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Comparative analysis of the meiotic effects of wheat *ph1b* and *ph2b* mutations in wheat×rye hybrids

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Abstract Wheat-wheat and wheat-rye homoeologous pairing at metaphase I and wheat-rye recombination at anaphase I were examined by genomic in situ hybridization (GISH) in wild-type (*Ph1Ph2*) and mutant *ph1b* and *ph2b* wheat × rye hybrids. The metaphase-I analysis revealed that the relative contribution of wheat-rye chromosome associations in *ph2b* wheat × rye was similar to that of the wild-type hybrid genotype but differed from the effect of the *ph1b* mutation. The greater pairing promotion effect of the *ph1b* mutation appears to be relatively more on distant homoeologous partner metaphase-I associations, whereas the lower promoting effect of *ph2b* is evenly distributed among all types of homoeologous associations. This finding reveals that distinct mechanisms are involved in the control of wheat homoeologous pairing by the two *Ph* genes. The frequency of wheat-rye recombination calculated from anaphase-I analysis was lower than expected from the metaphase-I data. A greater discrepancy was found in *ph2b* than in *ph1b* wheat × rye hybrids, which may suggest a more distal chiasma localization in the former hybrid genotype.

Key words Wheat × rye hybrids · *ph* mutations · Metaphase-I pairing · Anaphase-I recombination · GISH

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Introduction

The diploid-like meiotic behaviour of the allohexaploid wheat *Triticum aestivum* L. is regulated by a complex genetic system which prevents metaphase-I (MI) pairing between genetically related (homoeologous) chromosomes of the A, B and D genomes. This control is known to be exerted by two pairing homoeologous genes, *Ph1* (on 5BL) and *Ph2* (on 3DS), and several minor loci which act by either restricting or promoting homoeologous MI pairing (for a review, see Sears 1976). This meiotic control system has been manipulated for the genetic introgression of favourable traits from wild relatives into wheat. A large number of wheat × alien hybrids have been analysed in which the *Ph1* gene was absent either by nullisomy (e.g. Riley 1960; Naranjo et al. 1987) or by mutation (e.g. Dhaliwal et al. 1977; Orellana 1985). Less attention has been paid to the effect of $Ph2^-$ in the hybrids, even though its intermediate level of promotion is thought to be more appropriate for genetic transfers from closely related species (Sears 1982).

The majority of studies up to date have attempted to determine the level of wheat-alien MI chromosomal association promoted in a Ph^- background by means of either conventional staining techniques (e.g. Riley 1960), C-banding (e.g. Dhaliwal et al. 1977; Naranjo et al. 1987) or, more recently, genomic in situ hybridization (GISH) (e.g. Miller et al. 1994; Benavente et al. 1996).

The GISH analysis of wheat × rye hybrids presented here focussed primarily on examining whether the metaphase-I promoting effect of *ph* genotypes is evenly distributed among different combinations of pairing partners. The level of intergenomic recombination achieved by the lack of activity of *Ph* genes in the hybrids was also examined. A comparative analysis of *ph1b* and *ph2b* wheat × rye hybrids can be expected to disclose some insight on the mode of action of each

of the two major *Ph* genes controlling wheat homoeologous pairing.

Materials and methods

Three types of hybrids were used, namely *Ph1Ph2* wheat × rye (3 plants), *ph1b* wheat × rye (3 plants) and *ph2b* wheat × rye (2 plants), obtained by crossing either the wild-type, *ph1b* mutant (Sears' *ph* mutant) and *ph2b* mutant lines of hexaploid wheat cv 'Chinese Spring' as females and line 6Ri of diploid rye *Secale cereale* L. as male. Both *ph1b* (Sears 1977) and *ph2b* (Wall et al. 1971; Sears 1984) are deletion mutants.

Anthers of the emerging spikes containing pollen mother cells (PMCs) at metaphase I or anaphase I were fixed in 1 : 3 (v/v) acetic acid: ethanol and stored at −20°C for several months.

