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Determination of the frequency of wheat-rye chromosome pairing in wheat × rye hybrids with and without chromosome 5B

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Abstract Genomic in-situ hybridization (GISH) was used to determine the amount of wheat-rye chromosome pairing in wheat (*Triticum aestivum*) × rye (*Secale cereale*) hybrids having chromosome 5B present, absent, or replaced by an extra dose of chromosome 5D. The levels of overall chromosome pairing were similar to those reported earlier but the levels of wheat-rye pairing were higher than earlier determinations using C-banding. Significant differences in chromosome pairing were found between the three genotypes studied. Both of the chromosome-5B-deficient hybrid genotypes showed much higher pairing than the euploid wheat hybrid. However, the 5B-deficient hybrid carrying an extra chromosome 5D had significantly less wheat-rye pairing than the simple 5B-deficient genotype, indicating the presence of a suppressing factor on chromosome 5D. Non-homologous/non-homoeologous chromosome pairing was observed in all three hybrid genotypes. The value of GISH for assessing the level of wheat-alien chromosome pairing in wheat/alien hybrids and the effectiveness of wheat genotypes that affect homoeologous chromosome pairing is demonstrated.

Key words Chromosome pairing · Genomic in situ hybridization · Meiosis · Rye · Wheat

Introduction

In hybrids between wheat and related species, chromosome pairing during meiosis between homoeologous wheat and alien chromosomes is prevented by the action of the gene *Ph1* located on chromosome 5B (Riley et al. 1959). In the absence of chromosome 5B, and hence the *Ph1* gene, pairing between homoeologous chromosomes of the constituent wheat genomes, or between the wheat and alien genomes, can occur in wheat/alien hybrids.

When assessing the possibility of wheat improvement by genetic transfers from alien species, the ability to easily determine the amount of wheat-alien chromosome pairing, and hence recombination in hybrids, would be of great value. Earlier attempts have utilized C-banding (Mettin et al. 1976; Schlegel and Weryszko 1979; Hutchinson et al. 1983), however this technique has the disadvantage of not distinguishing the entire length of every alien chromosome, and in some species the entire C-banded karyotype is not clearly distinguishable from that of wheat. However, C-banding does have the advantage in some cases of identifying individual alien chromosomes.

Flourescent in-situ hybridization using total DNA of an alien species as a probe (GISH) has been shown to be a valuable technique for identifying alien chromosomes and chromosome segments in wheat (Le et al. 1989; Mukai and Gill 1991; Schwarzacher et al. 1992; Mukai et al. 1993), and recently King et al. (1993, 1994) have shown the technique to be a powerful tool in the study of chromosome pairing at meiosis in wheat/rye hybrids.

The present paper reports the use of GISH to study the amount and nature of wheat-rye chromosome pairing in wheat/rye hybrids with and without chromosome 5B.

Materials and methods

The following wheat (*Triticum aestivum* cv 'Chinese Spring') × rye (*Secale cereale* cv 'Petkus Spring') hybrids were studied:
'Chinese Spring' euploid × 'Petkus Spring' (CS × P)
'Chinese Spring' monosomic 5B × 'Petkus Spring' (CSM5B × P)
'Chinese Spring' nullisomic 5B tetrasomic 5D × 'Petkus Spring' (CSN5B-T5D × P)

Anthers at first metaphase of meiosis were fixed in a 3:1 mixture of ethanol and acetic acid, and stored in a refrigerator at 2–4 °C for up to

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3 weeks. Slides were prepared by placing the fixed anthers in a drop of 45% acetic acid on ethanol-washed slides; the pollen mother cells were squeezed from the anthers and the anthers discarded. A coverglass was added and the preparation was gently squashed beneath filter paper. The slides were screened using phase-contrast microscopy and those selected were then frozen on dry ice and the coverglasses removed. After air-drying, the slides were stored at − 20 °C in sealed containers until required.

Prior to GISH the slides were treated with proteinase K as described by King et al. (1994). The GISH procedures were as described by Schwarzacher et al. (1992) using rhodamine-labelled probe DNA except that a direct Flourored (Amersham International)-labelled probe (Leitch et al. 1994) was used to detect the rye chromosomes.

Five plants of each genotype were studied and 100 pollen mother cells at meiotic metaphase I of each plant were scored.

The chromosome pairing data were analysed using a contingency χ^2 test of bound versus unbound chromosome arms for wheat-wheat, wheat-rye, and rye-rye associations within and between genotypes.

