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The KL system in wheat permits homoeologous crossing over between closely related chromosomes

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ABSTRACT

The Chinese wheat landrace Kaixianluohanmai (KL) expresses the *ph*-like phenotype. A major QTL, *QPh.sicau-3A* (syn. *phKL*), responsible for this effect has been mapped to chromosome arm 3AL. This study presents some characteristics of homoeologous pairing and recombination induced by *phKL*. In KL haploids, the level of homoeologous pairing was elevated relative to *Ph1* Chinese Spring (CS) haploids. There was a clear preference for A–D pairing and less frequent for A–B and B–D, reflecting the higher levels of affinity between genomes A and D in wheat. The characteristics of pairing were affected by temperature and magnesium ion supplementation. The suitability of *phKL* for chromosome engineering was tested on three pairs of homoeologues: $2S^{v}$-2B, $2S^{v}$-2D, and 2RL-2BL. The recombination rates were 1.68%, 0.17%, and 0%, respectively. The *phKL* locus in KL induced a moderate level of homoeologous chromosome pairing and recombination when the *Ph1* locus of wheat was present, both in wheat haploids and hexaploids. The *Ph1*-imposed criteria for chromosome pairing and crossing over were relaxed to some degree, permitting homoeologous crossing over but only between closely related chromosomes; there was no crossing over between more differentiated chromosomes. Therefore, the *phKL* system (*QPh.sicau-3A*) can be a useful tool in chromosome engineering of wheat to transfer genes from closely related species with the benefit of reduced genomic chaos generated by the *ph1b* mutation.

1. Introduction

Polyploidisation plays a major role in evolution of higher organisms. In plants, approximately 70% of angiosperms have experienced one or more episodes of polyploidisation during their evolution [1] or domestication [2]. However, polyploidisation comes with a cost, and a major issue is meiotic chromosome pairing. Only bivalent pairing ensures regular reduction of chromosome number, and therefore efficient production of gametes. In polyploids, the presence of more than two sets of chromosomes offers a potential for pairing into configurations greater than two, and thus irregular disjunction in anaphase I. Autopolyploids, with only present of homologues, struggle with meiotic irregularities until homologues sufficiently differentiate to revert to bivalent pairing, and this is a very slow process [3]. However, allopolyploids contain two or more sets of chromosomes that are related by ancestral homology but have already differentiated, and they evolved genetic systems which recognise these differences and restrict pairing to strictly homologous; this guarantees diploid-like behaviour in the first meiotic division [4].

One of the most studied systems enforcing diploid-like behaviour in allopolyploids is the *Ph* system in common wheat (*Triticum aestivum* L.). It consists of several genes that act as either suppressors or promotors of homoeologous chromosome pairing. The major regulator of the diploid-like behaviour of polyploid wheat, *Ph1*, is located on the long arm of chromosome 5B [5,6]. *Ph1* prevents homoeologous pairing; it also regulates pairing of homologues by restricting pairing of differentiated chromosomes [7,8]. However, identical homologues, as in self-pollinating wheat, are

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not affected to any detectable degree when two doses of the locus are present.

The identity of *Ph1* remains controversial, with different candidates proposed by different groups: *Cdk* genes [9–11], *C-Ph1* [12,13], and *TaZip4-B2* [14,15]. All these candidates are associated with different parts of the crossover pathway after double-strand breaks in meiosis of mammals [16,17], yeast (*Saccharomyces cerevisiae*), rice (*Oryza sative*), and *Arabidopsis thaliana* [18]. All three are located in the deletion segment of *ph1b*, which is a deletion mutant of 5BL in Chinese Spring (CS) wheat that expresses a phenotype similar to the absence of 5BL. Mutations or deletions of single genes among *Cdk*, *C-Ph1*, and *TaZip4-B2* do not appear to fully mimic the effects of *ph1b* or nullisomic 5BL. Perhaps they may all contribute to the *Ph1* phenotype.

A less effective homoeologous pairing suppressor in wheat, *Ph2*, is located on the short arm of chromosome 3D [19–21]. A DNA mismatch repair gene, *TaMSH7* (*TraesCS3D02G119400*) is a promising candidate for *Ph2* [22]. *MSH7* was proposed to contribute to the suppression of homoeologous recombination in cultivated tomato (*Solanum lycopersicum*) [23] and *Arabidopsis thaliana* [24]. Suppressors with minor effects were also found on chromosome 4D [25] and the short arm of 3A [20]. Several pairing promotors in wheat are located on the short arms of group 2 [26], group 5 [27,28], and the long arms of group 3 chromosomes [19,20].

