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## Centromere association is an unlikely mechanism by which the wheat *Ph1* locus regulates metaphase I chromosome pairing between homoeologous chromosomes

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**Abstract** Chiasmata pairing between homoeologous chromosomes at metaphase I (MI) of meiosis in wheat is prevented by the activity of the *Ph1* locus on chromosome 5B. Several hypotheses have been proposed sharing the assumption that *Ph1* regulates MI chromosome pairing by regulating centromere-mediated chromosome alignment before the onset of meiosis. To test the relevance of the putative predetermination of chromosome pairing at MI by the centromere-mediated chromosome association prior to meiosis, a 2BL.2RL homoeoisochromosome was constructed and its MI pairing was assessed in the presence and absence of the *Ph1* locus. Although the 2BL and 2RL arms of the homoeoisochromosome paired with each other at MI in the absence of *Ph1*, they never paired with each other at MI in the presence of *Ph1*. Since the two arms were permanently associated in the homoeoisochromosome via a common centromere, it is unlikely that *Ph1* predetermines MI pairing between homoeologous chromosomes solely by controlling premeiotic association of centromeres. These findings are consistent with the idea that *Ph1* determines the chromosome pairing pattern at MI by scrutinizing homology across the entire chromosome.

### Introduction

Chromosomes are paired only homogenetically, i.e., homologs are paired but homoeologs are not, at meiotic metaphase I (MI) in allotetraploid *Triticum turgidum* L. ( $2n=4x=28$ ; genomes AABB) and allohexaploid *Triticum aestivum* L. ( $2n=6x=42$ ; genomes AABBDD). Metaphase I pairing between homoeologous chromosomes is low or

virtually absent in wheat haploids and most interspecific hybrids (Riley 1960; McGuire and Dvorak 1982) although extensive synaptic associations in meiotic prophase may be observed (Gillies 1987; Wang 1988). The lack of MI pairing between homoeologous chromosomes is primarily due to the activity of the *Ph1* locus on chromosome 5B (Okamoto 1957; Sears and Okamoto 1959; Riley and Chapman 1958) but, as pointed out by Gillies (1987), the locus may not preclude synapsis between homoeologous chromosomes.

Since the function of the *Ph1* locus is to prevent heterogenetic pairing at MI, its effect is unequivocally apparent at MI. The pattern of chiasmata chromosome pairing at MI has therefore been used to assess the activity of *Ph1* and other genes regulating heterogenetic chromosome pairing in tetraploid and hexaploid wheats, wheat haploids and hybrids. Chiasmata pairing between chromosomes at MI is the final effect of processes preceding it: homolog recognition, synapsis, crossing over, and chiasma formation. To minimize confusion of causes and effects, the term MI pairing will be used throughout this paper for the chiasmata associations between chromosomes at MI, with cognizance of the fact that a modification of MI pairing is an effect of modification of one or more processes occurring earlier.

Several hypotheses have been advanced to explain the mechanism by which *Ph1* precludes MI pairing between homoeologous chromosomes. Feldman and associates (Feldman et al. 1966; Feldman 1968, 1993; Avivi and Feldman 1973) have argued that MI chromosome pairing is predetermined by somatic association between chromosomes and that *Ph1* exerts its control by suppressing somatic association between homoeologs via modification of interactions between mitotic spindle microtubules and the centromeres. These assumptions agree with the observation that premeiotic application of colchicine phenocopies the effects of increased doses of *Ph1* (Driscoll et al. 1967; Dover and Riley 1973). Since the colchicine-sensitive period at which meiotic pairing is blocked appeared to be either during the last premeiotic mitosis or in the transition between the last mitosis

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and G1, Dover and Riley (1977) modified the somatic association hypothesis by suggesting that the elimination of heterogenetic pairing at MI is brought about by association of homologous chromosomes in the last premeiotic anaphase or telophase.

The idea that the decision whether or not homoeologs may be paired at MI is made prior to meiosis and that centromere behavior plays a key role in making that decision recently received new attention (Aragón-Alcaide et al. 1997a, b). It was observed that in the absence of *Ph1*, such as in the background of the *ph1b* mutation (Sears 1977), centromeres have a diffuse appearance relative to those in the *Ph1* background (Aragón-Alcaide et al. 1997b) leading to the suggestion that timing of the pairing of centromeric sequences predetermines chromosome pairing (Aragón-Alcaide et al. 1997b; Moore 1998). It should be noted that the *ph1b* mutation is in fact a large deletion (Gill et al. 1993) leaving the possibility that more than a single gene affecting chromosome pairing is absent (Roberts et al. 1999).

