Comparison of homoeologous chromosome pairing between hybrids of wheat genotypes Chinese Spring *ph1b* and Kaixian-luohanmai with rye

Ming Hao, Jiangtao Luo, Min Yang, Lianquan Zhang, Zehong Yan, Zhongwei Yuan, Youliang Zheng, Huaigang Zhang, and Dengcai Liu

Abstract: The *ph*-like genes in the Chinese common wheat landrace Kaixian-luohanmai (KL) induce homoeologous pairing in hybrids with alien species. In the present study, meiotic phenotypic differences on homoeologous chromosome pairing at metaphase I between hybrids of wheat genotypes Chinese Spring *ph1b* (CS*ph1b*) and KL with rye were studied by genomic in situ hybridization (GISH). The frequency of wheat–wheat associations was higher in CS*ph1b* × rye than in KL × rye. However, frequencies of wheat–rye and rye–rye associations were higher in KL × rye than in CS*ph1b* × rye. These differences may be the result of different mechanisms of control between the *ph*-like gene(s) controlling homoeologous chromosome pairing in KL and CS*ph1b*. Wheat–wheat associations were much more frequent than wheat–rye pairing in both hybriods. This may be caused by lower overall affinity, or homoeology, between wheat and rye chromosomes than between wheat chromosomes.

Key words: *Ph* gene, homoeologous metaphase I pairing, GISH.

Résumé : Les gènes de type *ph* chez la variété de pays chinoise Kaixian-luohanmai (KL) du blé induisent un appariement des homéologues chez des hybrides interspécifiques. Dans ce travail, au moyen d'hybridations génomiques in situ (GISH), les auteurs ont étudié les différences observées quant à l'appariement des homéologues au cours de la métaphase I chez des hybrides entre les blés Chinese Spring *ph1b* (CS*ph1b*) ou KL et le seigle. La fréquence des associations blé–blé était plus élevée chez les hybrides CS*ph1b* × seigle que chez les hybrides KL × seigle. Cependant, les d'associations blé–seigle et seigle–seigle étaient plus fréquentes chez les hybrides KL × seigle que CS*ph1b* × seigle. Ces différences découlent possiblement de différences quant aux mécanismes de contrôle entre les gènes de type *ph* qui régulent l'appariement des homéologues chez KL et le gène *ph1b*. Les associations blé–blé étaient beaucoup plus fréquentes que les appariements blé–seigle chez les deux hybrides. Ceci pourrait provenir d'une plus faible affinité globale, ou homéologie, entre les chromosomes du blé et du seigle qu'entre les chromosomes du blé.

Mots-clés : gène *Ph*, appariement des homéologues en métaphase I, GISH.

[Traduit par la Rédaction]

Introduction

Allopolyploids behave as cytological diploids during meiosis with only homologous chromosome pairing (Jenczewski and Alix 2004; Able et al. 2009). This ensures genome stability and fertility (Sánchez-Moran et al. 2001). The diploid-like meiotic behaviour of allopolyploids is thought to result from divergence of homoeologous chromosomes (Le Comber et al. 2010), probably supported by the activity of genes affecting different meiotic processes (Sears 1976; Cifuentes et al. 2010).

The diploid-like meiotic behaviour of common wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD) is regulated by a complex *Ph* (pairing homoeologous) system that prevents metaphase I (MI) pairing between genetically related (homoeologous) chromosomes of the A, B, and D genomes. This system includes a major pairing gene (*Ph1*) on chromosome 5B (Okamoto 1957; Riley and Chapman 1958), an intermediate pairing gene (*Ph2*) on chromosome 3D (Mello-Sampayo 1971; Sutton et al. 2003), and several minor loci (Sears 1976). The *Ph1* locus is related to a cluster of genes similar

M. Hao, J. Luo, M. Yang, L. Zhang, Z. Yan, Z. Yuan, and Y. Zheng. Triticeae Research Institute, Sichuan Agricultural University, Wenjiang, Sichuan 611130, P.R. China.
H. Zhang. Key Laboratory of Adaptation and Evolution of Plateau Biota, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 810001, P.R. China.
D. Liu. Triticeae Research Institute, Sichuan Agricultural University, Wenjiang, Sichuan 611130, P.R. China; Key Laboratory of Adaptation and Evolution of Plateau Biota, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 810001, P.R. China.