Disabling *Ph1* is an approach to induce homoeologous chromosome pairing, such as between wheat and its related species. The *ph1b* mutation is used in chromosome engineering for transferring desirable traits from wild relatives to cultivated wheat. In fact, it is a deletion of a ~60 Mb segment of 5BL harbouring the *Ph1* locus [29]. The resulting homoeologous chromosome pairing and consequent recombination make it possible to transfer small segments of chromatin with desirable traits and genes into wheat chromosomes, placing them in proper positions in the genome, or to remove some undesirable loci. Many such transfers have been accomplished [30].

Although the actual mechanism of the operation of *Ph1* remains unclear, it is evident that when it is absent, the metaphase I (MI) chromosome pairing becomes irregular, with frequent pairing of homoeologues producing multivalents and univalents. Consequently, there is uneven segregation of chromosomes in anaphase I, and therefore aneuploidy. During this stage, chromatin exchange between genomes takes place [31]. Thus, the *Ph1* mutant accumulates extensive chromosome and genome rearrangements over generations [32]. These rearrangements muddle the picture of the effects of *Ph1* absence; in practical applications, they are introduced into chromosome-engineered wheat, and greatly contribute to the chaos of genome organisation. This almost always has negative effects on agronomic traits, such as reduced fertility and late heading, and requires many generations of backcrosses to normal stocks to fix this issue. This slows the process of transferring engineered chromosomes from laboratory stocks to commercial cultivars.

In a landrace of hexaploid wheat, Kaixianluohanmai (KL), a gene (s) promoting homoeologous pairing in the presence of *Ph1* in wheat–alien hybrids was posited [33]. A moderate level of homoeologous pairing was present in hybrids of KL with rye (*Secale cereale* L.) and *Aegilops variabilis* [33–35]. The seed set in KL is normal, which indicates regular meiosis. A major QTL affecting this chromosome behaviour was detected on chromosome 3AL and designated as *QPh.sicau-3A* [36]. Flanking markers of this locus are physically located in a genomic interval of 696–725 Mb in chromosome 3A of the CS reference genome assembly (IWGSC version 1.0). However, there are major questions regarding the *ph*-like phenotype of KL (designated *phKL*). First, is *phKL* effective in promoting homoeologous pairing in haploids without any alien genome present? Second, is *phKL* effective in promoting homoeologous recombination in targeted pairs of homoeologues in the presence of functional *Ph1*? Third, if *phKL* is effective at both ploidy levels, what are the characteristics of this system at each ploidy level?

This study addresses these questions. We produced KL and CS haploids as controls to assess meiotic pairing in MI. We compared the ability of *phKL* to induce homoeologous recombination of three pairs of homoeologues with different levels of affinity (differentiation), 2S^v-2B, 2S^v-2D, and 2RL-2BL, with the presence of *Ph1* in the KL background. Then, we analyzed the physical distribution of recombination in the 2S^v-2B pair. As it is known that the temperature and Mg^{2+} concentration affect chromosome pairing in wheat [15,37], we assessed the effects of these two factors on chromosome pairing in haploids.

2. Material and methods

2.1. Plant materials

KL and CS haploids were produced by the *Hordeum bulbosum* method [38]. Briefly, several heads of KL and CS were emasculated and pollinated with *H. bulbosum*. Embryo rescue was performed 18–22 days after pollination, followed by embryo culture. The obtained plants were planted in soil and grown in a growth chamber at a constant temperature of 23 °C under a 16 h/8 h day/night regime.

The effect of KL on homoeologous recombination was tested on three pairs of chromosomes with different levels of affinity: 2S^v-2B, 2S^v-2D, and 2BL-2RL. Chromosome 2S^v was from *Ae. variabilis*, which was accessed from two common wheat lines, WJN2890 and WJN2896, with single chromosome substitutions 2S^v (2B) and 2S^v (2D), respectively [39]. The S^v genome is believed to be closely related to the B genome of wheat and less so to the D genome [40]. The chromosome arm 2RL, herein a centric translocation 2BS.2RL, originated from rye cv. Blanco. Although it showed the highest pairing affinity of all rye chromosome arms to wheat with the absence of *Ph1* [41], it was assumed to be the most distantly related homoeologue of wheat among the three pairs tested here.

The substitution lines 2S^v (2B) and 2S^v (2D) were crossed as female to KL, and F_1 hybrids were backcrossed to KL as male. The resulting BC_1F_1 hybrids were screened with the SSR marker *gwm539* to select plants with 2S^v [42]. Progeny of such selected BC_1F_1 hybrids (double monosomic lines) were screened to identify 2S^v-2B and 2S^v-2D recombinant chromosomes. The population with 2RL was created in a similar fashion: lines with homozygous translocation were crossed and backcrossed to KL or self-pollinated; screening was done by C-banding.