If the decision whether or not homoeologs may be paired at MI is imposed on chromosomes by the centromere-mediated timing of chromosome association, permanent association of homoeologous arms in an isochromosome composed of homoeologous arms ought to override the *Ph1* regulation and the arms should pair at MI regardless of the status of *Ph1*. However, if the presence or absence of pairing between homoeologous chromosomes at MI is not governed by centromere behavior prior to meiosis, homoeologous arms should not be paired at MI regardless of whether they are attached to each other at the centromere or not. To test this hypothesis, an homoeoisochromosome composed of wheat chromosome arm 2BL and rye chromosome arm 2RL (henceforth referred to as the 2BL.2RL homoeoisochromosome) was constructed and its chiasmate pairing at MI was investigated.

## Materials and methods

All manipulations and observations were performed in *T. aestivum* cv. Pavon 76. Homoeoisochromosome 2BL.2RL was recovered during the reconstruction of complete chromosomes 2B and 2R from centric translocations 2RS.2BL and 2BS.2RL (Brunell et al. 1999). Briefly, the two translocation lines of Pavon were crossed and the F1 plants self-pollinated. The progeny were screened by C-banding (Lukaszewski and Xu 1995) and plants with various misdivision products were recovered, among them 2B monosomics and a 2BL.2RL monohomoeoisomic (Fig. 1). These two stocks were crossed and plants with  $20''+2BL.2RL'$ ,  $20''+2BL.2RL'+iso2BL'$  and  $20''+2B'+2RL.2BL'$  (where ' and '' indicate monosome and disome, respectively) were selected among their progeny. The iso2BL must have been a product of misdivision of the 2B monosome. To remove *Ph1*, plants with  $20''+2BL.2RL'$  were crossed with the Pavon *ph1b*-mono 2B line and the F1 was backcrossed to the latter line. Plants with chromosome constitution  $19''+5Bph1b''+2BL.2RL'$  were selected. Pavon *ph1b*-mono 2B was created by backcrossing of the monosomic 2B line as a female to the Pavon *ph1b* line.

Anthers with a majority of the pollen mother cells (PMCs) at metaphase I from plants with the desired chromosome constitutions were collected and fixed in a 3:1 mixture of absolute alcohol



**Fig. 1** Development of the homoeoisochromosome 2BL.2RL (right) from two centric translocations: 2BS.2RL (left) and 2RS.2BL (middle)



**Fig. 2** Recombination of a wheat chromosome arm 2BL with rye chromosome arm 2RL in the absence of the *Ph1* locus in wheat. From left to right: wheat chromosome 2B, centric translocation 2BS.2RL, recombinant chromosome 2B with a terminal segment of 2RL on 2BL, recombinant chromosome 2BS.2RL with a terminal segment of 2BL on 2RL