Corresponding author: Dengcai Liu (e-mail: dcliu7@yahoo.com).

to Cdk2 (cyclin-dependent kinase) in mammals (Griffiths et al. 2006; Al-Kaff et al. 2008; Yousafzai et al. 2010*a*), and it has a downstream effect on the synapsis gene *TaASY1* by reducing its expression level (Boden et al. 2009). However, this does not readily explain the multiple cytological effects attributed to *Ph1*, including premeiotic chromosome condensation and arrangement, chromatin remodeling, chromosome synapsis, and recombination (Holm and Wang 1988; Feldman 1993; Luo et al. 1996; Dubcovsky et al. 1995; Mikhailova et al. 1998; Martinez-Perez et al. 2001; Prieto et al. 2004, 2005; Colas et al. 2008; Moore and Shaw 2009; Knight et al. 2010; Yousafzai et al. 2010*b*).

The intermediate pairing gene *Ph2* is involved in the progression of synapsis (Martinez-Perez et al. 2001; Prieto et al. 2005), although the gene responsible for the phenotype is still to be isoated (Sutton et al. 2003). Pairing restriction by *Ph1* and *Ph2* involves not only wheat homoeologues but also wheat–alien chromosomes in wide crosses containing a haploid set of related chromosomes. However, homoeologous chromosomes can pair in hybrids of Chinese Spring (CS) mutant lines *ph1b*, CS*ph2a*, and CS*ph2b* and related alien species enabling gene transfer from alien species to wheat (Wall et al. 1971; Sears 1982; Martinez-Perez and Moore 2008). Moreover, gene *Ph^I* from *Aegilops speltoides* can repress the action of *Ph1* and induce homoeologous chromosome pairing (Chen et al. 1994).

Phenotypic differences in homoeologous pairing have been reported among the hybrids of wheat and alien species (Driscoll and Quinn 1970; Dvorák and McGuire 1981; Farqoo et al. 1990; Ma et al. 1999; Ozkan and Feldman 2001) or in haploids from different common wheat cultivars (Martinez et al. 2005). These variations may be caused by allelic variants at the *Ph1* or *Ph2* loci that have not been identified or by other loci involved in pairing between homoeologous chromosomes. Variations in *Ph* genes are useful for further elucidation of the mechanisms of homoeologous pairing and gene transfer from alien species to wheat (Miller et al. 1998). Chinese common wheat landrace Kaixian-luohanmai (KL) exhibits homoeologous pairing in hybrids with *Secale cereale* L. ($2n = 2x = 14$, RR) and *Aegilops variabilis* Eig. ($2n = 4x = 28$, UUS^{L}S^L) at levels between those of hybrids involving CS*ph1b* or CS*ph2b*/CS*ph2a* (Luo et al. 1992; Liu et al. 1998, 2003; Xiang et al. 2005). However, KL × *Psathyrostachys huashanica* Keng ex Kuo ($2n = 2x = 14$, NsNs) hybrids showed significantly higher chromosome pairing than CS*ph1b* × *Psa. huashanica* (Kang et al. 2008). The lower pairing in CS*ph1b* × *Psa. huashanica* may be caused by a suppressor in *Psa. huashanica* (Sun and Yen 1994). These results suggested that the genetic action of KL on homoeologous pairing was different from those of CS mutant lines (Liu et al. 2003). However, all the studies on hybrids of KL with alien species were done by conventional staining techniques that did not distinguish wheat and alien chromosomes.

The objective of this study was to compare differences in homoeologous chromosome pairing between hybrids of KL and CS*ph1b* with rye using genomic in situ hybridization (GISH). The implications for genetic introgression between wheat and alien species are discussed.

Materials and methods

Plant materials

Four types of hybrids were used, namely CS*ph1b* × rye, CS*ph2a* × rye, CS × rye, and KL × rye. These hybrids were obtained by crossing CS*ph1b*, CS*ph2a*, CS, and KL as female with *Secale cereale* L. 'Qinling' as male. Wheat genotypes CS*ph1b*, CS*ph2a*, and CS were provided by E.R. Sears, University of Missouri, USA. KL was collected from Sichuan, China, and kept in the Triticeae Research Institute of Sichuan Agricultural University.

Chromosome preparations

Anthers of emerging spikes containing pollen mother cells (PMCs) at MI were fixed in 1:3 (*v/v*) acetic acid – ethanol and stored at 4 °C. For conventional staining, anthers were squashed in 2% acetocarmine. For GISH analysis, anthers were squashed in a drop of 45% (*v/v*) acetic acid and cover slips were removed by freezing with liquid nitrogen. The slides were air dried and stored at –20 °C until examined by GISH.

DNA probes

Genomic DNA isolated from leaves of rye 'Qinling' was labeled with digoxigenin-11-dUTP (Roche Diagnostics GmbH, Germany) by nick translation according to the manufacturer's instructions. Unlabelled CS DNA was used as blocking DNA.