Genomic DNA isolated from rye leaves was labelled with digoxigenin-11-dUTP (Boehringer Mannhein) by nick translation according to the manufacturer's instructions and used at a concentration of 10 ng/μl in the hybridization mixture. Unlabelled 'Chinese Spring' DNA (4× the concentration of the rye probe) was added as blocking DNA. In situ hybridization and immunological detection of the probe were carried out as described by Fernández-Calvín et al. (1995).

For the metaphase-I analysis, 150 PMCs of each hybrid genotype were scored. The anaphase-I (AI) analysis was based on observations of 22–50 PMCs per *Ph1Ph2* wheat × rye plant and 75 PMCs per *ph2b* hybrid plant. Data on *ph1b* wheat × rye AI cells are from Benavente et al. (1996).

Results and discussion

The frequencies of the different meiotic configurations observed in all plants examined are listed in Table 1. Calculations of MI associations were based on the minimum number of chiasmata necessary to explain each meiotic configuration. In agreement with all previous reports, *Ph1Ph2* and *ph1b* hybrid genotypes exhibited the lowest and highest levels of MI pairing, respectively, whereas *ph2b* wheat × rye showed an intermediate value. Student *t*-tests revealed that the frequency of MI associations per cell was always significantly different between genotypes [*Ph1Ph2* vs. *ph1b*: $t_{(4)} = 10.377$, $P < 0.001$; *Ph1Ph2* vs. *ph2b*: $t_{(3)} = 7.703$, $0.01 > P > 0.001$; *ph1b* vs. *ph2b*: $t_{(3)} = 6.343$, $0.01 > P > 0.001$].

Three types of MI chromosomal associations were distinguished by GISH (Fig. 1), i.e. wheat-wheat (w-w), wheat-rye (w-r) and rye-rye (r-r) (Table 1). The mean numbers of wheat-rye associations per cell are directly related to the overall level of MI pairing in the hybrids. Non-homologous rye-rye MI pairing was very infrequent. The latter has been described previously (Dhaliwal et al. 1977; Miller et al. 1994) and will not be discussed further at the present time.

Miller et al. (1994), using GISH, reported a relative frequency of w-r MI association that was close to 14% in wild-type wheat × rye hybrids, a value much higher than that found in earlier studies carried out using the C-banding technique (e.g. 3.2% in Orellana 1985 and 3.3% in Naranjo et al. 1987). These authors explained that GISH is more reliable in distinguishing between wheat and rye chromosomes (see also Fernández-Calvín et al. 1995). However, only 1% of w-r MI associations was found in the *Ph1Ph2* wheat × rye examined in the present GISH study. Furthermore, the percentage of w-r associations reported here for the *ph1b* hybrid genotype (4.4%) is slightly lower than those from the C-banding analyses of Dhaliwal et al. (1977) and Orellana (1985) (6.3% and 5.7%, respectively). This indicates that other factors, including the genotypes used, may constitute a much greater source of variation in w-w versus w-r associations than the cytological technique used.

Table 1 Frequencies of MI pairing configurations and MI associations in the wheat × rye hybrids examined. Mean values per cell for each hybrid genotype appear in brackets

Wheat × rye hybrid plant		MI pairing configurations[a]			MI associations			
	Number of cells	II	III	IV	Total	w-w	w-r	r-r
Ph1Ph2-1	50	56	–	–	58	57	–	1
Ph1Ph2-2	50	74	2	–	86	85	1	–
Ph1Ph2-3	50	56	1	–	60	59	1	–
Ph1Ph2	150	186	3	–	204	201	2	1
					(1.36)	(1.34)	(0.01)	(0.01)
ph1b-1	50	210	97	–	549	532	17	–
ph1b-2	50	225	113	4	604	558	43	3
ph1b-3	50	213	56	–	453	443	10	–
ph1b	150	648	266	4	1606	1533	70	3
					(10.71)	(10.22)	(0.47)	(0.02)
ph2b-1	75	186	29	–	270	263	7	–
ph2b-2	75	176	22	–	244	240	4	–
ph2b	150	362	51	–	514	503	11	–
					(3.43)	(3.35)	(0.07)	(0.00)