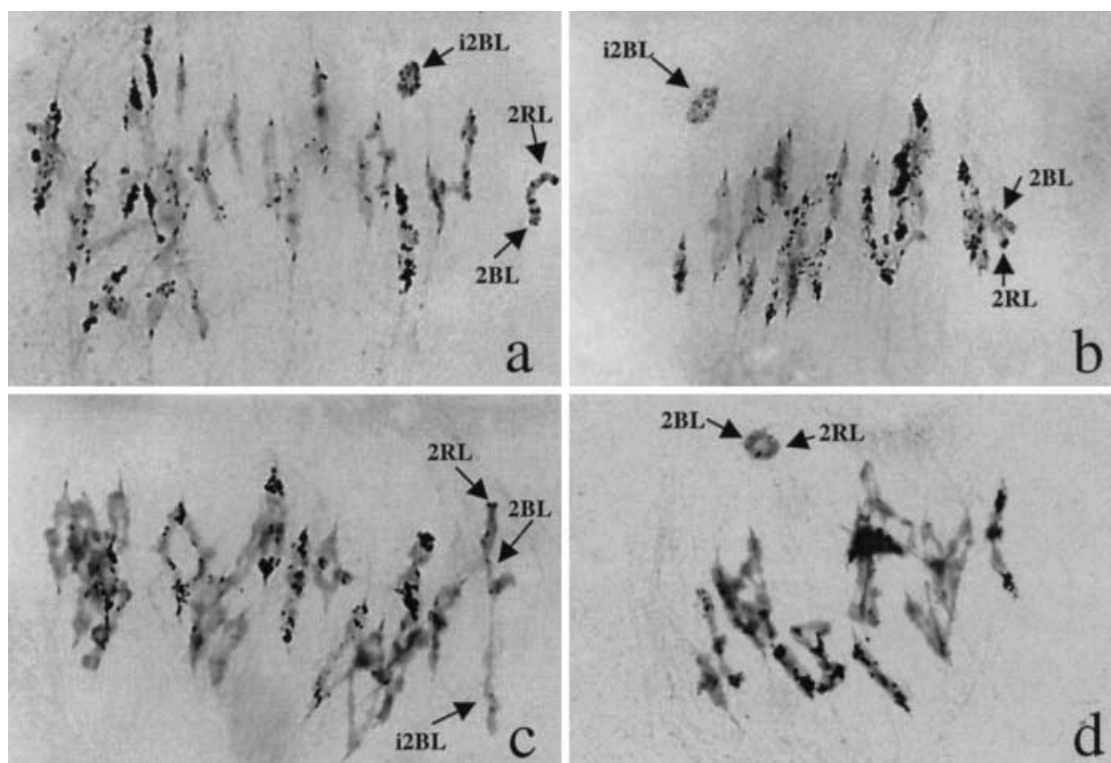
and glacial acetic acid (v/v) and refrigerated in that solution for several weeks. Meiotic C-banding was performed according to Giraldez et al. (1979). All PMCs available for analysis were scored for pairing of the chromosomes of interest.

Recombination of rye chromosome arm 2RL with wheat chromosome arm 2BL was analyzed in a sample of 430 progeny obtained by self-pollination of plants of Pavon 76 with chromosome constitution  $19''+2BS.2RL'+2B'+5B\ ph1b''$ . Recombinant wheat-rye chromosomes were identified by C-banding according to Lukaszewski and Xu (1995).

## Results

The recombination frequency of rye chromosome arm 2RL with wheat chromosome arm 2BL in the *ph1b* background was assessed by C-banding in the F2 progeny of plants with chromosome constitution  $19''+2B'+2BS.2RL'+5B\ ph1b''$ . Among 860 progeny chromosomes observed, 70 (8.1%) were wheat-rye recombined chromosomes (Fig. 2). The observed recombination frequency of 2RL with 2BL was close to that expected on the basis of their MI pairing frequency in the absence of *Ph1* (Naranjo and Fernandez-Rueda 1996). This finding clearly established that the 2BL and 2RL arms crossover in the absence of *Ph1*. On the other hand, no indication of recombination of 2RL with 2BL in the *Ph1* background has ever been obtained. For example, a total of 136 progeny of the 2BS.2RL translocation heterozygotes were screened by C-banding during the development of the translocation line 2BS.2RL in Pavon, involving a total of 11 backcrosses and selection of the 2BS.2RL translocation homozygotes at the conclusion of the backcross process. No recombination of 2RL with any of its wheat homoeologs was observed.

Pairing at MI of the arms of the 2BL.2RL homoeoisochromosome in the *Ph1* background was investigated in a total of 295 PMCs from plants with the following chromo-



**Fig. 3a–d** Behavior of the homoeoisochromosome 2BL.2RL in metaphase I of meiosis. **a, b** Pairing between the arms of isochromosome 2BL (designated *i2BL*) in a *Ph1* plant with  $20''+iso2BL'+2BL.2RL'$ . The homoeologous arms of the 2BL.2RL homoeoisochromosome are not pairing with each other. Compare the appearance of the homoeoisochromosome with

that of iso2BL in which the arms pair with each other. **c** Pairing between the 2BL arm of isochromosome 2BL (designated *i2BL*) and the 2BL arm of the 2BL.2RL homoeoisochromosome in a *Ph1* plant with  $20''+iso2BL'+2BL.2RL'$ . **d** Pairing of homoeologous arms of homoeoisochromosome 2BL.2RL in the *ph1b* background

**Table 1** The numbers of metaphase I pollen mother cells in which the arms of 2BL.2RL or 2BL.2BL homoeoisochromosomes were paired with each other or with another homologous arm, if present, in the *Ph1* and *ph1b* genetic backgrounds