Fluorescent in situ hybridization

The GISH mixture (15 µL per slide), containing 100% formamide ultra pure, 20× saline sodium citrate buffer (SSC), herring sperm (hs) DNA (Promega Corporation, Madison, USA) and 50% dextran sulphate (DS), 45 ng rye genomic probe, and 9 ug blocking DNA, was denatured at 80 °C for 10 min and stored on ice for 10 min. The slides were treated with 4% (*m/v*) paraformaldehyde for 10 min, equilibrated for 2× 5 min in 2× SSC, dehydrated in 70%, 95%, and 100% ethanol at room temperature (RT) for 5 min each, denatured at 80 °C for 2 min in 70% formamide / 2× SSC, dehydrated in –20 °C 70%, 95%, and 100% ethanol for 5 min each, and then air dried. Each slide was treated by 15 µL GISH mixture, covered with a 24 mm × 24 mm cover glass, sealed with rubber cement, and incubated overnight in a humid chamber at 37 °C. After hybridization, slides were washed in 2× SSC for 5 min at 42 °C, 5 min at RT, 7 min at 42 °C, 3 min at RT, and 3 min in 1× phosphate buffered saline (PBS) at RT. Slides were incubated at 37 °C for 50 min in 80 µL 0.5% BSA/1× PBS + 1 µL anti-digoxigenin-fluorescein, fab fragments (Roche Diagnostics GmbH) in a humid chamber, then washed 3 times for 3 min in 1× PBS and for 3 min in deionized water, and air dried. Propidium iodide (PI) was used to counterstain the chromosome preparations.

Meiotic observations were made and documented with an Olympus BX-51 microscope coupled with a Photometric SenSys Olympus DP70 CCD camera. Associations were calculated from the numbers of chromosome arms paired at MI per cell. Student's *t* test was applied for statistical evaluation of the differences between the means of the MI parameters in the hybrid genotypes.

Table 1. Mean chromosome pairing associations in hybrid genotypes.

Genotype	Staining technique	No. of cells	Bivalents (range)			
			Rod	Ring	Multivalent	Associations
KL × rye	CST[a]	36	4.14ab (1–7)	0.19b (0–1)	0.08c (0–2)	4.72b (1–8)
	GISH	45	4.73ab (1–7)	0.20b (0–1)	0.11c (0–2)	5.40b (3–9)
CSph1b × rye	CST	41	3.90b (1–7)	2.15a (0–5)	0.93a (0–3)	10.22a (7–16)
	GISH	53	4.85a (1–9)	1.87a (0–4)	0.47b (0–2)	9.53a (6–13)
CSph2a × rye	CST	58	1.74c (0–4)	0c	0.02c (0–1)	1.78c (0–4)
CS × rye	GISH	129	0.54d (0–4)	0c	0c	0.54d (0–4)

Note: Means within a column followed by different letters are significantly different at $p \leq 0.01$ following Student's t test.
[a]Conventional staining technique.

Fig. 1. Flourescence of meiotic metaphase I chromosomes in pollen mother cells from wheat–rye hybrids; Yellow-green fluoresce for rye and red propidium iodide flourescence for wheat chromosomes. (*a*) Six rod bivalents with wheat–wheat pairing in KL × rye, (*b*) a wheat–rye rod bivalent in KL × rye, (*c*) two wheat–wheat ring bivalents, and (*d*) one rye–rye rod bivalent in CSph1b × rye.
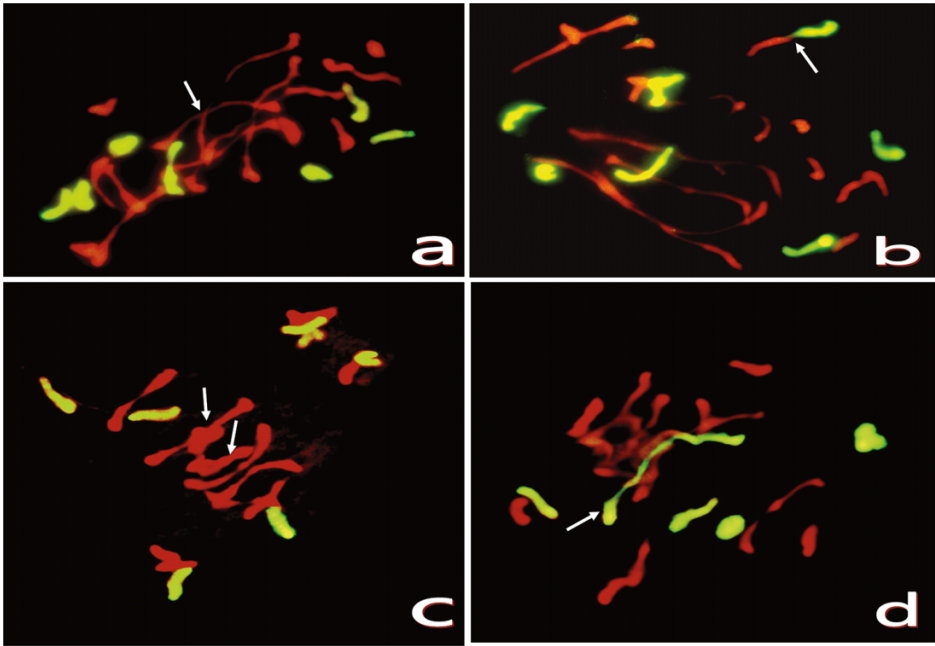
Results