Results and discussion

Chinese Spring euploid × rye hybrids

The low level of chromosome pairing observed in the CS × P hybrids (Table 1) was similar to that reported in earlier studies (Riley 1960; Mettin et al. 1976; Miller et al. 1983). Of the pairing observed, 82.8% was between wheat chromosomes, 13.8% between wheat and rye chromosomes and 3.4% between rye chromosomes (Table 2). Variation occurred between individual plants; this was not significant for the wheat-wheat pairing [$\chi^2_{(4)} = 9.123^{ns}$] but was significant for the wheat-rye [$\chi^2_{(4)} = 18.20^{**}$] and rye-rye [$\chi^2_{(4)} = 10.45^{*}$] pairing. The cause of this variation has not been determined but the most likely explanation is heterozygosity for factors affecting chromosome pairing in the out-breeding rye parent as found in similar studies of wheat/rye hybrids (Mettin et al. 1976; Miller et al. 1983).

Although the overall level of pairing was similar to that in earlier studies, the ratio of wheat-wheat to wheat-rye pairing was makedly different from that determined by C-banding (Mettin et al. 1976; Schlegel and Weryszko 1979). These earlier studies indicated respectively 97% and 95% wheat-wheat, 1.3% and 2.6% wheat-rye, and 1.7% and 2.4% rye-rye pairing. It is possible that there may have been much less wheat-rye pairing in these hybrids but it seems more likely that the GISH technique is a more reliable means of distinguishing the wheat and rye chromosomes, and thus detecting such pairing (Fig. 1).

Table 1 Mean chromosome pairing associations per pollen mother cell in the three hybrid genotypes

Genotype	Bivalent		Trivalent	Quadrivalent	Others
	Rod	Ring			
CS × P	0.55[a]	–	–	–	–
CSM5B × P	2.36	0.52	0.77	0.11	0.02[b]
CSN5B-5TD × P	2.77	1.05	0.61	0.06[c]	–

[a] Includes 1 ring ^{II} and 2^{III}
[b] Includes 6^{V} and 4^{VI}
[c] Includes 2^{V}

Table 2 Mean number of chromosome arm associations per pollen mother cell in the three hybrid genotypes

Genotype	Wheat-wheat	Wheat-rye	Rye-rye	Total
CS × P	0.48 (82.8%)	0.08 (13.8%)	0.02 (3.4%)	0.58 (100%)
CSM5B × P	5.11 (91.1%)	0.43 (7.7%)	0.07 (1.2%)	5.61 (100%)
CSN5B-T5D × P	4.19[a] (95.0%)	0.18 (4.1%)	0.04 (0.9%)	4.41 (100%)

[a] Not including the 5D bivalent

Chinese Spring monosomic 5B × rye hybrids

The overall level of pairing in the selected 27-chromosome CSM5B × P hybrids (Table 1) was close to that reported by Riley (1960) for a 5B-deficient wheat/rye hybrid. As expected, the pairing was significantly higher than that found in the CS × P hybrids [for wheat-wheat pairing $\chi^2_{(1)} = 2239.08^{***}$, wheat-rye pairing $\chi^2_{(1)} = 89.07^{***}$ and rye-rye pairing $\chi^2_{(1)} = 19.06^{***}$]. The majority of the paring was between wheat chromosomes (91.1%); the wheat-rye pairing (7.7%), although significantly higher than in the CS × P hybrids, was reduced in proportion to the wheat-wheat pairing as was rye-rye pairing (1.2%) (Table 2).

It is clear that, as previously reported by Riley (1960), the absence of chromosome 5B carrying the gene *Ph1* induces homoeologous chromosome pairing in wheat/rye hybrids. However, the wheat-wheat pairing is increased more than the wheat-rye pairing (Tables 1 and 2). This may partly be due to the higher number of wheat chromosomes present (20) than rye chromosomes (seven), but may also reflect a closer phylogenetic relationship between the A, B and D genomes of wheat than that between them and the R genome of rye.

The pairing between non-homologous/non-homoeologous chromosomes within the single R genome is less easy to explain (Fig. 1 c). It is possible that the low level of such pairing, also seen in the CS × P hybrids, is the result of pairing between common DNA sequences distributed throughout the rye chromosomes, similar to those reported by Bedbrook et al. (1980). Presumably similar intra-genomic pairing also occurred among the wheat chromosomes but could not be distinguished.

