Theor Appl Genet (1997) 94: 347–355

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Fluorescence in situ hybridization with multiple repeated DNA probes applied to the analysis of wheat-rye chromosome pairing

Received: 21 February 1996 / Accepted: 12 July 1996

Abstract Fluorescence in situ hybridization (FISH) with multiple probes has been applied to meiotic chromosome spreads derived from *ph1b* common wheat x rye hybrid plants. The probes used included pSc74 and pSc 119.2 from rye (the latter also hybridizes on wheat, mainly B genome chromosomes), the *Ae. squarrosa* pAs1 probe, which hybridizes almost exclusively on D genome chromosomes, and wheat rDNA probes pTa71 and pTa794. Simultaneous and sequential FISH with a two-by-two combination of these probes allowed unequivocal identification of all of the rye (R) and most of the wheat (W) chromosomes, either unpaired or involved in pairing. Thus not only could wheat-wheat and wheat-rye associations be easily discriminated, which was already feasible by the sole use of the rye-specific pSc74 probe, but the individual pairing partners could also be identified. Of the wheat-rye pairing observed, which averaged from about 7% to 11% of the total pairing detected in six hybrid plants of the same cross combination, most involved B genome chromosomes (about 70%), and to a much lesser degree, those of the D (almost 17%) and A (14%) genomes. Rye arms 1RL and 5RL showed the highest pairing frequency (over 30%), followed by 2RL (11%) and 4RL (about 8%), with much lower values for all the other arms. 2RS and 5RS were never observed to pair in the sample analysed. Chromosome arms 1RL, 1RS, 2RL, 3RS, 4RS and 6RS were observed to be exclusively bound to wheat chromosomes of the same homoeologous group. The opposite was true for 4RL (paired with 6BS and 7BS) and 6RL (paired with 7BL). 5RL, on the other hand, paired with 4WL arms or segments of them in more than 80% of the cases and with 5WL in the remaining ones. Additional cases of pairing involving wheat chromosomes belonging to more than one homoeologous group occurred with 3RL, 7RS and 7RL. These results, while adding support to previous evidence about the existence of several translocations in the rye genome relative to that of wheat, show that FISH with multiple probes is an efficient method by which to study fundamental aspects of chromosome behaviour at meiosis, such as interspecific pairing. The type of knowledge attainable from this approach is expected to have a significant impact on both theoretical and applied research concerning wheat and related Triticeae.

Key words Fluorescence in situ hybridization · Multiple probes · Chromosome pairing · Meiosis · Wheat · Rye

Introduction

In situ hybridization (ISH) techniques applied to plant meiotic chromosomes effectively combine the specificity and resolution of ISH with the chromosomal and genomic perspectives provided by meiotic cytogenetic analyses for studying fundamental genetic problems, such as chromosome pairing and recombination. Nonisotopic ISH and, in particular, fluorescence in situ hybridization (FISH), has greatly enhanced the potentiality of such an approach compared to the use of radioactive ISH. Recently, making use of total DNA from the genome of one species as a probe, together with an excess of unlabelled total DNA from the other parent (genomic in situ hybridization, GISH), King et al. (1994), Miller et al. (1994) and Xu et al. (1994) applied FISH to discriminate pairing partners in

Communicated by G. E. Hart

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meiotic metaphase I microsporocytes of wheat-alien hybrids. However, the use of simultaneous and/or successive visualization of several different probes on the same specimen, which is made possible by FISH, maximizes the amount of information attainable from a given chromosome preparation, either somatic or meiotic. FISH carried out employing different highly repeated DNA probes has recently allowed the discrimination of somatic wheat chromosomes belonging to the A, B and D genomes (Mukai et al. 1993) and of individual rye chromosomes in root-tip cells of wheat-rye hybrids (Cuadrado et al. 1995a).

In the study presented here, FISH was applied to meiotic metaphase I chromosome spreads derived from *ph1b* common wheat x rye hybrid plants using multiple repeated DNA probes both simultaneously and sequentially. All of the rye and most of the common wheat chromosomes could thus be identified, and hence their pairing behaviour, particularly that of rye chromosomes with those of the wheat genomes, studied in detail.