Table 1 shows the chromosome pairing configurations and associations at meiotic MI in the wheat × rye hybrids. In agreement with previous reports (Liu et al. 1998, 2003; Xiang et al. 2005), the number of chromosome associations in KL × rye was significantly lower than in CSph1b × rye but higher than in CSph2a × rye and CS × rye. Compared with CSph1b × rye, KL × rye showed a similar rod bivalent number although with less ring bivalents and multivalents. Both KL × rye and CSph2a × rye mainly formed rod bivalents. However, the number of rod bivalents in KL × rye was significantly higher than in CSph2a × rye.

The 21 wheat chromosomes and 7 rye chromosomes in F_1 hybrids were clearly distinguished by different fluorescent colors, i.e., yellow-green fluorescein for rye and red propidium iodide for wheat chromosomes (Fig. 1). Three types of chromosome pairing at MI were distinguished by GISH, viz. wheat–wheat (w–w), wheat–rye (w–r), and rye–rye (r–r) (Fig. 1, Table 2). Compared with CSph1b × rye, KL × rye showed less w–w associations per cell. However, KL × rye

showed similar w–r ($t = 0.09$, $p > 0.93$) and significantly higher r–r ($t = 2.7$, $p < 0.009$) associations per cell (Table 2). A few multivalents were observed. W–r chromosome pairing was totally attributed to one to three rod bivalents in both hybrids CSph1b × rye and KL × rye (Table 3). CSph1b × rye and KL × rye had similar w–r bivalent distributions among cells (Table 3). Ring r–r bivalents were not observed in either hybrid.

There were differences in distribution frequencies between CSph1b × rye and KL × rye for the three types of chromosome pairing (Table 2). The ratios of w–w for total associations were higher in CSph1b × rye than in KL × rye (87.7% vs. 73.3%, $t = 4.94 > t_{0.01} = 2.58$). However, ratios of w–r and r–r for total associations were higher in KL × rye than in CSph1b × rye (w–r, $t = 3.08 > t_{0.01} = 2.58$; r–r, $t = 632.46 > t_{0.01} = 2.58$) (Table 2). These results indicated that CSph1b × rye had a greater promoting effect on w–w pairing and a lower promoting effect on w–r and r–r pairing than KL × rye. Another difference was that the paired MI chromosomes in KL × rye seemed to be more slender than in CSph1b × rye (Fig. 1).

Table 2. Distributions of chromosome arm associations for three pairing types, viz. wheat–wheat (w–w), wheat–rye (w–r), and rye–rye (r–r).

Genotype	No. of cells	Total no. (frequency)				Mean no. per cell (range)		
		w–w	w–r	r–r	Total	w–w	w–r	r–r
KL × rye	45	178b (73.3%)	44a (18.1%)	21a (8.6%)	243	3.96b (2–6)	0.98a (0–3)	0.47a (0–1)
CS*ph1b* × rye	53	443a (87.7%)	51b (10.1%)	11b (2.2%)	505	8.36a (4–12)	0.96a (0–3)	0.21b (0–2)
CS × rye	129	62a (88.6%)	7b (10.0%)	1b (1.4%)	70	0.48c (0–4)	0.05b (0–1)	0.01c (0–1)

Note: Means within a column followed by different letters are significantly different at $p \leq 0.01$ following Student's *t* test.

Table 3. The distribution of wheat–rye pairing associations among cells.