Plant progeny with primary recombinants were screened to select homozygous recombinants. Common wheat cv. CS, WJN2890 (2S^v-2B substitution line), WJN2896 (2S^v-2D substitution line), and *Ae. variabilis* accession AS116 (genome UUS^{v}S^v) were used for marker development.

2.2. Development of KASP markers

To identify polymorphic SNPs between *Ae. variabilis* 2S^v and wheat homoeologous group 2 chromosomes, transcriptomic sequencing was performed with young leaves of line WJN2890 at tillering stage (stage 3) [43]. High-quality RNA from leaves of the 2S^v(2B) substitution line was used to generate PacBio IsoSeq libraries. Genomic RNA extraction and PacBio single-molecule long-read isoform sequencing were performed at Frasergen Co. (Wuhan, Hubei, China). The raw sequence data were filtered and polished by the iso-Seq3 workflow (https://www.pacb.com/software). The polished consensus sequences (PCSs) were BLASTed

against the wheat reference assembly CS v1.1 by TBtools v1.0986 [44] with default settings. The PCSs with hits on group 2 of CS were retained. Presumably, most PCSs had at least one hit on each group 2 chromosome (2A, 2B, and 2D) with varying bitscores. The PCSs with the highest bitscores against the 2B sequence were most likely derived from the 2S^v chromosome because of the close evolutionary relationship to the S^v and B genomes [45–48]. To detect primary recombinants, two pairs of markers on each arm were used, one distal and one proximal.

PCS sequences were randomly extracted by TBtools v1.0986 [44], and their 2B homoeologues were physically distributed at the distal and proximal regions of the short and long arms on the wheat reference map CS v1.1 [49]. These sequences were aligned with corresponding gene sequences of group 2 chromosomes of CS by DNAMAN (version 8; https://www.lynnon.com/) to identify-two types of SNPs for KASP marker development. First, triple-polymorphic SNPs between 2S^v, 2B, and 2A/2D alleles and between 2S^v, 2D, and 2A/2B alleles were selected (Fig. S1A). Second, double-polymorphic SNPs between 2S^v and the three wheat homoeologous alleles were selected (Fig. S2B). Using double-polymorphic SNPs as forward primers, SNPs specific for 2S^v-2B and 2S^v-2D were used to develop reverse primers. All KASP markers were designed such that the allele (A1) of 2S^v produced a HEX signal, whereas the 2B or 2D allele (A2) produced a FAM signal. Primer sequences are listed in Table S1. The KASP analysis followed a previously described procedure [36]. The Bio-Rad CFX96 real-time PCR system (Hercules, CA, USA) and Applied Biosystems (Carlsbad, CA, USA) were used for PCR amplification and genotyping.

2.3. Cytological observations

Homoeologous pairing in KL and CS haploids were scored at meiotic MI in pollen mother cells (PMCs). Anthers with PMCs at MI were collected and fixed in Farmer's fixative (3:1 ethanol:acetic acid) and stored at 4 °C. To assess chromosome pairing levels, anthers were squashed in aceto-carmine and observed under a light microscope. The pairing level for each individual was expressed as the mean number of paired chromosome arms in all scored PMCs. Preparations with most PMCs at MI were destained and used for genomic *in situ* hybridisation (GISH).

The putative recombinants 2S^v-2B selected with KASP markers were screened by fluorescent *in situ* hybridisation (FISH) and GISH. FISH probes Oligo-pSc119.2 and Oligo-pTa-713 were used to distinguish the wheat and 2S^v chromosomes [39,50,51]. These synthetic oligonucleotides were 5′ end-labelled with 6-carboxyfluorescein by Sangon Biotech Co. (Shanghai, China). The FISH probe mixture was in 2× SSC–1× TE buffer (10 μL per slide).

For the GISH procedure, genomic DNA of *Ae. longissima* (S^{l}S^l), rye (RR), and *Ae. tauschii* (DD) were labelled by the Atto550 NT labelling kit (Jena Bioscience, Jena, Germany). Genomic DNA of *T. urartu* (AA) was labelled by the Atto488 NT labelling kit. Genomic DNA of CS was used as blocking DNA for the S^l and R genome probes with a probe-to-block ratio of 1:350, whereas the genomic DNA of *Ae. speltoides* (SS) was used as blocking DNA for the A and D genome probes at the same ratio. The *in situ* hybridisation procedure was as follows: slides were crossed linked twice in the ultraviolet cross linker for 12 s. The FISH probe mixtures were added to each slide and covered with plastic cover slips. Slides were denatured at 85 °C (2 min) and transferred to 37 °C for 2 h. For GISH, the probe mixture was denatured at 90 °C for 10 min and applied to the slides. Slides were incubated at 50 °C overnight, washed in 2× SSC at room temperature for 5 min for FISH and for 20 min at 52 °C for GISH. Chromosome preparations were counterstained with DAPI (4′,6-diamidino2-phenylindole) or PI (Propidium Iodide) in Vectashield (Vector Laboratories, Burlingame, CA, USA). Images were acquired using a BX-63 microscope (Olympus, Japan) coupled with a Photometric SenSys Olympus DP70 CCD camera.

