Original Article

Cytogenet Genome Res 119:263–267 (2007)
DOI: 10.1159/000112072

Cytogenetic and Genome Research

Condensation of rye chromatin in somatic interphase nuclei of *Ph1* and *ph1b* wheat

D. Kopecky[a] D.C. Allen[b] M. Duchoslav[c] J. Dolezel[a] A.J. Lukaszewski[b]

[a]Institute of Experimental Botany, Olomouc (Czech Republic)
[b]Department of Botany and Plant Sciences, University of California, Riverside, CA (USA)
[c]Department of Botany, Faculty of Sciences, Palacky University, Olomouc (Czech Republic)

Accepted in revised form for publication by B. Friebe, 17 August 2007.

Abstract. The *Ph1* locus in hexaploid wheat (*Triticum aestivum* L.) enforces diploid-like behavior in the first metaphase of meiosis. To test the hypothesis that this chromosome pairing control is exercised by affecting the degree of chromatin condensation, the dispersion of rye chromatin in interphase nuclei in somatic tissues of wheat-rye chromosome translocations 1RS.1BL, 2RS.2BL, 2BS.2RL, 3RS.3DL and 5RS.5BL was compared in *Ph1* and *ph1b* isogenic backgrounds. No significant differences in rye chromatin condensation that could be attributed to the *Ph1* locus were detected. Regardless of the *Ph1* status, each rye chromosome arm tested conformed to the general Rabl's orientation and occupied portions of the nuclei proportional to their length. Earlier observations that indicated the involvement of *Ph1* locus in rye chromatin condensation in wheat could have been due either to specific loci on the studied 5RL rye arm that control the chromosome condensation process or to damage to the genetic system controlling chromatin condensation in the existing *ph1b* stocks of wheat. That damage might have been caused by homoeologous recombination and uneven disjunction of chromosomes from multivalents.

Efficient production of balanced gametes depends on regular bivalent formation in the first metaphase (MI) of meiosis. Any deviation from bivalent pairing introduces some degree of instability, hence lowers the efficiency of the entire process. While bivalent formation is natural in a normal diploid organism, polyploidy may lead to the formation of multivalents and hence, unequal disjunction in the first anaphase (AI). Perhaps for this reason, many polyploid species evolved genetic systems that enforce bivalent pairing in MI (for review, see Jenczewski and Alix, 2004). The most studied of these is the *Ph (pairing homoeologous)* system of polyploid wheat. It consists of at least two loci, *Ph1* and *Ph2*, located on chromosomes 5BL and 3DS, respectively (Riley and Chapman, 1958; Sears and Okamoto, 1958; Sears, 1977).

Request reprints from Adam J. Lukaszewski
Department of Botany and Plant Sciences, University of California
Riverside, CA 92521 (USA)
telephone: +1 951 827 3946; fax: +1 951 827 4437
e-mail: adam.lukaszewski@ucr.edu

Wheat (*Triticum aestivum* L., 2n = 6x = 42) is an allopolyploid with the nuclear genome composed of three related genomes A, B and D derived from its three diploid ancestors. A chromosome in each genome has its genetic equivalent in the two other genomes to the extent that most nulli-tetrasomic combinations, where a pair of chromosomes from one genome is replaced by an additional pair of chromosomes from one of the other two genomes, are fully functional and fertile (Sears, 1966). These genetically related chromosomes from different genomes are called homoeologues. With the *Ph* system disabled, homoeologues in wheat are capable of frequent meiotic pairing. Of the two loci, *Ph1* has a far stronger effect (Sears, 1984). In its absence, but in the presence of *Ph2*, homoeologous recombination takes place, not only among the three genomes of wheat but also among any of those and other chromosomes introduced into wheat from related species. This feature facilitates chromosome engineering and introgressing alien chromatin into wheat (Sears, 1981).

Even though the effects of the *Ph1* locus have been known for 50 years now (Riley and Chapman, 1958; Sears