[a] II, bivalents; III, trivalents; IV, quadrivalents

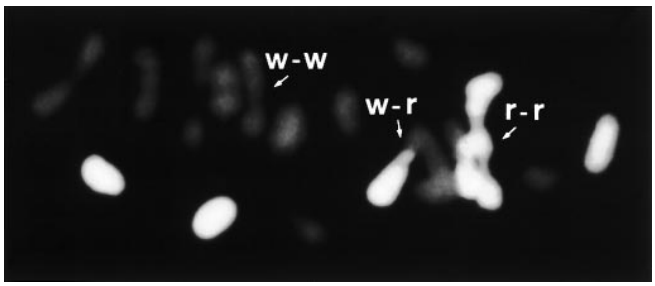

Fig. 1 Fluorescent micrograph of a *ph1b* wheat × rye hybrid metaphase-I cell. Examples of the three types of MI associations revealed by GISH are marked by *arrows*

To examine whether the promoting effect of each *ph* mutation on chromosomal association is evenly distributed among all types of MI homoeologous pairing, we compared the ratio of w-w and w-r associations between genotypes (Table 2). The contingency chi-square tests performed revealed that the ratio of w-w vs w-r MI association in *ph1b* wheat × rye significantly differs from that of either of the *Ph1Ph2* and *ph2b* hybrids. Further, our results indicate that the pairing promotion effect of the *ph1b* mutation was not uniform on all types of MI association but was relatively less on those involving two wheat homoeologous partners (w-w). Similar conclusions are made after statistical comparison between the wild-type and the *ph1b* wheat × rye hybrids analyzed by Orellana (1985) (see Table 2). A comparison between *Ph1Ph2* and *ph2b* by means of a contingency chi-square test may not be appropriate because one of the expected values was lower than 5.0, which can result in overestimation of the real difference between observed and expected frequencies. Nevertheless the corresponding χ^2 value given in Table 2, even if overestimated, indicates that the proportions of wheat-wheat and wheat-rye MI associations in *ph2b* wheat × rye are not significantly different from those of the *Ph1Ph2* hybrid genotype.

Table 2 Comparison of the frequencies of wheat-wheat (w-w) and wheat-rye (w-r) MI associations between wheat × rye hybrid genotypes. Expected values appear in brackets

Wheat × rye genotype	w-w	w-r	Contingency χ^2 ($df = 1$)
Ph1 Ph2	201 (194.9)	2 (8.1)	5.391*
ph1b	1533 (1539.1)	70 (63.9)	
Ph1Ph2	201 (199.3)	2 (3.7)	1.112[b]
ph2b	503 (504.7)	11 (9.3)	
ph1b	1533 (1541.7)	70 (61.3)	5.279*
ph2b	503 (494.3)	11 (19.7)	
Ph1Ph2[a]	664 (650.0)	22 (36.0)	7.015**
ph1b[a]	2928 (2942.0)	177 (163.0)	

* $0.05 > P > 0.01$; ** $0.01 > P > 0.001$
[a] Observed values from data in Orellana (1985)
[b] See text