2.4. Genotyping

A proportion of 2S^v-2B recombinants selected by KASP markers (those rejected by *in situ* hybridisation were excluded) along with WJN2890 and WJN2896 were genotyped by 55K SNP array to estimate the size of the 2S^v segments. Genomic DNA was extracted from young leaves using the CTAB method [52]. The 55K SNP array was performed by China Golden Marker Co. (Beijing, China). The 660K SNP array genotyping data of KL [36] were also used in marker analysis.

2.5. Physical mapping

Markers that were heterozygous in WJN2890, KL, and WJN2896 were discarded. To generate a physical map with better resolution using the recombinants, two approaches were used for further marker filtering. Each approach generated an independent physical map. The first approach was to select polymorphic markers between chromosomes 2B and 2S^v. Homologous markers located on group 2 chromosomes and polymorphic markers between KL and the two substitution lines (WJN2890 and WJN2896) were assumed to be polymorphic markers between chromosomes 2B and 2S^v. The second approach was to select 2B markers of KL. Markers missing in WJN2890 and present in KL were assumed to be specific to the 2B chromosome of KL.

After filtering, all conserved markers were aligned against the CS assembly v2.1 [53] by blastn-2.9.0 (https://www.ncbi.nlm.nih.gov/books/NBK279680). Markers were ordered by their physical positions. For the map generated by the first approach, the genotyping data of recombinants were assigned to the S^v allele when identical to the WJN2890 and WJN2896 genotypes, or to the 2B allele when identical with the KL genotype, representing the B genome. For the map generated by the second approach, the missing genotyping data (NA) of recombinants were assigned to the S^v allele, representing the S^v genome, and the genotype data which were identical to the KL genotype were assigned to the 2B allele, representing the B genome.

2.6. Temperature treatment and Mg^+ supplementation

Five KL haploids were grown in a growth chamber as listed above, and watered with Hoagland solution, which was composed of 1 mmol L^{-1} KNO_3, 0.5 mmol L^{-1} $CaCl_2$, 0.5 mol L^{-1} NH_4Cl, 0.15 mmol L^{-1} $MgSO_4$, 0.001 mmol L^{-1} KH_2PO_4, 100 mmol L^{-1} FE-EDTA, and 2 μmol L^{-1} $FeCl_3$.

For temperature treatment, one of the haploids judged to be about one day before meiosis was transferred to a growth chamber set at 28 °C. After 24 h, meiotic chromosome pairing of three tillers of that haploid was scored in PMCs. For the Mg^{2+} treatment, one haploid in the stem elongation stage was irrigated with a modified Hoagland solution in which the Mg^{2+} concentration was increased to 2 mmol L^{-1}. The rest of the haploids were grown under standard conditions (constant temperature of 23 °C, 16 h/8 h day/night regime, and watering with the standard Hoagland solution) and used as controls.

2.7. Statistical analysis

The Student's *t*-test and chi-square (χ^2) test were used in this study. The Student's *t*-test was used for comparing the paired arms frequency of KL and CS haploid, as well as for comparing the paired arms frequency of KL haploid under 28 °C and 23 °C condition. The χ^2 test was used to compare the number of rod bivalents of KL

haploid under 2 mmol L^{-1} and 0.15 mmol L^{-1} Mg^{2+} irrigation. The R3.6.1 software (https://cran.rstudio.com/) was used to carry out statistical analysis.

3. Results

3.1. Pairing patterns in KL haploids

Meiotic pairing in the PMCs of three KL haploids and two CS haploids were scored in MI (Fig. 1). The mean numbers of paired chromosome arms per PMC in KL and CS haploids were 3.15 (n = 189) and 0.825 (n = 100), respectively, with most pairing in rod bivalents. The difference in the paired arms frequency between KL and CS was significant (t-test, P = 0.0028). Additionally, the mean number of rod bivalents in KL was significantly higher than that in CS (t-test, P = 0.0006). Using *in situ* hybridisation with the A and D genome probes, genomic identity of paired arms was scored in 14 PMCs in a KL haploid. Among 27 paired arms, 20 were A–D associations, six were B–D, and one was A–B. There was a very clear preference for the A–D pairing.

With high temperature treatment (28 °C) applied to one KL haploid for 24 h before meiosis, the mean number of paired arms in anthers from three different tillers tested was 2.14 (n = 42), 1.98 (n = 41), and 2.14 (n = 21), respectively (mean, 2.08; Fig. 1C). Pairing was significantly reduced by elevated temperature (t-test, P = 0.0079). The rod bivalent frequency was also significantly reduced (23 °C: 28 °C = 3.15:2.09, t-test, P = 0.0072), and the univalent frequency was significantly higher (23 °C: 28 °C = 15.05:16.92, t-test, P = 0.0088).