Prior to the advent of ISH techniques, the assignment of chromosomes to either the wheat or rye genome was carried out in C-banded meiocytes of wheat-rye hybrids (Mettin et al. 1976). This technique, however, only enabled ready identification of individual rye (R) chromosomes 1R and 5R (Naranjo et al. 1987; 1988), the others only being able to be discriminated when present as cytologically marked members (Naranjo and Fernandez-Rueda 1991). As a result of the present research, not only could the contribution to the total pairing of the wheat-wheat, wheat-rye and rye-rye associations be determined, but also the type and frequency of each wheat-rye association established unequivocally. Preliminary data on the same material have been presented by Cuadrado et al. (1995c).

Materials and methods

Plant material

Six F_1 hybrid plants between the *T. aestivum* cv 'Chinese Spring' *ph1b* mutant (Sears 1977) and a self-pollinated selection from the Portuguese rye landrace 'Centeio do Alto', named H148-4, H148-6, H152-2, H152-4, H165-2 and H178-3, were used as the source of the material – both root tips and anthers – for cytological preparations to be subjected to FISH. All plants were grown in a controlled environment at 18°–20°C throughout the meiotic stage, following a period of about 2 months at a lower temperature (8°–12°C) regime.

The *ph1b* mutant was employed to allow homoeologous pairing to occur among the wheat (A, B and D) and rye (R) genomes present in the hybrid combinations. 'Centeio do Alto' is characterized by a generalized reduced amount of C-heterochromatin with respect to other *S. cereale* cultivars (T. Mello-Sampayo, personal communication; Ceoloni and Bernardo, unpublished; Cuadrado et al. 1995a).

DNA probes and labelling

Two probes isolated from *S. cereale*, pSc74, which contains part of the 480-bp highly repeated DNA family (Bedbrook et al. 1980), and pSc 119.2, containing the 120-bp family subclone isolated by McIntyre et al. (1990), were used. In addition, the pAs1 clone isolated from the D genome of *Ae. squarrosa* (Rayburn and Gill 1986) and pTa71, containing the repeat unit of 25S-5.8S-18S rDNA from *T. aestivum* (Gerlach and Bedbrook 1979), were employed. Hybridization with the wheat pTa794 probe, which corresponds to the complete 5S gene unit (Gerlach and Dyer 1980), was occasionally carried out. In addition to other evidence, including pairing partnership, use of the latter probe represents an useful aid to discriminate chromosome 3R, which carries a 5S locus on its short arm (Alonso-Blanco et al. 1994; Cuadrado et al. 1995b), from the morphologically similar 2R in cases where results with other sequences are not discriminating.

Probes were labelled either with digoxigenin-11-dUTP or rhodamine-4-dUTP. The polymerase chain reaction (PCR) and, occasionally, nick translation were used for sequence labelling.

Cytological preparations and FISH

Anthers extracted from freshly collected young spikes and root tips pretreated in ice-cold water for 26 h were fixed in 3:1 (v/v) ethanol–acetic acid and stored at −20°C. Both materials were subjected to the same enzymatic treatment – about a 1-h digestion in a 2% (w/v) cellulase (1:4 Onozuka RS and Calbiochem) and 4% liquid pectinase (Sigma) in a 0.01 *M* citric acid-sodium citrate buffer at pH 4.8. Chromosome spreads were made from enzyme-softened materials squashed in a drop of 45% acetic acid. Subsequent pretreatments were according to Leitch et al. (1994). FISH with multiple probes was carried out as described in Cuadrado and Jouve (1994). Two probes, each labelled and detected with a different fluorochrome, were routinely combined in the same hybridization mixture (30 µl/slide). After washing in 4 × SSC containing 0.2% (v/v) Tween-20, rehybridization was carried out with either one or two additional probes. No modification of the denaturation time or temperature was adopted in reprobing. No appreciable alteration was detected in chromosome morphology after the second and even third probing.

A programmable thermal controller (PT-100, M.J. Research) was used for denaturation and in situ hybridization of spread chromosome preparations.

Pairing analysis

A total of 171 pollen mother cells at the metaphase I stage were analysed. These included a small fraction of incomplete cells in which the presence of the seven rye chromosomes could always still be ascertained. To evaluate the pairing frequency, we calculated the average number ($\pm$SEM) of total metaphase I bonds per cell as well as the relative contribution (%) to this figure of wheat-wheat (W-W), wheat-rye (W-R) and rye-rye (R-R) associations for each of the six hybrid plants. The number of bonds per cell corresponded, as in Naranjo et al. (1989) and Naranjo (1994), to the minimum number of chiasmata that explained each meiotic configuration (i.e. given the type of configurations observed, one chiasma per open bivalent; two chiasmata per closed bivalent and chain trivalent; three per chain quadrivalent and frying-pan trivalent and four per chain pentavalent).

