More frequent wheat-wheat chromosome pairing was also reported by Miller et al. [1994] and Cuadrado et al. [1997] in wheat-rye hybrids with and without the *Ph1* gene. All of these chromosome pairing results reflect the fact that the constituent genomes of hexaploid wheat are more closely related to each other than to the rye genome.

In agreement with this chromosome pairing data, restriction fragment length polymorphism (RFLP)-based comparative mapping of rye and wheat also revealed that chromosome arms 2RS, 3RL, 4RL, 5RL, 6RS, 6RL, 7RS, and 7RL have all been involved in at least one translocation relative to wheat [Devos et al., 1993]. Naranjo and Fernández-Rueda [1996] also reported reduced wheat-rye pairing frequency for the above-mentioned rye arms, which was attributed to evolutionary rearrangements. Many studies confirmed that telomeres facilitate homologue recognition in a process known as 'telomere-bouquet' formation in early meiosis [Bass et al., 1997; Martinez-Perez et al., 2003; Prieto et al., 2004]. Together with many other factors, synchronized chromatin remodelling also enables related chromosomes to become competent to pair and recombine [Colas et al., 2008]. Evolutionary rearrangements in the chromosome structure, such as translocations and inversions, change the relative position of the telomeric regions and the conformational pattern of the chromosomes, which may be responsible for the low pairing frequency of rearranged and non-rearranged chromosomes [Devos et al., 1995; Lukaszewski et al., 2012] and of wheat and rye chromosomes.

Interestingly, the pairing level of wheat chromosomes in wheat euhaploids was found to be 0.62–1.05 rod bivalents per PMC [Jauhar et al., 1991], which is quite similar to the level of wheat-wheat chromosome pairing in wheat-rye hybrids observed in the present study (table 2). These data suggest that the rye genome does not significantly influence the formation of wheat-wheat chromosome associations.

The discrimination of the A, B and D genome chromosomes by FISH showed the predominance of A-D associations followed by B-D and A-B associations (table 2). Earlier studies on the pairing behaviour of wheat chromosomes in euhaploids and wheat-alien hybrids also confirmed the much higher pairing affinity of the A and D genomes. N-banding studies on the chromosome pairing relationship between the A, B and D genomes of bread wheat euhaploids demonstrated that about 80% of the metaphase I associations occurred between the A and D genomes in the presence of *Ph1b*, followed by A-B and B-D associations with lower frequency [Jauhar et al., 1991]. With the use of C-banding, Naranjo et al. [1987] found that A-D associations were the most frequent in 3 different *T. aestivum* 'Chinese Spring' × rye combinations (5B-deficient, 3D-deficient and wild type), while in the case of group 3 and 6 chromosomes, the B-D pairing affinity was greater than that of A-B. Further studies on the chromosome pairing of wheat-rye hybrids [Cuadrado et al., 1997] and wheat-*Aegilops geniculata* hybrids [Cifuentes and Benavente, 2009] using FISH confirmed the preferential A-D pairing. The higher frequency of A-D associations relative to B-D and A-B suggests that the A and D genomes are more closely related to each other than to the B genome. In this context, C-banding and RFLP analysis on wheat and wheat-alien genetic stocks showed that the B genome of wheat is more prone to genome rearrangements than are the A and D genomes [Qi et al., 2006; Badaeva et al., 2007]. It can be assumed that this leads to structural differences which influence the intergenomic chromosome pairing of wheat.

In the present study, a tendency was observed for long arms to pair more frequently than short arms within a given type of wheat-wheat chromosome pair (table 3), confirming previous observations by Naranjo et al. [1987]. The more frequent association of long arms compared to short arms was especially conspicuous in the case of 3A-3D pairing, where the association frequency for the 3AL and 3DL arms was much higher (0.048) than for 3AS-3DS (0.007). On the other hand, the most frequent chromosome association within the whole set of wheat-wheat associations was found between the short

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arms of the 3B and 3D chromosomes (0.113). These latter results were not confirmed by Naranjo et al. [1987], who observed the highest frequency of associations within the group 3 chromosomes for 3AL-3DL. However, the increased pairing ability of the 3B and 3D chromosomes observed here may be associated with the anomalies found in the physical positions of the expressed sequence tag (EST) sequences between the group 3 chromosomes of hexaploid wheat. Among the 12 multi-EST anomalies observed by Munkvold et al. [2004] during the bin mapping of 996 ESTs on the group 3 chromosomes of wheat, 7 were between the 3B and 3D chromosomes. The authors suggested that one possible reason for these anomalies could be the occurrence of rearrangements between the 3B and 3D chromosomes. The increased frequency of 3BS-3DS associations might be the reason for rearrangements between these chromosomes. As mentioned above, telomeres have an important role in homologous recognition in the early zygotene phase [Martinez-Perez et al., 2000; Prieto et al., 2004]. The pattern of constitutive heterochromatic knobs in telomeric and subtelomeric positions [Gill et al., 1991] could indicate the similar structure of the A and D genome chromosomes and also of the 3BS and 3DS arms, which may be responsible for the increased pairing affinity. However, further experiments will be required if the background of the increased pairing ability of the 3B and 3D chromosomes is to be more precisely determined.

The present study proved that sequential FISH and GISH is a powerful technique for the systematic analysis of chromosome pairing in the meiotic metaphase I of wheat-alien hybrids, aimed at determining the homoeologous relationships between the different genomes. The pairing frequencies between the wheat genomes suggested the stronger homoeology of the A and D genomes relative to the B genome, especially in the case of the group 3 chromosomes. The only exception was the short arms of the 3B and 3D chromosomes, which showed the highest pairing affinity of all the arm combinations detected.

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