With high Mg^{2+} concentration (2 mmol L^{-1}) applied to one KL haploid starting at the stem elongation stage, the average number of paired arms among 55 PMCs was 3.64, whereas that of the three haploids grown in the standard Hoagland solution was 3.15 (Mg^{2+} concentration, 0.15 mmol L^{-1}). This difference was not statistically significant, which indicated that the Mg^{2+} supplementation did not affect homoeologous pairing frequency in KL. However, the crossover positions appeared to be more interstitial than in the control. In the control, essentially all bivalents were in end-to-end associations (Fig. 1B) with 19 bivalents out of 245 showing an interstitial site of a chiasmata. The proportion of rod bivalents with interstitial crossovers in the Mg^{2+}-irrigated haploid was significantly higher (44/197, χ^2 = 18.99 > 0.01) (Fig. 1D).

3.2. Homoeologous recombination in the KL background

The crossing scheme used to generate populations for homoeologous recombinant screening is illustrated in Fig. 2. Of the 20 screened plants, 13 had chromosome $2S^v$, as indicated by the $2S^v$ specific SSR marker *gwm539*. These 13 BC_1F_1 plants generated 2522 BC_1F_2 progeny, whereas eight BC_1F_2 plants that were double monosomic for $2S^v$ and 2B (as indicated by KASP markers) produced 722 BC_1F_3 progeny. The 3244 progeny (2522 BC_1F_2 and 722 BC_1F_3) were screened for recombinants of $2S^v$ with 2B.

A total of 23,728,849 subreads were obtained with an average read length of 1287 bp. These were polished using iso-seq3, generating 14,197 PCSs, with a mean length of 1361 bp. A total of 4357 PCSs were aligned to the group 2 (2A, 2B, and 2D) assemblies of CS. With four possible types of primary recombinants, the PCSs which were mapped to the distal parts of an arm (i.e., distally and proximally) were used to identify polymorphic SNPs between $2S^v$-2B. In total, seven KASP markers were developed and located at 10, 152.1, 161.4, 224.6, 442.7, 790.7, and 799 Mb of the 2B chromosome assembly (Table S1).

The $2S^v$-2B population was genotyped by at least four pairs of $2S^v$-2B-specific KASP markers (two pairs for each pair of arms) (Table S2). Among 3244 screened progenies, 98 appeared to contain a recombinant chromosome, of which 28 appeared to be recombined in the short arms and 69 recombined in the long arms. One individual appeared to have recombined in both arms. Among non-recombinants, 220 progeny appeared to carry misdivision products, such as telocentric and centric translocations. There were 127 plants for which no genotype could be reliably established. There were 382 progeny plants of addition lines, as indicated by cytology or transmission rate. Taken together, the recombination