The total frequency of association (in percentage) of each rye chromosome arm with wheat chromosomes as well as the type and frequency of each association were also evaluated in relation to the total and per plant number of W-R associations observed.

Results and discussion

Chromosome identification by simultaneous and sequential FISH

Simultaneous and sequential FISH with a two-by-two combination of highly repeated DNA probes (see Materials and methods) allowed unequivocal identification of all the rye and several of the wheat chromosomes present in metaphase I spreads of six plants of the same ABDR hybrid combination. This permitted both easy discrimination between W-W and W-R pairing associations and identification of the individual pairing partners.

The rye line used had previously shown polymorphism for C-banding (Bernardo and Ceoloni unpublished) and FISH (Cuadrado et al. 1995a) in somatic preparations. Therefore, the chromosomes of the hybrid plants to be used for meiotic analyses were first screened in somatic tissues using FISH with the pSc74 and pSc119.2 probes to detect the particular hybridization pattern present. This knowledge facilitated the identification of individual R chromosomes at meiotic metaphase I, where observations can be complicated by stretching and other morphological alterations. Similar to the finding of Cuadrado et al. (1995a), the observed variation involved relatively few sites, as most of the ISH bands for both pSc74 and pSc119.2 appeared rather highly conserved (see Table 1 of the quoted study).

Additional information was gained by simultaneous and/or sequential use of different probes. However, pSc74 by itself gave a clear differentiation of rye chromosomes from those of wheat, thereby allowing immediate identification of situations where the seven rye chromosomes occurred as univalents (Fig. 1c–e) or were partly involved in bivalent (Fig. 1a, b, f–i) or more complex associations (Fig. 1a, b, j–l) with those of the wheat complement. Furthermore, rye chromosomes 5R and 6R could be individually identified because as well as having a distinctive morphology they also exhibit specific and highly conserved landmarks when hybridized with pSc74. Thus, this probe is more advantageous in wheat x rye pairing studies than total rye genomic DNA (King et al. 1994; Miller et al. 1994).

Simultaneous hybridization with pSc74 and pSc119.2 allowed all of the R chromosomes and several of the wheat complement, mainly those belonging to the B genome, to be identified (Fig. 1a, c, j). Furthermore, reprobing the same chromosome preparations with probes pAs1 and pTa71 enabled identification of wheat D genome chromosomes (pAs1, Fig. 1b, e, i, l) and confirmed previous identifications of chromosome arms bearing NOR sites (see pTa71 on 1RS, 1BS, 6BS in Fig. 1b, d and also on 5DS in Fig. 1b).