Genotype	Total no. (frequency)			No. of cells with different bivalent numbers			
	Rod	Ring	Multivalent	0	1	2	3
KL × rye	42 (95%)	0	2 (5%)	16 (34.8%)	15 (33.3%)	13 (28.9%)	1 (2.2%)
CS*ph1b* × rye	50 (98%)	0	1 (2%)	20 (37.7%)	17 (32.1%)	14 (26.4%)	2 (3.8%)
CS × rye	7 (100%)	0	0	122 (94.6%)	7 (5.4%)	0	0

Discussion

The present results indicated that CS*ph1b* × rye and KL × rye hybrids had phenotypically different effects on homoeologous pairing. Differences between KL and CS*ph1b* were also observed in their hybrids with *Psa. huashanica*, a more distantly related species. Homoeologous pairing was significantly higher in KL × *Psa. huashanica* than CS*ph1b* × *Psa. huashanica* attributable to the suppressing action of *Psa. huashanica* on *ph1b* (Sun and Yen 1994; Kang et al. 2008). Moreover, wheat genotype CS*ph1b* allows homoeologous pairing among its own chromosomes leading to reduced seed setting (Sears 1976; Ceoloni and Donini 1993) and an unstable karyotype (Sánchez-Morán et al. 2001), whereas KL chromosomes pair normally (Liu et al. 2003). These differences indicate a different mechanism controlling *ph*-like activity in KL compared with CS*Ph1b*. Monosomic analysis suggested that one locus on chromosome 6A in KL may promote homoeologous pairing (Liu et al. 1997).

The *Ph1* locus on chromosome 5B enforces strict bivalent pairing in common wheat. Homoeologous chromosome pairing in wheat–alien hybrids and wheat haploids is also restricted when *Ph1* is functional. When *Ph1* is disrupted, as in mutant *ph1b*, homoeologous chromosomes can pair with each other (Martinez-Perez and Moore 2008). If sets of four chromosomes from each of seven homoeologous groups have the same potential to pair in CS*ph1b* × rye with 28 homoeologous chromosomes, a similar association value between w–w and w–r is expected as they have the same probability of pairing (e.g., (1A-1B, 1A-1D, and 1B-1D) vs. (1A-1R, 1B-1R, and 1D-1R) in group one). However, the average mean association of w–w (8.36) was much higher than for w–r (0.96) in CS*ph1b* × rye. Similar results were also reported by Miller et al. (1994) and Benavente et al. (1998). This may be caused by lower overall affinity, or homoeology, between wheat and rye chromosomes than between wheat chromosomes. These results suggested that even with a no function situation for *Ph1*, only chromosomes with high homoeology can associate with each other. It is not clear as to the threshold of homoeology leading to homoeologous association.

Table 4. Bivalents distribution wheat–wheat pairing associations.

Genotype	No. of cells with w–w bivalents (frequency)	
	≤ 7	8
CS*ph1b* × rye	47 (88.7%)	6 (11.3%)
KL × rye	45 (100%)	0
CS × rye	129 (100%)	0

Although our results did not allow us to determine which rye chromosomes engaged in pairing, w–r associations occur in particular homoeologous chromosome arms with close genetic affinity, such as 1BL-1RL and 2BL-2RL as previously reported (Naranjo 1992; Naranjo and Fernandez-Rueda 1996; Dvorak and Lukaszewski 2000). On the other hand, assuming that only homoeologous pairing can take place, the maximum bivalent number should be seven for wheat–wheat pairing and zero for rye–rye pairing. However, more than seven w–w bivalents were observed in some PMCs of CS*ph1b* × rye (Table 4). This indicated that pairing between nonhomoeologous chromosomes also occurs. Similarly, r–r association in both CS*ph1b* × rye and KL × rye indicated the existence of nonhomoeologous pairing (Table 2). This may be related to nonhomoeologous translocations that occurred during the evolution of each species (Liu et al. 1992; Naranjo 1992; Miller et al. 1994) or to the accumulative effects of nonhomoeologous translocations in CS*ph1b* itself (Sánchez-Moran et al. 2001).

References

Able, J.A., Crismani, W., and Boden, S.A. 2009. Understanding meiosis and the implications for crop improvement. Funct. Plant Biol. **36**(7): 575–588. doi:10.1071/FP09068.

Al-Kaff, N., Knight, E., Bertin, I., Foote, T., Hart, N., Griffiths, S., and Moore, G. 2008. Detailed dissection of the chromosomal region containing the *Ph1* locus in wheat *Triticum aestivum*: with deletion mutants and expression profiling. Ann. Bot. (Lond.), **101**(6): 863–872. doi:10.1093/aob/mcm252. PMID:17951583.

Benavente, E., Orellana, J., and Fernández-Calvín, B. 1998. Comparative analysis of the meiotic effects of wheat *ph1b* and *ph2b* mutations in wheat × rye hybrids. Theor. Appl. Genet. **96**(8): 1200–1204. doi:10.1007/s001220050857.