Chromosome constitution <sup>a</sup>	<i>Ph1</i> status	Cells	Pairing within isochromosome 2BL.2BL		Pairing between chromosomes
$20''+2BL.2RL'$	+	179	0	N/A	N/A <sup>b</sup>
$20''+2BL.2RL'+2B'$	+	71	0	N/A	64 (2BL-2BL.2RL) <sup>c</sup>
$20''+2BL.2RL'+iso2BL.2BL'$	+	45	0	28	13 (2BL.2BL-2BL.2RL)
$20''+2BL.2RL'$	-	23	3	N/A	N/A

<sup>a</sup> '' indicates disomes and ' indicates monosomes

<sup>b</sup> N/A (not applicable) indicates that the specific pairing could not have occurred because of chromosome absence

<sup>c</sup> The dash indicates arms of two chromosomes involved in pairing, e.g., 2B-2BL.2RL indicates that the pairing between chromosome 2B and 2BL.2RL isochromosome involved the 2BL arm

some constitutions:  $20''+2BL.2RL'$ ,  $20''+2BL.2RL'+2B'$  and  $20''+2BL.2RL'+iso2BL'$  (Table 1). In none of the cells scored were the homoeologous arms 2BL and 2RL paired with each other. Arm 2RL always remained unpaired (Fig. 3); the 2BL arm of the 2BL.2RL homoeoisochromosome was paired with a high frequency whenever a homologous arm was available. In plants with chromosome constitution  $20''+2B'+2BL.2RL'$ , arm 2BL of the 2RL.2BL homoeoisochromosome was paired with the 2BL arm of chromosome 2B in 90.1% of the cells. In plants with chromosome constitution  $20''+2BL.2RL'+iso2BL'$ , the 2BL arms of the two isochromosomes were paired with each other in

28.9% of MI PMCs analyzed (Fig. 3); in 62% of the cells, two arms of iso2BL were paired and the 2BL arm of the 2BL.2RL homoeoisochromosome was not paired (Table 1, Fig. 3).

The *ph1b* lines of Pavon have poor vigor and tend to be sterile. The MI pairing of the arms of the 2BL.2RL homoeoisochromosome could be assessed in only 23 PMCs. In three of these cells, the arms of the 2BL.2RL homoeoisochromosome were paired with each other (Fig. 1), suggesting an MI pairing frequency of about 13%.

## Discussion

The homoeologous arms 2BL and 2RL of normal chromosomes 2B and 2R do not pair at MI in the presence of the *Ph1* locus but they frequently do so in its absence (Naranjo and Fernandez-Rueda 1996). Attachment of the 2RL arm to a wheat chromosome arm, such as 2BS, at the centromere had no negative impact on the ability of 2RL to crossover with the homoeologous 2BL arm in the absence of *Ph1*. In the 2RL.2BL homoeoiso-chromosome, no MI pairing between 2RL and 2BL was observed among 295 cells scored in the *Ph1* background. In the *ph1b* background, only 23 cells could be scored but they provided clear evidence that MI pairing between 2RL and 2BL was taking place. Therefore, permanent association of the homoeologous arms at the centromere in the homoeoiso-chromosome did not alter their MI pairing response to the activity of the *Ph1* locus; whether they were independent or permanently associated, they were not paired at MI in the *Ph1* background. In the absence of the *Ph1* locus, the two arms paired at MI with comparable frequencies when they were permanently associated in the homoeoiso-chromosome or present in separate chromosomes. These observations are inconsistent with the assumption that modification of centromere behavior by *Ph1* prior to meiosis is sufficient to prevent crossing over and MI pairing between homoeologous chromosomes in wheat.