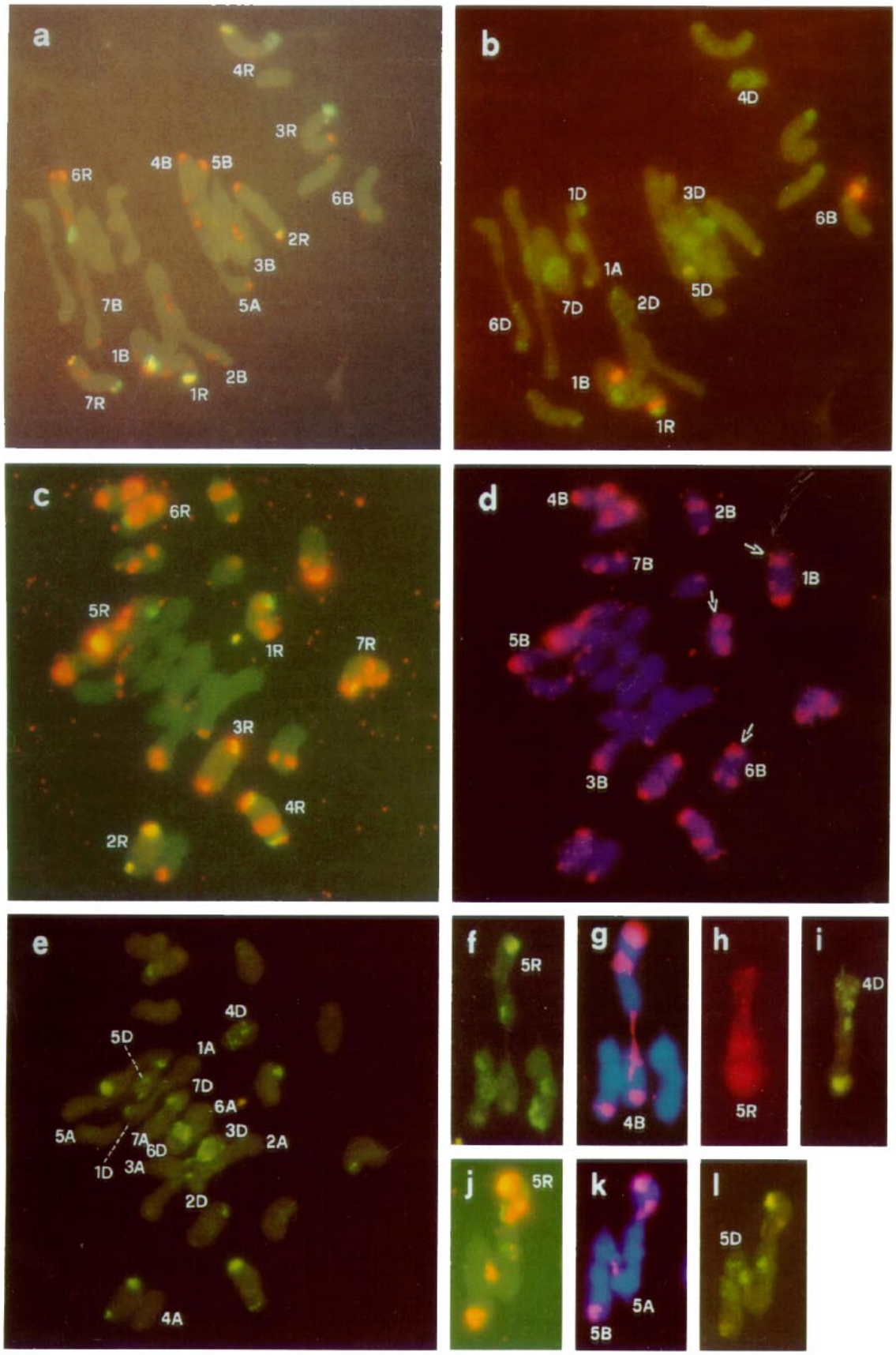
The A genome chromosomes, only a few of which possess hybridization sites for the pSc119.2 (4A and 5A) and the pAs1 (1A and 4A) probes (Mukai et al. 1993), and for which no specific, discriminating probe is available for use in ISH analyses, were assigned to this genome essentially for being non-Bs and non-Ds. Their assignment to a given homoeologous group was then inferred on the basis of the expected pairing partnerships (Fig. 1a, b, e, k). Thus, in a considerable number of cells, either directly or indirectly, all of the chromosomes could be identifed (see Fig. 1c–e).

Total and intergenomic pairing frequency

The six hybrid plants deriving from the same wheat cv 'Chinese Spring' *ph1b* x rye cv 'Centeio do Alto' cross combination were compared on the basis of their total amount of pairing (mean number of bonds per cell $\pm$SEM) and of the relative contribution to this figure of the wheat-wheat, wheat-rye and rye-rye pairing (Table 1). Some between-plant variation, commonly observed in similar studies on wheat-rye hybrids (see Ceoloni and Donini 1993; Miller et al. 1994), was detected. Such differences, which are likely to be due to a heterozygosity for factors affecting chromosome pairing in the out-breeding rye parent, are of particular concern in hybrids H148-4 and H148-6. H148-4 showed the lowest total pairing frequency (7.83 bonds/cell), which appears to result mainly from a decrease in W-W pairing (7.24 bonds/cell, ranging from 8.21 to 9.23 in the other plants), but also from a decrease in the W-R pairing (0.55 bonds/cell, ranging from 0.65 to 1.13 in the others). H148-6, on the other hand, apparently owes its highest overall pairing frequency to a considerable increase in W-R pairing, representing almost 11% of the total pairing (from 6.5 to 7.9% in the other plants). The remaining four plants exhibited rather similar values of total pairing frequency and of bond formation within the wheat, and between the wheat and rye genomes (Table 1).

Rye-rye pairing, probably resulting from non-homologous/non-homoeologous pairing between DNA sequences common to several R chromosomes, was detected at a very low frequency in only two of the hybrids analysed, namely H148-4 and H178-3 (Table 1).