Boden, S.A., Langridge, P., Spangenberg, G., and Able, J.A. 2009. TaASY1 promotes homologous chromosome interactions and is affected by deletion of *Ph1*. Plant J. **57**(3): 487–497. doi:10.1111/j.1365-313X.2008.03701.x. PMID:18826431.

Ceoloni, C., and Donini, P. 1993. Combining mutations for the two homoeologous pairing suppressor genes *Ph1* and *Ph2* in common wheat and in hybrids with alien Triticeae. Genome, **36**(2): 377–386. doi:10.1139/g93-052. PMID:18469995.

Chen, P.D., Tsujimoto, H., and Gill, B.S. 1994. Transfer of *Ph*^I genes promoting homoeologous pairing from *Triticum speltoides* to common wheat. Theor. Appl. Genet. **88**(1): 97–101. doi:10.1007/BF00222400.

Cifuentes, M., Eber, F., Lucas, M.O., Lode, M., Chèvre, A.M., and Jenczewski, E. 2010. Repeated polyploidy drove different levels of crossover suppression between homoeologous chromosomes in *Brassica napus* allohaploids. Plant Cell, **22**(7): 2265–2276. doi:10.1105/tpc.109.072991. PMID:20639447.

Colas, I., Shaw, P., Prieto, P., Wanous, M., Spielmeyer, W., Mago, R., and Moore, G. 2008. Effective chromosome pairing requires chromatin remodeling at the onset of meiosis. Proc. Natl. Acad. Sci. U.S.A. **105**(16): 6075–6080. doi:10.1073/pnas.0801521105. PMID:18417451.

Driscoll, C.J., and Quinn, C.J. 1970. Genetic variation in *Triticum* affecting the level of chromosome pairing in intergenetic hybrids. Can. J. Genet. Cytol. **12**(2): 278: 278–282. doi:10.1139/g70-041.

Dubcovsky, J., Luo, M.C., and Dvorak, J. 1995. Differentiation between homoeologous chromosomes 1A of wheat and 1A^m of *Triticum monococcum* and its recognition by the wheat *Ph1* locus. Proc. Natl. Acad. Sci. U.S.A. **92**(14): 6645–6649. doi:10.1073/pnas.92.14.6645. PMID:11607556.

Dvorak, J., and Lukaszewski, A.J. 2000. Centromere association is an unlikely mechanism by which the wheat *Ph1* locus regulates metaphase I chromosome pairing between homoeologous chromosomes. Chromosoma, **109**(6): 410–414. doi:10.1007/s004120000093. PMID:11072796.

Dvorák, J., and McGuire, P.E. 1981. Nonstructural chromosome differentiation among wheat cultivars, with special reference to differentiation of chromosomes in related species. Genetics, **97**(2): 391–414. PMID:17249077.

Farqoo, S., Iqbal, N., and Shah, T.M. 1990. Intergenetic hybridization for wheat improvement. III. Genetic variation in *Triticum* species affecting homoeologous chromosome pairing. Cereal Res. Commun. **18**(3): 233–237.

Feldman, M. 1993. Cytogenetic activity and mode of action of the pairing homoeologous (*Ph1*) gene of wheat. Crop Sci. **33**(5): 894–897. doi:10.2135/cropsci1993.0011183X003300050003x.

Griffiths, S., Sharp, R., Foote, T.N., Bertin, I., Wanous, M., Reader, S., et al. 2006. Molecular characterization of *Ph1* as a major chromosome pairing locus in polyploid wheat. Nature, **439**(7077): 749–752. doi:10.1038/nature04434. PMID:16467840.

Holm, P.B., and Wang, X. 1988. The effect of chromosome 5B on synapsis and chiasma formation in wheat, *Triticum aestivum* cv. Chinese Spring. Carlsberg Res. Commun. **53**(2): 191–208. doi:10.1007/BF02907179.

Jenczewski, E., and Alix, K. 2004. From diploids to allopolyploids: the emergence of pairing control genes. Crit. Rev. Plant Sci. **23**(1): 21–45. doi:10.1080/07352680490273239.

Kang, H.Y., Zhang, H.Q., Wang, Y., Jiang, Y., Yuan, H.J., and Zhou, Y.H. 2008. Comparative analysis of the homoeologous pairing effects of *phKL* gene in common wheat × *Psathyrostachy huashanica* Keng ex Kuo. Cereal Res. Commun. **36**(3): 429–440. doi:10.1556/CRC.36.2008.3.7.