Assuming that only homoeologous pairing can take place, the maximum size of pairing configuration possible should be a trivalent for wheat-wheat pairing and a quadrivalent for wheat-rye pairing (Fig. 1 b, d). However, wheat-wheat quadrivalents and pentavalents (0.09 per cell) and wheat-rye pentavalents and hexavalents

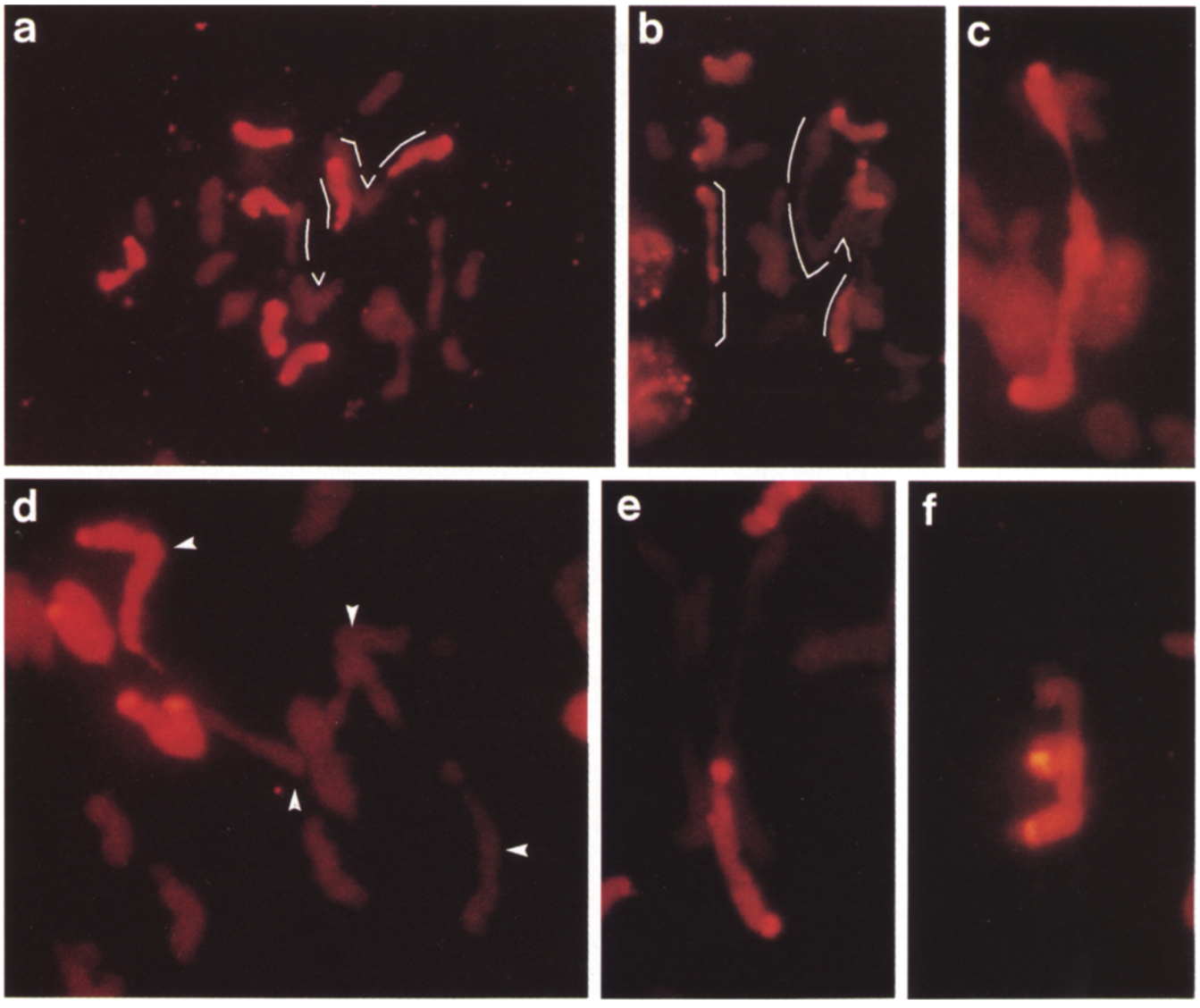

Fig. 1a–f Fluorescent in-situ hybridization of chromosomes at metaphase I of meiosis in pollen mother cells of wheat/rye hybrids (the rye chromosomes are bright red). **a** A cell of the CSM5B × P hybrid showing two wheat-wheat-rye V-shaped trivalents. **b** A partial cell of the CSM5B × P hybrid showing a wheat-rye rod bivalent and a wheat-wheat-wheat-rye chain quadrivalent. **c** A bivalent between two non-homologous/non-homoeologous rye chromosomes. **d** A wheat-wheat-wheat-rye chain quadrivalent. **e** A single chiasmate wheat-rye bivalent. **f** A wheat-rye bivalent with a clear chiasma loop

(0.01 per cell) were observed. These larger-than-expected associations probably arise from the presence of non-homoeologous translocations of the type reported by Naranjo et al. (1987) and Liu et al. (1991), which could give rise to pairing configurations involving the chromosomes of more than one homoeologous group. Support for this interpretation comes from the observation of a wheat-rye chain pentavalent in which the two terminal chromosomes were rye and the central three were wheat.