The relative proportions of wheat-wheat, wheat-rye and also rye-rye pairing observed in the present study are of the same order of magnitude as those reported for 'Chinese Spring' mono-5B $\times$ 'Petkus Spring' rye (Miller et al. 1994). On the other hand, the overall amount of pairing exhibited by the present material is considerably higher than that reported in the above quoted study and in earlier studies (Riley 1960; Riley et al. 1959). The different experimental conditions adopted in these studies may account for the differences in pairing frequency. However, with the wheat genotype being essentially the same, some contribution of the rye parent to such a difference cannot be excluded. The


a
4R
3R
6R
4B
5B
6B
2R
3B
7B
5A
1B
2B
7R
1R
b
4D
1D
3D
6B
1A
5D
7D
2D
6D
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c
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4D
5D
1A
7D
6A
5A
7A
3D
2A
6D
1D
3A
2D
4A
f
5R
g
4B
h
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i
4D
j
5R
k
5A
5B
l
5D

Table 1 Different types of pairing involving the wheat (W) and rye (R) genomes in six *ph1b* common wheat x rye hybrid plants. Mean number of bonds per cell and relative frequency (%) of each type of pairing are given

Hybrid	Type of pairing						
	W-W		W-R		R-R		Total
	M ± SEM	% Total	M ± SEM	% Total	M ± SEM	% Total	M ± SEM
H148-4	7.24 ± 0.08	92.46	0.55 ± 0.03	7.02	0.03 ± 0.01	0.38	7.83 ± 0.09
H148-6	9.17 ± 0.06	89.03	1.13 ± 0.04	10.97	0	0	10.30 ± 0.08
H152-2	8.89 ± 0.10	92.99	0.67 ± 0.04	7.01	0	0	9.56 ± 0.10
H152-4	8.21 ± 0.17	91.94	0.71 ± 0.03	7.95	0	0	8.93 ± 0.17
H165-2	9.21 ± 0.04	92.28	0.77 ± 0.02	7.72	0	0	9.98 ± 0.04
H178-3	9.23 ± 0.05	92.58	0.65 ± 0.02	6.52	0.09 ± 0.01	0.90	9.97 ± 0.05

'Centeio do Alto' selection used here might contain genetic factors particularly effective in promoting chromosome pairing in hybrids with wheat. Its reduced amount of C-heterochromatin compared to other rye cultivars (T. Mello-Sampayo, personal communication; Ceoloni and Bernardo, unpublished; Cuadrado et al. 1995a) might then be an additional promoting factor as far as the wheat-rye pairing is concerned, although this matter remains controversial (Schlegel 1979; Naranjo and Lacadena 1980; Kaltsikes et al. 1983).

Type and frequency of wheat-rye chromosome associations

Data about type and frequency, both total and per plant, of the wheat-rye associations observed in the 'Chinese Spring' *ph1b* wheat × 'Centeio do Alto' rye hybrids are reported in Table 2. In all cases the rye chiasmatic arm and in most cases also the wheat member(s) and the corresponding bound arm(s) were identified. Although for the trivalent and higher rank configurations only the rye arm and that of the wheat partner bound to it are specified in the Table, pairing of the identified, additional wheat partners followed the expected arm-to-arm sequence.

R chromosomes paired most frequently with those of the wheat B genome (about 70%), and much less with D and A genome members (about 17% and 14%, respectively). Pairing competition can largely account for this outcome, as highly preferential A-D pairing, observed in the present (Fig. 1e) and in previous studies (Naranjo et al. 1987; 1988), is expected to leave the R and B genome chromosomes more prone to establish associations. In the sample analysed here, R-B associations, which were the only ones observed with 6RL, 6RS and 7RL, were predominant in all homoeologous groups, with a few exceptions. The most notable of these concern plant H148-4, in which 1R paired preferentially (L arm) or even exclusively (S arm) with the D wheat homoeologue, and plant H178-3, where 5RL bonds were much more frequent with 5AL (i.e. with its distal 4AL translocated segment, see ahead) than with 4BL (Table 2). Such deviations from the general pairing trend might be due to the existence of translocations present in the *ph1b* wheat parent, of which different plants were employed in developing the F_1 hybrids with rye, although these were not detected with the available probes. One such translocation, however, was clearly observed in plant H148-6. This consisted of a 4B-4D exchange resulting in a 4BL-4DL.4DS chromosome in which the breakage point was proximal to the pSc119.2 hybridization sites typical of the 4BL arm (not shown).