Knight, E., Greer, E., Draeger, T., Thole, V., Reader, S., Shaw, P., and Moore, G. 2010. Inducing chromosome pairing through premature condensation: analysis of wheat interspecific hybrids. Funct. Integr. Genomics, **10**(4): 603–608. doi:10.1007/s10142-010-0185-0. PMID:20676714.

Le Comber, S.C., Ainouche, M.L., Kovarik, A., and Leitch, A.R. 2010. Making a functional diploid: from polysomic to disomic inheritance. New Phytol. **186**(1): 113–122. doi:10.1111/j.1469-8137.2009.03117.x. PMID:20028473.

Liu, C.J., Atkinson, M.D., Chinoy, C.N., Devos, K.M., and Gale, M.D. 1992. Nonhomoeologous translocations between group 4, 5 and 7 chromosomes within wheat and rye. Theor. Appl. Genet. **83**(3): 305–312. doi:10.1007/BF00224276.

Liu, D.C., Luo, M.C., Yang, J.L., Yen, C., Lan, X.J., and Yang, W.Y. 1997. Chromosome location of a new paring promoter in natural populations of common wheat. Xi Nan Nong Ye Xue Bao, **10**: 10–15. [In Chinese.]

Liu, D.C., Luo, M.C., Yen, C., Yang, J.L., and Yang, W.Y. 1998. The promotion of homoeologous pairing in hybrids of common wheat cv Kaixianluohanmai with alien species. *In* Proceedings of the 9th International Wheat Genetics Symposium. *Edited by* A.E. Slinkard. University Extension Press, University of Saskatchewan, Saskatoon, Sask. Vol. 4. pp. 377–378.

Liu, D.-C., Zheng, Y.-L., Yan, Z.-H., Zhou, Y.-H., Wei, Y.-M., and Lan, X.-J. 2003. Combination of homoeologous pairing gene *phKL* and *Ph2*-deficiency in common wheat and its mitotic behaviors in hybrids with alien species. Acta Bot. Sin. **45**(9): 1121–1128.

Luo, M.C., Yang, Z.L., Yen, C., and Yang, J.L. 1992. The cytogenetic investigation on F₁ hybrid of Chinese wheat landrace. *In* Exploration of crop breeding. *Edited by* Z.L. Ren and J.H. Peng. Science and Technology Press of Sichuan, China. pp. 169–176. [In Chinese.]

Luo, M.C., Dubcovsky, J., and Dvorák, J. 1996. Recognition of homology by the wheat *Ph1* locus. Genetics, **144**(3): 1195–1203. PMID:8913760.

Ma, R., Zheng, D.S., and Fan, L. 1999. The possibility of *ph* genes existing spontaneously in common wheat. Acta Agron. Sin. **25**(1): 99–104. [In Chinese.]

Martinez, M., Cuadrado, C., Laurie, D.A., and Romero, C. 2005. Synaptic behaviour of hexaploid wheat haploids with different effectiveness of the diploidizing mechanism. Cytogenet. Genome Res. **109**(1–3): 210–214. doi:10.1159/000082402. PMID:15753579.

Martinez-Perez, E., and Moore, G. 2008. To check or not to check? The application of meiotic studies to plant breeding. Curr. Opin. Plant Biol. **11**(2): 222–227. doi:10.1016/j.pbi.2008.01.001. PMID:18294901.

Martinez-Perez, E., Shaw, P., and Moore, G. 2001. The *Ph1* locus is needed to ensure specific somatic and meiotic centromere association. Nature, **411**(6834): 204–207. doi:10.1038/35075597. PMID:11346798.

Mello-Sampayo, T. 1971. Genetic regulation of meiotic chromosome

pairing by chromosome 3D of *Triticum aestivum*. Nat. New Biol. **230**(1): 22–23. PMID:5283628.

Mikhailova, E.I., Naranjo, T., Shepherd, K., Wennekes-van Eden, J., Heyting, C., and de Jong, J.H. 1998. The effect of the wheat *Ph1* locus on chromatin organisation and meiotic chromosome pairing analyzed by genome painting. Chromosoma, **107**(5): 339–350. doi:10.1007/s004120050316. PMID:9880767.

Miller, T.E., Reader, S.M., Purdie, K.A., and King, I.P. 1994. Determination of the frequency of wheat–rye chromosome pairing in wheat × rye hybrids with and without chromosome 5B. Theor. Appl. Genet. **89**(2–3): 255–258. doi:10.1007/BF00225150.