1424–8581/07/1194–0263$23.50/0

and Okamoto, 1958), its mode of action still remains unknown. The proposed hypotheses have ranged from *Ph1* controlling spatial disposition of chromosomes in all tissues of a plant (Feldman and Avivi, 1988) via the control of centromeres (Martínez-Perez et al., 2001, 2003) to the control of stringency of crossing over (Dubcovsky et al., 1995). Recently it has been postulated that the *Ph1* locus controls chromosome pairing by affecting the level of chromosome condensation (Mikhailova et al., 1998; Maestra et al., 2002). In its absence, alien chromatin appeared more diffuse in somatic interphase nuclei and in early meiosis, and so, presumably, it was more open to surveys by molecular machinery attempting to identify and juxtapose DNA segments amenable to crossing over. This effect was clearly visible both in the meiocytes and in somatic tissues tested. Because condensation differences are postulated as a mechanism in the recognition of homology (Prieto et al., 2004) and invoked in the putative identification of the DNA sequence of the *Ph1* locus (Griffiths et al., 2006) we have initiated a survey of a collection of alien introgressions into some crop species to test if such easily detectable differences in the levels of the interphase chromatin condensation could indeed be responsible for large differences in the ability of homoeologous chromosomes to pair, including large differences in pairing affinity of the same chromosomes in different genetic backgrounds. In the first step, observations of Mikhailova et al. (1998) and Maestra et al. (2002) were verified using several wheat-rye centric translocations in the *Ph1*+ and *Ph1*– backgrounds in wheat. Here we report that no measurable differences in chromatin condensation attributable to the status of the *Ph1* locus could be detected.

Materials and methods

The plant material consisted of lines of hexaploid spring wheat (*T. aestivum* L.) cv. 'Pavon 76', homozygous for centric wheat-rye translocations 1RS.1BL, 2RS.2BL, 2BS.2RL, 3RS.3DL and 5RS.5BL. These translocations were either produced in 'Pavon 76' or were transferred to it by at least seven backcrosses. In the course of engineering wheat-rye centric translocations (Lukaszewski, 2000; Lukaszewski et al., 2004) or in anticipation of such attempts, these translocations were combined with the *ph1b* mutation line of 'Pavon 76'. This line was produced by seven backcrosses of the original *ph1b* mutation from cv. 'Chinese Spring' (Sears, 1977) to 'Pavon 76', followed by selfing and selection of homozygotes. The exception to this protocol was the 3RS.3DL *ph1b* line for which a double homozygote was not available and was selected specifically for this study. One double homozygote was identified among the progeny of a plant 19' + 3RS.3DL + 3D + 5B*ph1b*', where ' denotes disomy/homozygosity.

Seeds of the appropriate stocks were germinated on wet filter paper in Petri dishes, root tips were collected to ice water for 26–30 h and fixed in a mixture of absolute alcohol:glacial acetic acid (3:1) at 37°C for seven days and then stored at –18°C. Cytological preparations and in situ hybridization with labeled DNA were made according to Massoudi-Nejad et al. (2002). In all experiments, genomic in situ hybridization (GISH) was done with a probe prepared from total genomic DNA of rye. The probe was labeled with digoxigenin by DIG-nick translation and detected with anti-DIG fluorescein (FITC) using standard kits from Roche Applied Science (USA) following the manufacturer's instructions. The hybridization mix contained unlabeled genomic DNA of wheat sheared to ca. 200–500 bp fragments at 1:150 ratio (probe: blocking DNA). Following hybridization, preparations were counterstained with 1.5% propidium iodide (PI) in VectaShield antifade (Bio-Rad, UK), mounted and observed under a microscope.

To minimize experimental error, all observations were made on slides each with two preparations of the same translocation on it: one in the *Ph1* and one in *ph1b* background. Squashes were made side by side using 18 × 18 mm cover slips; GISH was performed on both preparations with the same hybridization mixture under single 22 × 40 mm cover slips.