As in the CS × P hybrids, there was again between-plant variation; this time it was significant for the wheat-wheat [$\chi^2_{(4)} = 75.923^{***}$] and the rye-rye [$\chi^2_{(4)} = 9.66^{**}$] pairing but not for the wheat-rye pairing [$\chi^2_{(4)} - 7654^{ns}$].

Chinese Spring nullisomic 5B-tetrasomic 5D hybrids

The overall level of pairing in CSN5B-5D hybrids (Table 1) was similar to that observed by Hutchinson et al. (1983) but, as with euploid wheat × rye hybrids, the proportion of wheat-rye pairing determined by C-banding was lower (1.69%) than that determined in this study by GISH (4.1%, Table 2). This again indicates the greater efficiency of the GISH technique in distinguishing wheat and rye chromosomes at meiosis.

The CSN5B-T5D × P data in Table 2 have been adjusted to take account of the presence of the two homologous 5D chromosomes; as these are known to form a regular bivalent in a wheat/rye hybrid situation (Miller and Reader 1985), one wheat-wheat bivalent per cell has, therefore, been subtracted from the data. The

level of pairing was significantly higher than in the CS × P hybrids for wheat-wheat [$\chi^2_{(1)} = 1684.78$***] and wheat-rye [$\chi^2_{(1)} = 24.21$***] pairing but not for the rye-rye pairing [$\chi^2_{(1)} = 3.71^{ns}$]. Although the CSM5B × P and CSN5B-5D × P hybrids both had higher pairing than the CS × P hybrids, there were significant differences between them. The CSN5B-T5D × P hybrids had significantly lower wheat-wheat [$\chi^2_{(1)} = 32.68$***], wheat-rye [$\chi^2_{(1)} = 24.21$***] and rye-rye [$\chi^2_{(1)} = 6.40$*] pairing. The proportion of wheat-rye pairing was also lower (Table 2) and indicated a marked drop in the number of higher-order configurations (quadri-, penta- and hexavalents), especially those involving rye chromosomes; 17 such configurations involving wheat and rye chromosomes were observed in the CSM5B × P hybrids but only a single quadrivalent occurred in the CSN5B-T5D hybrids.

As the CSM5B × P and CSN5B-T5D × P hybrids differ only by the presence of one extra chromosome 5D, the differences in pairing must be attributed to the effect of the extra dose of this chromosome. Genes on the long arms of chromosomes 5B and 5R are known to decrease homoeologous chromosome pairing while genes on the short arms of chromosomes 5A, 5B and 5R have been shown to increase it (for a review see Gale and Miller 1987). The presence of a pairing suppressor on chromosome 5D is therefore highly probable. The extra dose of 5D aparently has a greater suppressive effect on wheat-rye pairing than on wheat-wheat pairing (Table 2).

In general the wheat-rye pairing observed was of a single chiasmate nature (Fig. 1e) but rare associations showing a distinct chiasma loop (Fig. 1f) were seen in the high-pairing genotypes.

From the data presented here it is clear that genomic in-situ hybridization (GISH) provides a reliable technique for determining the frequency of wheat-alien chromosome pairing in wheat/alien hybrids. This study only involved hybrids between wheat and rye but the effectiveness of the technique has also been demonstrated in a wheat/*Thinopyrum* hybrid (King et al. 1993).

Our study also shows the value of the technique for assessing the relative merits of the different genotypes that influence homoeologous chromosome pairing. It is clear from these results that the simple chromosome-5B-deficient 'Chinese Spring' genotype is more effective than the compensating nullisomic 5B-tetrasomic 5D genotype at inducing wheat-rye chromosome pairing in hybrids. Also using the GISH technique, King et al. (1994) studied chromosome pairing in a 'Chinese Spring' tetrasomic 3B × rye hybrid. The level of wheat-rye pairing observed was similar to that found by this study in the CSM5B × P hybrids. Thus it would appear that the monosomic 5B or the tetrasomic 3B genotypes are better parents than the nullisomic 5B-tetrasomic 5D genotyope for inducing wheat-rye chomosome pairing.

The GISH technique provides wheat cytogeneticists with a reliable means of assessing the potential for wheat-alien recombination by determining the level of wheat-alien chromosome pairing in interspecific and intergeneric hybrids. In addition, by identifying which wheat genotypes are the most effective for inducing homoeologous chromosome pairing the technique assists in selecting the best strategy for obtaining alien introgression into wheat.

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