Miller, T.E., Reader, S.M., Shaw, P.J., and Moore, G. 1998. Towards an understanding of the biological action of the *Ph1* locus in wheat. *Edited by* A.E. Slinkard. *In* Proceedings of the 9th International Wheat Genetics Symposium, University Extension Press, University of Saskatchewan, Sask., Canada. Vol. 1. pp. 17–19.

Moore, G., and Shaw, P. 2009. Improving the chances of finding the right partner. Curr. Opin. Genet. Dev. **19**(2): 99–104. doi:10.1016/j.gde.2009.02.006. PMID:19327980.

Naranjo, T. 1992. The use of homoeologous pairing in the identification of homoeologous relationships in Triticeae. Hereditas, **116**: 219(3): 219–223. doi:10.1111/j.1601-5223.1992.tb00145.x.

Naranjo, T., and Fernandez-Rueda, P. 1996. Pairing and recombination between individual chromosomes of wheat and rye in hybrids carrying the *phlb* mutation. Theor. Appl. Genet. **93**(1–2): 242–248. doi:10.1007/BF00225752.

Okamoto, M. 1957. Asynapsis effect of chromosome V. Wheat Inf. Serv. **5**: 6.

Ozkan, H., and Feldman, M. 2001. Genotypic variation in tetraploid wheat affecting homoeologous pairing in hybrids with *Aegilops peregrine*. Genome, **44**(6): 1000–1006. doi:10.1139/g01-100. PMID:11768203.

Prieto, P., Shaw, P., and Moore, G. 2004. Homologue recognition during meiosis is associated with a change in chromatin conformation. Nat. Cell Biol. **6**(9): 906–908. doi:10.1038/ncb1168. PMID:15340450.

Prieto, P., Moore, G., and Reader, S. 2005. Control of conformation changes associated with homologue recognition during meiosis.

Theor. Appl. Genet. **111**(3): 505–510. doi:10.1007/s00122-005-2040-6. PMID:15895201.

Riley, R., and Chapman, V. 1958. Genetic control of the cytologically diploid behaviour of hexaploid wheat. Nature, **182**(4637): 713–715. doi:10.1038/182713a0.

Sánchez-Morán, E., Benavente, E., and Orellana, J. 2001. Analysis of karyotypic stability of homoeologous-pairing (*ph*) mutants in allopolyploid wheats. Chromosoma, **110**(5): 371–377. doi:10.1007/s004120100156. PMID:11685537.

Sears, E.R. 1976. Genetic control of chromosome pairing in wheat. Annu. Rev. Genet. **10**(1): 31–51. doi:10.1146/annurev.ge.10.120176.000335. PMID:797311.

Sears, E.R. 1982. A wheat mutant conditioning an intermediate level of homoeologous pairing. Can. J. Genet. Cytol. **24**(6): 715–719. doi:10.1139/g82-076.

Sun, G.L., and Yen, C. 1994. The ineffectiveness of the *ph1b* gene on chromosome association in the F_1 hybrid *Triticum aestivum* × *Psathyrostachys huashanica*. Wheat Inf. Serv. **79**: 28–32.

Sutton, T., Whitford, R., Baumann, U., Dong, C.M., Able, J.A., and Langridge, P. 2003. The *Ph2* pairing homoeologous locus of wheat (*Triticum aestivum*): identification of candidate meiotic genes using a comparative genetics approach. Plant J. **36**(4): 443–456. doi:10.1046/j.1365-313X.2003.01891.x. PMID:14617076.

Wall, A.M., Riley, R., and Chapman, V. 1971. Wheat mutants permitting homoeologous meiotic chromosome pairing. Genet. Res. **18**(03): 311–328. doi:10.1017/S0016672300012714.

Xiang, Z.G., Liu, D.C., Zheng, Y.L., Zhang, L.Q., and Yan, Z.H. 2005. The effect of *phKL* gene on homoeologous pairing of wheat–alien hybrids is situated between gene mutants of *Phl* and *Ph2*. Hereditas (Beijing), **27**: 935–940. [In Chinese.]

Yousafzai, F.K., Al-Kaff, N., and Moore, G. 2010*a*. Structural and functional relationship between the *Ph1* locus protein 5B2 in wheat and CDK2 in mammals. Funct. Integr. Genomics, **10**(2): 157–166. doi:10.1007/s10142-010-0170-7. PMID:20422243.

Yousafzai, F.K., Al-Kaff, N., and Moore, G. 2010*b*. The molecular features of chromosome pairing at meiosis: the polyploid challenge using wheat as a reference. Funct. Integr. Genomics, **10**(2): 147–156. doi:10.1007/s10142-010-0171-6. PMID:20422242.