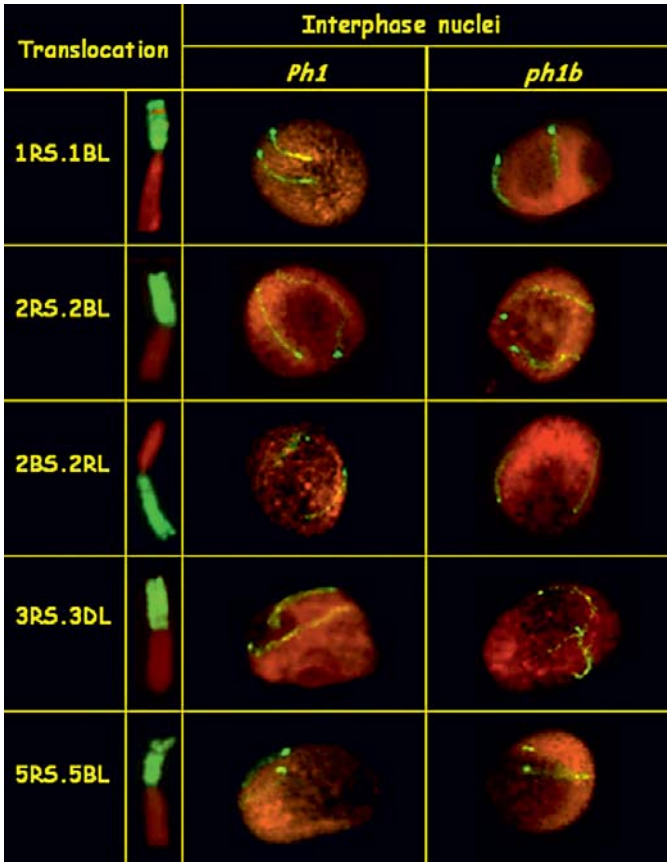
Samples were collected and measurements were done in two laboratories in Riverside (CA, USA) and Olomouc (Czech Republic). For the measurements of chromatin dispersion, images of individual nuclei or groups of nuclei were captured using Meridian InSight Point Confocal attachment mounted on a Zeiss Axioscope 20 microscope (Riverside) or by SensiCam B/W CCD camera attached to Olympus AX70 microscope (Olomouc). The resulting images were analyzed for the total area of each nucleus, as visualized by the red color of PI, and the area of green fluorescence (FITC), occupied by rye chromatin within each nucleus. The percentage of the total nucleus area occupied by rye chromatin was taken as a measure of its dispersion. Samples of nuclei analyzed per plant ranged from 25 to 52. The proportions thus obtained in individual lines were compared for each translocation separately in the *Ph1* and *ph1b* backgrounds using Nested ANOVA with plants nested within *Ph1*/*ph1b* (NCSS 2001 software, www.ncss.com).

Results and discussion

Visual screening of all lines failed to detect any obvious differences either in the arrangement or dispersion of rye chromatin in wheat interphase nuclei, between different translocations and between the *Ph1* and *ph1b* backgrounds (Fig. 1). In all cases, labeled rye chromosome arms conformed to the general Rabl's orientation and tended to run parallel across the nucleus. Some deviations from this predominant arrangement were evident but they were relatively infrequent and no translocation-specific or *Ph1*-specific pattern was obvious. The same general pattern of chromosome arrangement and chromatin dispersion was observed in the nuclei of somatic tissues of anthers in the *Ph1* and *ph1b* lines of the 1RS.1BL and 2BS.2RL translocations analyzed for meiotic pairing (data not shown). The conclusion from these cursory observations was that there was no gross difference in the arrangement or the state of dispersion among different translocations analyzed, nor between the *Ph1* and *ph1b* backgrounds, in somatic tissues of root tips or anthers. This is in striking contrast to the observations of Mikhailova et al. (1998) where the disposition of a rye chromosome arm in the *ph1b* background appeared haphazard and disorganized, in all tissues analyzed.

Despite some variation in signal intensities between different runs of GISH, the measurements of the dispersion of rye chromatin in wheat nuclei produced fairly uniform results (Table 1, Fig. 2). The average proportion of the nuclear area occupied by rye chromatin for individual translocations ranged from ca. 4% to almost 8.5% of the total area of a nucleus. Given that each nucleus contained two labeled chromosome arms among the total of 42 chromosomes present, and that the domains occupied by the labeled rye arms overlapped with the counterstained wheat arms, the area proportions were as expected, considering size differ-

1



2

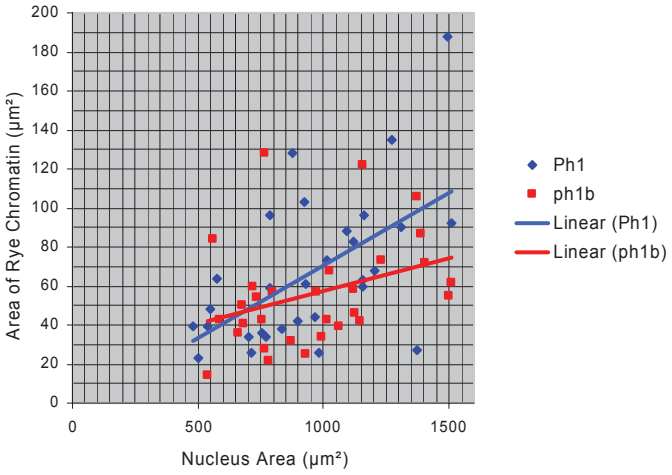


Fig. 1. Centric wheat-rye translocation in mitotic metaphase and in interphase nuclei in *Ph1* and *ph1b* wheat as visualized by in situ hybridization with total rye genomic DNA labeled with FITC and counterstained with propidium iodide.