Holm (1986) observed multivalent associations during early phases of synapsis in *Ph1* hexaploid wheat and assumed that they involved homoeologous chromosomes. He therefore argued that if homoeologs associate into multivalents in early meiosis, the decision on the absence of pairing between homoeologs at MI could not possibly be made before meiosis. It needs to be pointed out, however, that it has never been determined which chromosomes engaged in multivalent synaptic associations in *Ph1* wheat. They may or may not be homoeologous. Extensive synapsis was observed in *Ph1* interspecific hybrids of wheat and in wheat haploids although little pairing occurred at MI (Gillies 1987; Wang 1988). Since synapsis in these plants did not reflect the great differences in pairing between homoeologous chromosomes at MI in the *Ph1* and *ph1* backgrounds, Gillies (1987) and Holm and Wang (1988), like Holm (1986) previously, argued against the predetermination of synapsis and MI pairing by positioning of chromosomes prior to meiosis. A large body of evidence exists showing that nonhomologous synapsis may occur in the absence of homologs. Therefore, arguments against the predetermination of MI pairing by premeiotic chromosome positioning based on synaptic configurations should be treated with caution. The need for caution is exemplified by considerable differences in the fraction of the wheat chromosome complement synapsed in *Ph1* haploids derived from different *T. aestivum* cultivars with similar low levels of homoeolog pairing at MI (Wang 1988).

Nevertheless, the contentions of Holm (1986), Gillies (1987) and Holm and Wang (1988) were upheld by studies utilizing another approach: recombination of chromo-

somes composed of homologous and homoeologous segments in *Ph1 T. aestivum*. In the presence of *Ph1*, recombination was consistently absent from homoeologous segments but normal levels of recombination took place in juxtaposed homologous segments. This pattern was observed irrespective of the length of the homoeologous segment, its location on the centromere-telomere axis and homology of both the telomeric and centromeric regions (Dubcovsky et al. 1995; Luo et al. 1996). In another experiment, MI pairing frequencies were assessed for a large number of 4B/4D recombinant chromosomes, each having a terminal 4D segment of variable length and a 4B proximal segment with 4B centromere, with chromosome 4D in *Ph1 T. turgidum* (Dvorak et al. 1995). The MI pairing frequencies ranged from 26% PMCs to 98% PMCs, reflecting the lengths of the 4D homologous segments. Since the centromeric region was invariably homoeologous, none of the 4B/4D chromosomes should have paired at MI with 4D if MI pairing between homoeologs is predetermined by centromere-mediated premeiotic alignment. Likewise, wheat midget chromosomes were paired at MI with the wheat chromosome from which they were derived in spite of having homoeologous centromeres or centromeres from unrelated chromosomes (Lukaszewski 1997). The results of these experiments, like those reported in this paper, are inconsistent with the idea that MI pairing between homoeologous chromosomes is prevented by the *Ph1* locus via centromere-mediated alignment of chromosomes prior to meiosis. They are consistent with the idea that *Ph1* determines the chromosome pairing pattern at MI by scrutinizing homology across the entire chromosome in one or more stages prior to MI (Luo et al. 1996).

In trying to reconcile observations on the behavior of premeiotic chromosomes and what is seen at MI in the *Ph1* and *ph1* states, it should be noted that no evidence has so far been obtained for causality between premeiotic chromosome behavior and MI chromosome pairing in wheat; hypotheses postulating premeiotic predetermination of MI homoeologous pairing have relied on various correlated observations. However, it cannot be excluded that altered premeiotic chromosome behavior observed in the *Ph1* genetic background compared with the *ph1* genetic background is not pleiotropy of the *Ph1* activity. In the genetic background of hexaploid wheat, strong dominant genes, exemplified by *Ph1*, tend to be regulatory. If *Ph1* is a regulatory rather than a structural locus, correlated effects that are not causally related are likely and could account for the conflicting inferences on the nature of the action of *Ph1*.

## References

- Aragón-Alcaide L, Reader S, Beven A, Shaw P, Miller T, Moore G (1997a) Association of homologous chromosomes during floral development. *Curr Biol* 7:905–908  
 Aragón-Alcaide L, Reader S, Miller T, Moore G (1997b) Centromeric behaviour in wheat with high and low homoeologous chromosomal pairing. *Chromosoma* 106:327–333