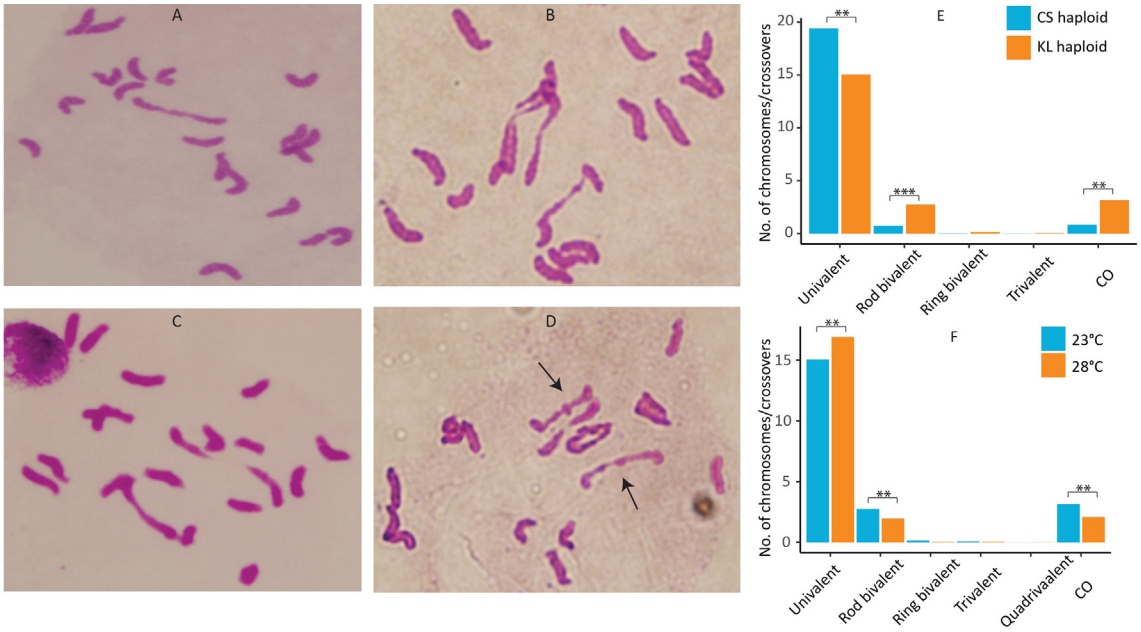

Fig. 1. Chromosome pairing in the PMCs of CS and KL haploids under normal conditions (A, B, and E) and of KL haploids under high temperature (C, F) and supplemented with 2 mmol L^{-1} Mg^{2+} ions (D). (A) CS haploid; (B) KL haploid; (C) KL haploid at 28 °C; (D) KL haploid with 2 mmol L^{-1} Mg^{2+} applied. (E) Chromosome pairing frequencies in KL (yellow) and CS haploids (blue) under normal conditions. **, P < 0.01; ***, P < 0.001. (F) Chromosome pairing of the KL haploid in normal (23 °C, blue) and elevated (28 °C, yellow) temperatures. Arrows show the proximal crossovers sites.

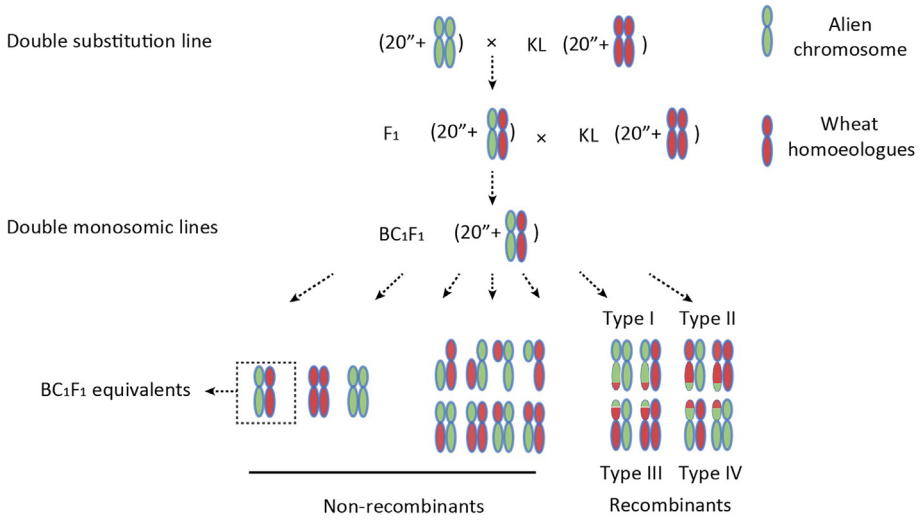

Fig. 2. Production of recombinants in the KL background. Green, alien chromosomes or arms. Red, wheat chromosomes or arms. The double substitution lines, which may also be translocation lines, were pollinated by KL. The F_1 hybrids were backcrossed to KL as female. Double monosomic lines were selected by molecular markers or by cytology in BC_1F_1 to produce recombinant screening populations. Double monosomic lines of BC_1F_2 were identified by progeny screening in BC_1F_3.

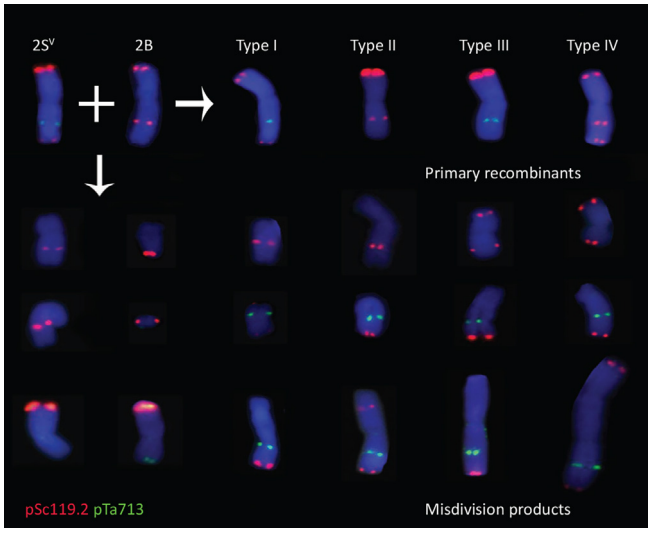

Fig. 3. FISH karyotyping of 2S^v and 2B chromosomes, the four primary types of recombinants, and misdivision products. Red, pSc119.2; green, pTa-713. Top row, left to right, chromosomes 2S^v and 2B, and four types of their recombinants. Rows 2–4, misdivision products of 2S^v and 2B.

rate for 2S^v-2B was 3.62% (99 crossover points among 2735 successfully screened plants). The recombination rates of the short arms and long arms were 1.02% and 2.52%, respectively.