Fig. 2. The relationship between the total nucleus area and the area occupied by rye chromatin in the *Ph1* and *ph1b* lines of the 'Pavon 76' 2BS.2RL translocation line.

Table 1. Proportion of the total nucleus area occupied by rye chromatin in several *Ph1* and *ph1b* wheat-rye translocation lines of cv. 'Pavon 76' (mean ± standard error)

Chromosome translocation	Number of nuclei evaluated		Proportion of rye chromatin in total nucleus area, %		Probability (ANOVA)
	Ph1	*ph1b*	*Ph1*	*ph1b*	
1RS.1BL	51	50	5.04 ± 0.21	5.41 ± 0.22	0.222
2RS.2BL	177	176	6.27 ± 0.14	6.19 ± 0.14	0.860
2BS.2RL	82	82	7.07 ± 0.28	6.93 ± 0.30	0.902
3RS.3BL	25	25	7.83 ± 0.43	8.44 ± 0.50	0.361
5RS.5BL	150	150	4.06 ± 0.10	5.07 ± 0.14	0.001
Total	485	483	6.05 ± 0.18	6.41 ± 0.18	0.292

ences among rye chromosomes. In the rye genome, chromosome 2R is the longest and has the highest DNA content (Lukaszewski et al., 1982); it has an arm ratio of about 1.2. The shortest arm in the rye genome is 5RS; in this study, it occupied the lowest proportion of the nuclear area. The nuclei sampled varied in size, either due to differences in the mitotic cycle or degree of squashing, and the proportions of the area occupied by rye chromatin changed with changes in the total nucleus area (Fig. 2).

With one exception, no significant differences were found in the proportions of nuclei occupied by rye chromatin in any of the pairwise comparisons of the *Ph1* and *ph1b* for individual translocations (Table 1, Fig. 1). The exception was the 5RS translocation where in the *ph1b* line rye chromatin occupied significantly larger proportions of the nuclei. This is not surprising, as the 5RS.5BL translocations in the *Ph1* and *ph1b* backgrounds are not identical. Given that *Ph1* is located on 5BL and tightly linked to the centromere (Sears, 1984), the two lines had to be produced separately: transfer of 5RS.5BL from the *Ph1* to the *ph1b* background or vice versa would have required screening large samples to identify rare recombinants between the centromere and the *Ph1* locus. The translocation breakpoint in the 5RS.5BL *Ph1* line is in the centromere; in the *ph1b* background it is on the

long arm, 10–12% of the relative arm's length away from the centromere. Consequently, the rye segment in the latter is longer and so rye chromatin occupies a larger proportion of the nuclei.

In the final test, all *Ph1* lines were compared to all *ph1b* lines and no statistically significant difference was observed. The only conclusion that can be drawn from these results is that among the lines analyzed in this study, the *Ph1* locus did not affect rye chromatin condensation in somatic tissues of wheat. This is in clear contrast to the observations of Mikhailova et al. (1998) where the differences in the appearance of the 5RL rye arm between *Ph1* and *ph1b* lines were so obvious, both in the somatic tissue and in meiocytes, that no attempts to quantify the effect were made. Based on those differences and using the same stocks, Maestra et al. (2002) felt justified to state that 'Our results…are strongly in favor of the putative effect of the *Ph1* locus on chromatin condensation and organization'. This putative effect of *Ph1* on chromatin condensation was invoked by Griffiths et al. (2006) in an attempt to identify the coding DNA sequence of *Ph1* in the sequenced segment of chromosome 5B known to contain the locus.

The difference between the studies of Mikhailova et al. (1998) and Maestra et al. (2002) on the one hand and this study on the other may lay in the nature of the stocks used. The 5RL ditelosomic in the *ph1b* background that was used by Mikhailova et al. (1998) and Maestra et al. (2002) was not analyzed in this study. Assuming that the difference was not due the environment in which the respective studies were conducted, two explanations seem plausible: either the 5RL arm of rye affects chromatin condensation in wheat in some interaction with the *Ph1* locus, or the *ph1b* lines used in the two experiments were different.