Rye chromosome arms 1RL and 5RL showed the highest pairing frequency (32.3% and 31.5%, respectively), followed by 2RL (11%) and 4RL (7.9%). Much lower values were exhibited by the other rye arms, and 2RS as well as 5RS did not establish any association

Fig. 1a–l Fluorescence in situ hybridization onto meiotic metaphase I chromosome spreads of PMCs from *ph1b T. aestivum* cv 'Chinese Spring' × *S. cereale* cv 'Centeio do Alto' hybrid plants. **a** Simultaneous hybridization with digoxigenin-labelled pSc74, detected by fluorescein conjugate (*green*), and rhodamine-labelled and directly detected (*red*) pSc119.2 permits individual identification of all the rye chromosomes (5R is missing from the plate) and of the B genome members, present either as univalents or involved in pairing associations. Note the 6R-7B bivalent and the pentavalent involving 1R. **b** Reprobing of the same preparation with pAs1 (*green*) and pTa71 (*red*) allows identification of D genome and NOR-bearing chromosomes (1R, 1B, 6B and also 5D), respectively. **c** Simultaneous hybridization with pSc74 (*green*) and pSc119.2 (*red*) shows the presence of the seven rye chromosomes as univalents. **d, e** Reprobing carried out hybridizing simultaneously pTa71 (*red*, **d**) and pAs1 (green, **e**): while pSc119.2 sites from the first FISH run are still visible in **d**, additional red signals compared to those present in **c** mark NOR sites (*arrowed*). The pAs1 hybridization pattern (**e**) allows identification of the D genome chromosomes, most of which are involved in pairing with A genome members (as deduced by identification of all the other chromosomes present in the cell). Note the 5B-5D-5A and 3A-3D-3B trivalents and the 1A-1D, 2A-2D, 6A-6D and 7A-7D bivalents. **f–l** Portions of cells in which the 5RL arm, carrying a distal 4RL translocation, was observed to pair with wheat group 4 chromosomes (4B in **f–g** and 4D in **h–i**) or translocated segments of them (4AL/5AL.5AS in **j–l**; see text for comments)

Table 2 Type and frequency (%) of metaphase I associations between arms of rye (R) and wheat (W) chromosomes in six *ph1b* common wheat x rye hybrids. Chromosome arms 2RS and 5RS, having shown no association with wheat chromosomes, are not included

R chromosome arm	Total pairing per R chromosome arm	Type of R-W association	Per plant and total frequency of each R-W association						
			H148-4	H148-6	H152-2	H152-4	H165-2	H178-3	Total
1RL	32.3	1RL-1BL	12.5	23.1	25.0	40.0	29.3	13.6	23.6
		1R-1BL-2A-2D-2B			0.8				0.8
		1RL-IDL	18.8	3.8			4.9		4.7
		1RL-1DL-1A-1B						4.5	0.8
		1RL-1AL		3.8			2.4		1.6
		1RL-1AL-1D					2.4		0.8
1RS	3.1	1RS-1BS		3.8					0.8
		1RS-1BS-W[a]		3.8					0.8
		1RS-1DS	6.3						0.8
		1RS-1DS-1A	6.3						0.8
2RL	11.0	2RL-2BL	12.5	3.8		10.0		13.6	5.5
		2RL-2BL-2A-2D						4.5	0.8
		2RL-2DL			8.3				0.8
		2RL-2AL-2D		3.8					0.8
		2RL-2AL-2D-2B		3.8					0.8
		2RL-W(A or D)		3.8		10.0	2.4		2.4
3RL	1.6	3RL-3BL-W(A or D)		3.8					0.8
		3RL-6BL	6.3						0.8
3RS	4.0	3RS-3BS	6.3				2.4	4.5	2.4
		3RS-3AS						4.5	0.8
		3RS-W					2.4		0.8
4RL	7.9	4RL-6BS		7.7	8.3		2.4		3.1
		4RL-7BS			8.3		2.4		1.6
		4RL-7BS-W					2.4		0.8
		4RL-W(A or D)					2.4	9.1	2.4
4RS	3.1	4RS-4BS					2.4		0.8
		4RS-4DS			8.3				0.8
		4RS-4DS-4B					2.4		0.8
		4RS-4DS-W					2.4		0.8
5RL	31.5	5RL-4BL	12.5	3.8[b]	25.0	20.0	9.8	4.5	10.2
		5RL-4DL		3.8		10.0		4.5	7.9
		5RL-4DL-4B					2.4		0.8
		5RL-5AL		3.8				18.2	3.9
		5RL-5AL-5D-5BL				10.0	2.4	13.6	3.9
		5RL-5BL	6.3	7.7			4.9	4.5	4.7
		5RL-5DL					2.4		0.8
		5RL-W	6.3	3.8			7.3		3.9
6RL	1.6	6RL-7BL	6.3		8.3				1.6
6RS	0.8	6RS-6BS					2.4		0.8
7RL	1.6	7RL-2BS-2D					2.4		0.8
		7RL-7BL		3.8					0.8
7RS	1.6	7RS-5BL		3.8					0.8
		7RS-W					2.4		0.8