- Avivi L, Feldman M (1973) Mechanism of non-random chromosome placement in common wheat. In: Sears ER, Sears LMS (eds) Proc 4th Int Wheat Genet Symp, Columbia, Missouri Agric. Agric Exp Stat, College of Univ Missouri, pp 627–633
- Brunell MS, Lukaszewski AJ, Whitkus R (1999) Development of arm specific RAPD markers for rye chromosome in wheat. *Crop Sci* (in press)
- Dover GA, Riley R (1973) The effect of spindle inhibitors applied before meiosis on meiotic chromosome pairing. *J Cell Sci* 12:143–161
- Dover G, Riley R (1977) Inferences from genetical evidence on the course of meiotic chromosome pairing in plants. *Philos Trans R Soc Lond B Biol Sci* 277:313–326
- Driscoll CJ, Darvey NL, Barber HN (1967) Effect of colchicine on meiosis of hexaploid wheat. *Nature* 216:687–688
- Dubcovsky J, Luo M-C, Dvorak J (1995) Differentiation between homoeologous chromosomes 1A of wheat and 1A<sup>m</sup> of *Triticum monococcum* and its recognition by the wheat *Ph1* locus. *Proc Natl Acad Sci U S A* 92:6645–6649
- Dvorak J, Dubcovsky J, Luo M-C, Devos KM, Gale MD (1995) Differentiation between wheat chromosomes 4B and 4D. *Genome* 38:1139–1147
- Feldman M (1968) Regulation of somatic association and meiotic pairing in common wheat. In: Finley KW, Shepherd KW (eds) Proc 3rd Int Wheat Genet Symp, Canberra, Australia. Austral Acad, Canberra, pp 169–178
- Feldman M (1993) Cytogenetic activity and mode of action of the pairing homoeologous (*Ph1*) gene of wheat. *Crop Sci* 33: 894–897
- Feldman M, Mello-Sampayo T, Sears ER (1966) Somatic association in *Triticum aestivum*. *Proc Natl Acad Sci USA* 56:1447–1453
- Gill KS, Gill BS, Endo TR, Mukai Y (1993) Fine physical mapping of *Ph1*, a chromosome pairing regulator gene in polyploid wheat. *Genetics* 134:1231–1236
- Gillies CB (1987) The effect of *Ph* gene alleles on synaptonemal complex formation in *Triticum aestivum* × *T. kotschyi* hybrids. *Theor Appl Genet* 74:430–438
- Giraldez R, Cermeno MC, Orellana J (1979) Comparison of C-banding pattern in the chromosomes of inbred lines and open pollinated varieties of rye. *Z Pflanzenzucht* 83:40–48
- Holm PB (1986) Chromosome pairing and chiasma formation in allohexaploid wheat, *Triticum aestivum* analyzed by spreading of meiotic nuclei. *Carlsberg Res Commun* 51:239–294
- Holm PB, Wang X (1988) The effect of chromosome 5B on synapsis and chiasma formation in wheat, *Triticum aestivum* cv. Chinese Spring. *Carlsberg Res Commun* 53:191–208
- Lukaszewski AJ (1997) Construction of mid-gene chromosomes in wheat. *Genome* 40:566–569
- Lukaszewski AJ, Xu X (1995) Screening large populations of wheat hybrids by C-banding. *Cereal Res Commun* 23:9–13
- Luo M-C, Dubcovsky J, Dvorak J (1996) Recognition of homoeology by the wheat *Ph1* locus. *Genetics* 143:1195–1203
- McGuire PE, Dvorak J (1982) Genetic regulation of heterogenetic chromosome pairing in polyploid species of the genus *Triticum* sensu lato. *Can J Genet Cytol* 24:57–82
- Moore G (1998) To pair or not to pair: chromosome pairing and evolution. *Curr Opin Plant Biol* 1:116–122
- Naranjo T, Fernandez-Rueda P (1996) Pairing and recombination between individual chromosomes of wheat and rye in hybrids carrying the *ph1b* mutation. *Theor Appl Genet* 93:242–248
- Okamoto M (1957) Asynaptic effect of chromosome V. *Wheat Inf Serv* 5:6
- Riley R (1960) The diploidization of polyploid wheat. *Heredity* 15:407–429
- Riley R, Chapman V (1958) Genetic control of the cytologically diploid behaviour of hexaploid wheat. *Nature* 182:713–715
- Roberts MA, Reader SM, Dalgliesh C, Miller TE, Foote TN, Fish LJ, Snape JW, Moore G (1999) Induction and characterization of *Ph1* wheat mutants. *Genetics* 153:1909–1918
- Sears ER (1977) Induced mutant with homoeologous pairing in common wheat. *Can J Genet Cytol* 19:585–593
- Sears ER, Okamoto M (1959) Intergenomic chromosome relationships in hexaploid wheat. In: Proc X Int Congress Genet. Univ Toronto Press, Toronto, Canada pp 258–259
- Wang X (1988) Chromosome pairing analysis in haploid wheat by spreading of meiotic nuclei. *Carlsberg Res Commun* 53:135–166