Data of Naranjo and Maestra (1995) on the frequencies of the three different types of pairing which can be distinguished by means of C-banding in wheat × *Ae. longissima* hybrids (namely, A–D, B–S^l and 'others', which includes A–B, A–S^l, D–B and D–S^l) were used to perform the contingency chi-square tests shown in Table 3. Results from these comparisons are in agreement with those of the wheat × rye hybrids reported here. The relative proportions of the different types of MI homoeologous association are similar to those of the wild-type genotype in *ph2b* hybrids but differ under the *ph1b* mutation effect. Wheat × *Ae. longissima* hybrids exhibit two types of preferential MI pairing, i.e., A–D and B–S^l, (Naranjo and Maestra 1995). This indicates the closer evolutionary relationship between the A and the D genomes of wheat, and between the B genome of wheat and the S^l genome of *Ae. longissima*. A further assessment of results in Tables 2 and 3 reveals that in both wheat × alien *ph1b* hybrids deviation from the ratio of the wild-type genotype is due to the excess of MI associations between less similar homoeologous partners, i.e. w-r in wheat × rye hybrids, and those pooled in the type 'others' in wheat × *Ae. longissima* hybrids.

All data seem to indicate that the MI pairing promotion achieved by the *ph1b* mutation does not affect all types of pairwise combinations to the same extent but that the effect is relatively greater between distant homoeologous partners. The promoting effect of the *ph2b* mutation on the other hand is evenly distributed among all possible types of homoeologous association.

This finding could be explained in terms of the quantitative difference in the promoting effect between $Ph1^-$ and $Ph2^-$ mutations in wheat × alien hybrids. Thus, in MI cells having fewer chiasmata, the frequency of each type of homoeologous pairing association will exclusively depend upon the affinity for pairing and crossing-over between the two genomes concerned. However, in cells having a high number of chiasmata, some proportion of them must necessarily involve less-related homoeologues. As a consequence, the relative frequency of the less-abundant types of MI pairing (presumably involving less-related homoeologous partners) can be expected to increase with higher levels of crossing-over.

Alternatively, the differences in pairing patterns reported here between *ph1b* and *ph2b* wheat × alien hybrids can indicate that *Ph1* and *Ph2* genes exert control on synapsis and/or chiasma formation between

Table 3 Comparison of the frequencies of the different types of MI associations between wheat × *Ae. longissima* (ABDS^{l}) hybrid genotypes from data in Naranjo and Maestra (1995). Expected values appear in brackets

ABDS^{l} genotype	A–D	B–S^{l}	Others[a]	Contingency χ^2 ($df = 1$)
Ph1 Ph2	244 (204.4)	168 (138.4)	130 (199.1)	41.738***
ph1b	2029 (2068.6)	1371 (1400.6)	2084 (2014.9)	
Ph1Ph2	244 (226.5)	168 (181.1)	130 (134.4)	3.037[ns]
ph2b	915 (932.5)	759 (745.9)	558 (553.6)	
ph1b	2029 (2092.4)	1371 (1513.9)	2084 (1877.8)	131.555***
ph2b	915 (851.6)	759 (616.1)	558 (764.2)	

[ns] $P > 0.05$, ***$P < 0.001$

[a] includes A–B, A–S^{l}, D–B and D–S^{l} MI associations

homoeologous chromosomes by distinct mechanisms. If so, mutations at these genes may differ in their consequences in the hybrids, not only at the quantitative but also at the qualitative level.

Ultrastructural analysis of synaptonemal complex formation in nulli-5B wheat × rye (see Holm and Wang 1988) and *ph1b* wheat × *Ae. kotschyi* hybrids (Gillies 1987) demonstrated that the *Ph1* locus does not affect the ability of homoeologues to form synaptonemal complexes since the degree of synapsis achieved in the two *Ph1*$^{-}$ hybrid combinations was similar to that found in the corresponding wild-type hybrid genotypes. Both studies addressed the effect of the *Ph1* gene to some mechanism that influences the ability of crossing-over among homoeologous pairing partners. Holm and Wang (1988) proposed an additional effect on the stringency of synapsis so that the lack of activity of this pairing control gene causes a relaxation in the requirements of sequence similarity for extended synapsis. With respect to this point, it has recently been demonstrated that the *Ph1* gene can distinguish homologous from homoeologous regions between the 1A chromosome of wheat and a 1A wheat-*Triticum monococcum* recombinant chromosome (Dubcovsky et al. 1995; Luo et al. 1996). If under the effect of *ph1b*, the degree of chromosomal divergence between potential pairing partners can not be properly discriminated, then MI association will tend to be more randomly established among all homoeologues. This could explain the relative proportion of chromosome association between less-related homoeologues being increased in *ph1b* wheat × alien hybrids.