[a] W stands for an unidentified wheat chromosome
[b] This pairing involved the distal portion of a 4BL-4DL.4DS translocated chromosome detected in this plant (see text)

with the wheat chromosomes (Table 2). These findings are in general agreement with those of Naranjo et al. (1988), where, however, only 1RL, 5RL and, indirectly, 2RL could be identified, and also with those of Naranjo and Fernandez-Rueda (1991), where identification of all rye chromosome arms was made possible by the use of cytological markers (C-bands, telocentrics and translocations).

The observed relative frequencies of pairing per R chromosome arm were generally consistent for all six hybrid plants. Depending on the R chromosome, the observed W-R associations involved wheat chromosomes belonging to homoeologous and/or non-homoeologous groups. In particular, rye arms IRL, 1RS, 2RL, 3RS, 4RS and 6RS established pairing with wheat homoeologous chromosomes only (Table 2). The

opposite was true for 4RL (paired with 6BS and 7BS) and 6RL (paired with 7BL, Fig. 1a). 5RL, on the other hand, paired with 4WL arms or segments of them (see ahead) in more than 80% of the cases (Fig. 1f–l), and with 5WL in the remaining ones.

The occurrence of W-R associations apparently involving wheat chromosomes belonging to non-homoeologous groups is consistent with the existence of chromosomal rearrangements in the *S. cereale* genome relative to that of wheat, previously suggested on the basis of meiotic observations of cytologically marked materials (Naranjo and Fernandez-Rueda, 1991) and of the locations of molecular and biochemical markers (Liu et al. 1992; Rognli et al. 1992; Devos et al. 1993). Thus, a postulated 5RL/4RL distal translocation allows pairing of 5RL with the L arms of wheat homoeologous group 4 chromosomes. Pairing with 5AL (Table 2, Fig. 1j–l) is also of this kind, as it possesses a distal 4AL portion resulting from a 4AL/5AL translocation, which is postulated to have originally occurred at the diploid wheat level (Devos et al. 1995) and is present both in *T. monococcum* (Devos et al. 1995) and polyploid wheats (Naranjo et al. 1987; Naranjo 1990; Liu et al. 1992; Devos et al. 1993, 1995). Similarly, the observed 3RL-6BL, 4RL-6BS, 4RL-7BS, 6RL-7BL, 7RL-2BS and 7RS-5BL associations can be accounted for by corresponding R-R translocations that occurred during the evolution of *S. cereale* (see above quoted citations).

An overall examination of the types and frequencies of rye-wheat associations detected here using FISH with multiple repeated DNA probes corroborates previous cytogenetic and molecular evidence on the existence of several causative factors determining the final pairing outcome. Among these, chromosome size has certainly an influence on pairing frequency, as proven in classical (Sallee and Kimber 1979) and more recent studies (e.g. Bernardo et al. 1988). Thus, when complete colinearity and arm correspondence exist between the rye and wheat partners, which is limited at the whole chromosome level to the group 1 chromosomes (Devos et al. 1993), arm length favours the longer arm in chiasmata formation (Table 2). The short size of 5RS, coupled with the presence, even in 'Centeio do Alto' rye, of a prominent block of repeated sequences at its telomere (see Fig. 1c, f–l and Cuadrado et al. 1995a) can similarly account for the lack (these data) or very low level (Naranjo and Fernandez-Rueda 1991) of pairing with its wheat homoeologous arm. On the other hand, other factors appear to determine the pairing pattern of other rye chromosomes. For example, metacentric 3R showed higher pairing in its short than in its long arm (Table 2 and Naranjo and Fernandez-Rueda 1991). Although no biochemical or molecular evidence is available for the presence of a translocated rye segment to 3RL (Devos et al. 1993), these pairing data suggest the existence of a 3L/6L translocation, probably distally located (see also Naranjo and Fernandez-Rueda 1991). Thus, a chromosomal rearrangement involving terminal regions evidently corresponds to a considerable reduction in the arm pairing ability. This same reasoning can be applied to the case of 2RS, where a sequence of a 2S/7S and a 2S/6S distal translocations (Devos et al. 1993) causes complete (these data, Table 2) or almost complete (Naranjo and Fernandez-Rueda 1991) suppression of pairing of this rye arm with those of the wheat complement sharing partial homoeology. Similarly, a terminal deletion of 6RS (Devos et al. 1993) is responsible for the low pairing frequency of the whole arm (Table 2 and Naranjo and Fernandez-Rueda 1991). Lower pairing frequencies with respect to non-rearranged chromosome arms of comparable or even smaller length are shown by 4RL which, however, had some pairing in both the distal (6S) and more proximal (7S) translocated portions (Table 2), as well as by 6RL, which exhibited only pairing of the most distal 7L translocation in the present sample (Table 2 and Fig. 1a), and by both arms of 7R (Table 2), probably the most rearranged chromosome of the rye genome (Rognli et al. 1992).