It is known that rye chromosome 5R interferes with the diploidizing system in wheat and that this effect appears to be dosage-dependent (Riley et al., 1973; Lelley, 1976). Perhaps the effect observed by Mikhailova et al. (1998) and Maestra et al. (2002) in the disomic 5RL addition to the *ph1b* line of wheat was not a consequence of the absence of the *Ph1* locus but rather a full expression of the loci on 5RL, uninterrupted by *Ph1*. No stocks suitable for a critical comparison are currently available. It must be noted, however, that Riley et al. (1973) associated *Ph1* suppression by 5R with the short and not the long arm which contradicts numerous observations of the last author (Lukaszewski, unpublished data).

It has been recently illustrated (Sanchez-Moran et al., 2001) that the *ph1b* lines of wheat suffer major genome rearrangements. Initially, this must be a consequence of homoeologous pairing that takes places in the absence of *Ph1*. This is, after all, why *Ph1* is considered so critical for the stability and fertility of wheat. As the genome damage due to homoeologous exchanges (and resulting unequal disjunction of chromosomes in AI) accumulate other effects of other chromosomes and chromosome regions may also start playing a role in the chromosome behavior and further contribute to the general genome instability. Several wheat chromosomes are known to affect meiotic behavior, such as the asynaptic effect of nullisomy 3B (Sears, 1954). Because homoeologous recombination events are unpredictable, it is likely that the *ph1b* lines currently available in many laboratories around the world, over 30 years after the first isolation of the *ph1b* mutant (Sears, 1977), differ substantially in their chromosome constitution and hence their genetics. While they are suitable for the induction of homoeologous pairing in chromosome engineering efforts, they may be quite unsuitable for any studies of the mode of action of the *Ph1* locus itself. At best, they represent the combined effects of the absence of *Ph1* and all consequences of its absence that have accumulated since the line was created. In this sense, many effects now attributed to the *Ph1* locus may in fact be due to the presence of various genome rearrangements that have accumulated since the *Ph1* locus had been removed.

No reports have been published that would suggest or imply that efforts have been undertaken to clean up the existing *ph1b* lines or to maintain them with the compensating presence of *Ph1* so that no further damage to the genome occurs. The *ph1b* lines used in this study were newly developed by repeated backcrosses to euploid cv. 'Pavon 76'. This approach guaranteed that in every backcross generation a functional dominant *Ph1* allele was present thus preventing homoeologous recombination. At the same time, the seven backcrosses used to develop the *ph1b* line of cv. 'Pavon 76' plus a cross and a backcross to each of the translocations lines must have removed most, if not all, chromosome aberrations that might have been present in the starting Chinese Spring *ph1b* line obtained from Dr. E. R. Sears. None of the lines of the present study had more than two generations in the *ph1b* status making chromosome rearrangements less likely than in a line maintained by selfing for 30 years.

This study does not shed any new light on the mode of action of the *Ph1* locus in wheat. However, it provides evidence that the locus does not affect plant-wide chromatin condensation to any appreciable effect. Disorganized early meiosis in the 5RL *ph1b* line of Mikhailova et al. (1998) and Maestra et al. (2002) was likely a consequence of altered chromatin organization in the entire plant including the germ line, and not the other way around. Meiotic behavior was not the object of this study; however, MI pairing of the two translocations studied in detail, 1RS.1BL and 2BS.2RL, was perfectly normal for rye introgressions in wheat and resulted in wheat-rye recombined chromosomes among progeny (Lukaszewski, 2000; Lukaszewski et al., 2004).

In maize, chromosomes undergo a structural conformation change as they enter meiosis (Dawe et al., 1994) and this change may be related to the homology search. A similar change is postulated to also take place in wheat (Mikhailova et al., 1998). Perhaps this stage may be affected by the *Ph1* locus. However, as this stage is short and transient there appears no reliable method of comparing the *Ph1*+ and *Ph1*– lines in sufficiently large and homogenous samples to determine if *Ph1* affects this stage. Still, given all accumulated evidence, primarily from the observations of the synaptonemal complex formation (Holm, 1988) and crossing over in introgressed alien segments (Dubcovsky et al., 1995; Luo et al., 1996), the argument seems difficult to dismiss that the *Ph1* locus in wheat affects the stringency of recombination.

References

Dawe RK, Sedat JW, Agard DA, Cande WZ: Meiotic chromosome pairing in maize is associated with a novel chromatin organization. Cell 76: 901–912 (1994).

Dubcovsky J, Luo MC, Dvorak J: Differentiation between homoeologous chromosomes 1A of wheat and $1A^m$ of *Triticum monococcum* and its recognition by the *Ph1* locus. Proc Natl Acad Sci USA 92:6645–6649 (1995).