The KASP marker-identified recombinant chromosomes were verified and characterised by direct cytological observation and/or genotyped using the 55K SNP array. Out of 98 putative recombinants, 70 were examined by two FISH probes, pSc119.2 and pTa-713. Karyotyping indicated that 39 of those were indeed recombined chromosomes and the remaining 31 were not; they were aberrant chromosomes, such as deletions (Fig. 3). GISH was performed on 2S^v-2B using the genomic DNA of *Ae. longissima* as a probe. Few chromosomes hybridised clearly to show recombined segments of 2S^v (Fig. S2).

To further verify the 2S^v-2B putative recombinants, out of 67 KASP marker-identified recombinants, which excluded 31 that were rejected by FISH, 60 were genotyped by the 55K SNP array. In total, 2598 SNPs physically located on chromosome 2B (CS assembly v2.1) were used. Two marker filtering methods (see details in Material and methods section) were applied. The first approach produced 157 polymorphic markers between 2S^v and 2B. The second approach produced a higher density of SNPs (1260) and was used for further analysis. In genotyping of the putative recombinants, missing data were assumed to indicate the presence of 2S^v; homozygous markers were assumed to be the 2B genotype. The phase change of the 2S^v and 2B genotypes was detected by ordering all 2B specific makers (1260 SNPs) by their physical locations (Table S3). Among 60 genotyped plants, 44 had clearly detectable crossover points in SNP genotyping, whereas 16 plants did not show any evidence of a phase change. The 44 plants included 37 recombinants previously confirmed by FISH and seven for which FISH did not detect recombination (Table S4). The 16 plants with no phase change detected by SNPs mostly failed to perform FISH karyotyping except one plant which was indicated as a recombinant by FISH (Table S4).

Taken together, for the 2S^v-2B population, three approaches (KASP markers, FISH, and the SNP array) detected 46 *bona fide* recombinants, including 37 detected by KASP markers and FISH, seven detected only by the SNP array, and two detected by FISH karyotyping (Table S4). Therefore, the overall recombination rate of 2S^v-2B in the KL system was 1.68% (46/2735).

The 44 recombinants confirmed by SNP genotyping were used to characterise the crossover patterns between 2S^v and 2B. With the 2S^v assembly not available, only the 2B chromosome assembly of CS v2.1 was used as a reference. The 44 crossovers were allocated into 26 bins, with four bins on the short arm and 22 on the long arm (Fig. 4; Table S5). The general pattern of recombination for the entire chromosome was typical for wheat, with concentration in the distal regions of chromosome arms. More than 92% (38/41) of crossovers occurred in less than 30% of the chromosome length. There was an exception in the long arm, where one crossover appeared to have occurred in the centromeric region; this may be a misdiagnosed centric breakage–fusion.

In the BC_1F_1 2S^v-2D population, six plants out of 30 showed the presence of 2S^v, as indicated by the 2S^v-specific SSR marker *gwm539*. These six plants generated 630 BC_1F_2 progeny. Three of the BC_1F_2 plants which were double monosomic 2S^v and 2D, as indicated by KASP markers, produced 162 BC_1F_3 progeny. These