The overall picture emerging from the present and previous (Naranjo and Fernandez-Rueda 1991) studies clearly indicates that wheat-rye pairing is much affected by the occurrence of the various chromosomal rearrangements which differentiate the genomes of the two species. Such events apparently interfere not only with pairing initiation at the telomeres (Holm 1986) but also with the establishment of subsequent pairing sites in relatively more proximal portions (Abirached-Darmency et al. 1983; Holm 1986), where repeated changing of the homoeologous relationships may further hinder chromosome allignment and synapsis. In this respect, an apparent exceptional behaviour is that of 5RL. Its total pairing with wheat chromosomes (31.5%), including a major fraction of its distal portion with group 4 chromosomes or chromosomal segments (4BL, 4DL and 4AL on 5AL) and a minor one of the more proximal segment with 5WL chromosomes, is in fact only inferior to that of the fully homoeologous 1RL (32.3%; see also Naranjo et al. 1988; Naranjo and Fernandez-Rueda 1991). The physical location of a translocation breakpoint, particularly its distance from the highly recombinogenic telomeric regions (reviewed in Devos et al. 1995), as well as the length of the translocated segment(s) are likely to affect the overall arm pairing. With the tools presently available, the physical position of the breakpoints of the various rearrangements which differentiate the rye from the wheat genome cannot be precisely determined. However, the pairing behaviour of 5RL may be related to the 4L/5L translocation, which has been identified in several Triticeae species and thought to be an extremely primitive event, with virtually identical breakpoints in rye and in wheat (Devos et al. 1993). This would result in 5RL having a degree of correspondence in the segmental homoeology relative to wheat that, unlike

more recent translocations, practically coincides with a whole arm homoeology (e.g. of the 1R type).

Conclusions

The discrimination of pairing partners is of great importance in the study of hybrid combinations of wheat chromosomes with those of related Triticeae species. The ability of chromosomes to pair with each other is in fact one of the criteria on which their degree of relatedness can be evaluated. This information becomes fundamental when the wheat-alien transfer of genetic material through induced homoeologous recombination is desired. In cases such as that of wheat-rye combinations, a knowledge of the physical position and length of the chromosomal rearrangements present in the rye genome relative to that of wheat, and of their consequent effect on pairing, is an essential prerequisite for designing the best transfer strategy and predicting the eventual outcome.

The results presented here show that, even with the current technology and probes available, FISH, allowing simultaneous visualization of multiple DNA targets on the same specimen, is an efficient and highly reproducible technique by which to study fundamental aspects of chromosome behaviour at meiosis, such as interspecific pairing. In this, FISH can not only complement the information provided by other, both conventional and molecular, cytogenetic methods of analysis, but also broaden the overall knowledge attainable for theoretical and applied research, particularly when little prior knowledge is available on the structure or genetic relationships between the alien and wheat chromosomes.

The potential of such an approach will be greatly enhanced by the increased availability of informative probes and by the eventual development of procedures which will allow single and low-copy DNA probes to be efficiently used in ISH analyses of plant chromosomes.

Acknowledgements The authors thank M. O'Dell and B.S. Gill for gifts of clones. Financial support to C. Ceoloni and N. Jouve from an EEC-AIR3-CT93-1137 Concerted Action contract is gratefully acknowledged.

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