Feldman M, Avivi L: Genetic control of bivalent pairing in common wheat: the mode of Ph1 action, in Brandham PE (ed): Kew Chromosome Conference III, pp 269–279 (HMSO, London 1988).

Griffiths S, Sharp R, Foote TN, Bertin I, Wanous M, et al: Molecular characterization of *Ph1* as a major chromosome pairing locus in polyploid wheat. Nature 439:749–752 (2006).

Holm PB: Chromosome pairing and synaptonemal complex formation in hexaploid wheat, nullisomic for chromosome 5B. Carlsberg Res Comm 53:91–110 (1988).

Jenczewski E, Alix K: From diploids to alloploids: the emergence of efficient pairing control genes in plants. Critical Rev Plant Sci 23:21–45 (2004).

Lelley T: Induction of homoeologous pairing in wheat by genes of rye suppressing chromosome 5B effect. Can J Genet Cytol 18:485–489 (1976).

Lukaszewski AJ: Manipulation of the 1RS.1BL translocation in wheat by induced homoeologous recombination. Crop Sci 40:216–225 (2000).

Lukaszewski AJ, Wozna J, Apolinarska B, Naganowska B: The DNA content of the individual chromosomes of rye. Theor Appl Genet 62:145–148 (1982).

Lukaszewski AJ, Rybka K, Korzun V, Malyshev SV, Lapinski B, Whitkus R: Genetic and physical mapping of homoeologous recombination points involving wheat chromosome 2B and rye chromosome 2R. Genome 47:36–45 (2004).

Luo MC, Dubcovsky J, Dvorak J: Recognition of homology by the wheat *Ph1* locus. Genetics 144: 1195–1203 (1996).

Maestra B, de Jong JH, Shepherd K, Naranjo T: Chromosome arrangement and behavior of two homologous telosomes at the onset of meiosis in disomic wheat-5RL addition lines with and without the *Ph1* locus. Chromosome Res 10: 655–667 (2002).

Martínez-Pérez E, Shaw P, Moore G: The *Ph1* locus is needed to ensure specific somatic and meiotic centromere association. Nature 411:204–207 (2001).

Martínez-Pérez E, Shaw P, Aragón-Alcaide L, Moore G: Chromosomes form into seven groups in hexaploid and tetraploid wheat as a prelude of meiosis. Plant J 36:21–29 (2003).

Massoudi-Nejad A, Nasuda S, McIntosh RA, Endo TR: Transfer of rye chromosome segments to wheat by gametocidal system. Chromosome Res 10:349–357 (2002).

Mikhailova EI, Naranjo T, Shepherd K, Wennekes-van Eden J, Heytig C, de Jong JH: The effect of the wheat *Ph1* locus on chromatin organization and meiotic chromosome pairing analyzed by genome painting. Chromosoma 107:339–350 (1998).

Prieto P, Shaw P, Moore G: Homologous recognition during meiosis is associated with a change in chromatin conformation. Nat Cell Biol 6: 906–908 (2004).

Riley R, Chapman V: Genetic control of the cytologically diploid behaviour of hexaploid wheat. Nature 182:713–715 (1958).

Riley R, Chapman V, Miller TE: The determination of meiotic chromosome pairing, in Sears ER, Sears LMS (eds): Proc 4^{th} Int Wheat Genet Symp, pp 731–738 (Agric Exp Station, University of Missouri, Columbia, 1973).

Sanchez-Moran E, Benavente E, Orellana J: Analysis of karyotypic stability of homoeologous-pairing (*ph*) mutants in alloploid wheats. Chromosoma 110:371–377 (2001).

Sears ER: The aneuploids of common wheat. Res Bull Univ Mo 572:1–59 (1954).

Sears ER: Nulli-tetrasomic combinations in hexaploid wheat, in Riley R, Lewis KR (eds): Chromosome Manipulation in Plant Genetics, pp 29–45 (Oliver and Boyd, Edinburgh 1966).

Sears ER: An induced mutant with homoeologous pairing in wheat. Can J Genet Cytol 19:585–593 (1977).

Sears ER: Transfer of alien genetic material to wheat, in Evans LT, Peacock WJ (eds): Wheat Science – Today and Tomorrow, pp 75–89 (Cambridge University Press, New York 1981).

Sears ER: Mutations in wheat that raise the level of meiotic chromosome pairing, in Gustafson JP (ed): Gene Manipulation in Plant Improvement, pp 295–300 (Plenum Press, New York 1984).

Sears ER, Okamoto M: Intergenomic relationships in hexaploid wheat. Proc Xth Inter Congress Genet 2:258–259 (1958).