There is no information on the effect of the *Ph2* locus on synaptonemal complex formation. Ji and Langridge (1994) have identified a wheat cDNA clone that maps within the region lost in a *Ph2* deletion mutant. This clone represents early meiosis-specific expressed genes. According to the derived protein sequence, it has been suggested that the product encoded by the *Ph2* gene could play some structural role in the construction of synaptonemal complex. It can be inferred from our study that, whatever the mechanism actually affected by mutation at this locus, the ability to discriminate chromosome similarity among related partners seems not to be influenced.

GISH analysis of anaphase-I cells in wheat × rye hybrids allowed us to examine for the presence of wheat-rye recombinant chromosomes, which can be cytologically distinguished from the wheat and rye homogeneously coloured 'parental' types (Fig. 2) (see also Benavente et al. 1996). Although the number of recombinant chromosomes in each hybrid genotype is related to its level of wheat-rye MI pairing (see Tables 1 and 4), the frequency of w-r MI associations exceeds the frequency of w-r AI recombination in both *ph* mutant hybrid genotypes. Such a discrepancy is particularly remarkable for *ph2b* wheat × rye where the frequency of w-r recombination accounts for less than 10% of the frequency of w-r MI associations, whereas this ratio is of 35.5% in the *ph1b* genotype.

For rye homologous partners, it has been demonstrated that the lower the level of MI association the more distal chiasma localization (John 1990; see also Benavente and Orellana 1992). If extended to wheat-rye chromosome association, chiasmata can be expected to occur more distally in *ph2b* than in *ph1b* hybrids. Then, following Benavente et al. (1996), the resolution limits of GISH for detecting wheat-rye recombinants resulting from extremely distally located crossingovers and/or a greater proportion of chromosomal associations that do not represent chiasma formation in the *ph2b* hybrid genotype could explain

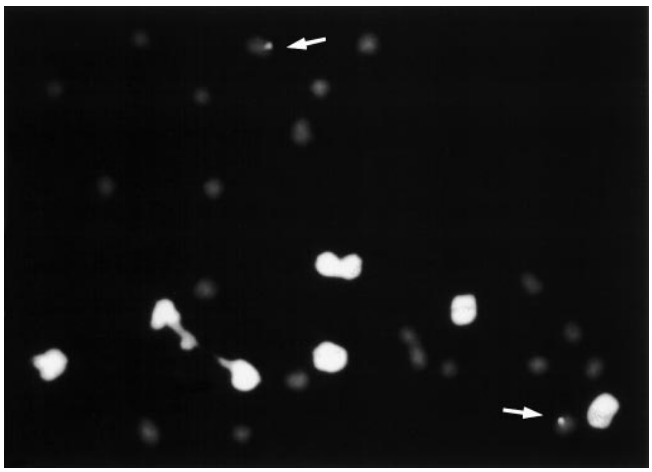

Fig. 2 Fluorescent micrograph of a *ph1b* wheat × rye hybrid anaphase-I cell. Arrows indicate two wheat^{rye} recombinant chromosomes

Table 4 Frequency of wheat-rye recombination in AI cells in the wheat × rye hybrids examined. Mean values per cell appear in brackets

Wheat × rye genotype	Number of cells	pairs of w-r recombinants
Ph1Ph2	119	– (0.000)
ph1b	132	22 (0.167)
ph2b	150	1 (0.007)

the relatively greater excess of MI association over AI recombination detected in *ph2b* wheat × rye. Whatever the reason, it remains to be elucidated whether such a difference in the ratio of w-r AI recombination revealed by GISH between both *ph* mutations is related to their reported distinct effects at the MI stage.

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