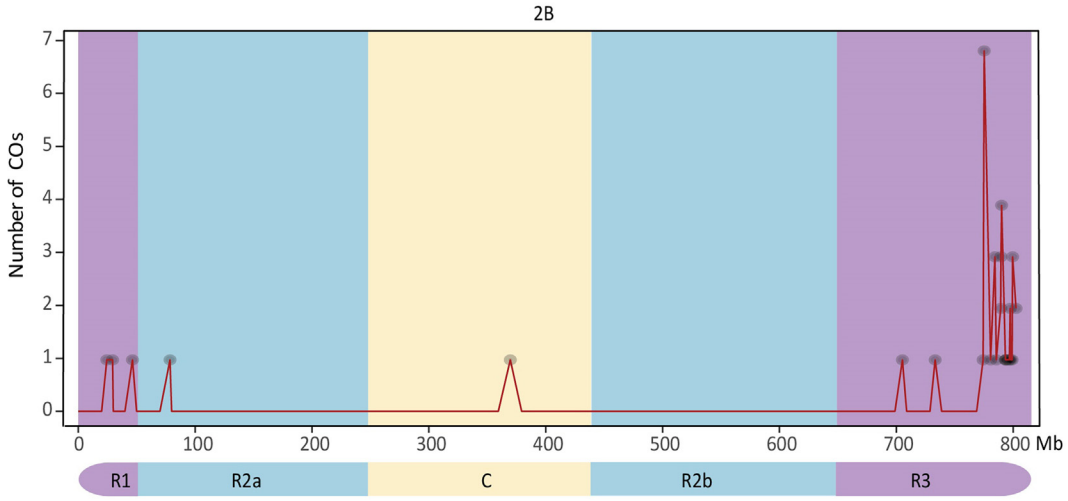

Fig. 4. Physical distribution of crossover points among $2S^v$ and 2B recombinants induced by KL, projected on the 2B chromosome DNA assembly. Black dots represent the crossovers points. The five main regions of wheat chromosomes are represented: R1 and R3 are distal regions (purple), R2a and R2b are pericentromeric regions (blue), C is the centromeric region (yellow).

792 plants (630 BC_1F_2 and 162 BC_1F_3) comprised the screening population. Four pairs of $2S^v$-2D KASP markers were developed and were located at 0.3, 160.6, 373, and 651.6 Mb of the 2D assembly, respectively (Table S1). These findings indicated the presence of a recombined chromosome in seven out of 792 screened progeny (Table S2). Among those, five were recombined in the short arms and two in the long arms. Among the remaining progeny, 35 appeared to carry misdivision products and 35 plants did not produce reliable data. Based on the transmission rates of $2S^v$ and 2D, 160 plants were determined to be progeny of addition lines. Therefore, the actual size of the population was 597. The overall recombination rate was 1.17% (7/597), with 0.84% in S and 0.33% in L. Three of the seven KASP marker-identified recombinants were analysed by *in situ* hybridisation using the A and D genome DNAs as probes. Only one clearly showed translocation (Fig. S2C).

In the 2RL-2BL population, 14 plants with a chromosome constitution of 20″+T2BS.2RL+2B (translocation heterozygotes) were identified by C-banding. These plants produced numerous seeds, of which 1000 were screened by C-banding for 2RL-2BL recombinants. Screening relied on differences in the banding patterns between 2RL and 2BL based on two points; specifically, the presence of a readily identifiable telomeric C-band on 2RL and an absence of such a band on 2BL as one point, and the centromere as the other point. No recombinants of 2RL and 2BL were identified. Meiotic pairing of 2RL with 2BL was scored by GISH using rye total genomic DNA as a probe and the wheat genomic DNA as a block. Among 1246 scored PMCs, very few cases were observed that had convincing MI associations of 2RL with 2BL (Fig. 5B), even when 2RL appeared to be associated with wheat chromatin (Fig. 5D).

A QTL, *QPh.sicau-3A* located in the 696–725 Mb genomic interval on 3AL of KL, was previously identified as contributing to the increased level of homoeologous pairing. To verify its contribution to homoeologous pairing, we genotyped 10 BC_1F_1 plants of the $2S^v$-2B population by the KASP marker (AX-109895327) linked to *QPh.sicau-3A* [36]. Among those 10 plants, four were homozygous for the KL allele and six were heterozygous. The average recombination rate among the progeny of the four KL homozygotes was significantly higher than that among the progeny of heterozygotes (2.67% vs. 0.91%, respectively). This demonstrates that *QPh.sicau-3A* significantly contributes to homoeologous recombination with a dosage-dependent manner.

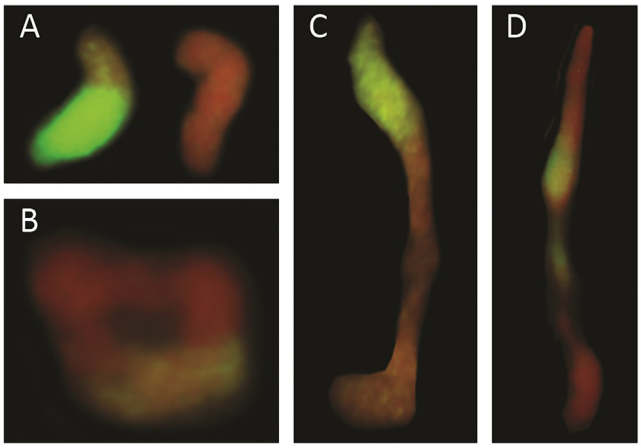

Fig. 5. Types of chromosome associations of T2BS.2RL with 2B in MI of meiosis. (A) Univalent; (B) A ring bivalent; 2RL-2BL paired; (C) Rod bivalent paired 2BS-2BS; (D) Rod bivalent, 2RL is paired but with a chromosome much smaller than 2B. Green signal, 2RL; Red signal, 2B, 2BS.

4. Discussion

4.1. The KL system induces homoeologous recombination between closely related genomes at the haploid and hexaploid levels

Meiotic pairing and recombination of homoeologues in haploids or wide hybrids of hexaploid wheat is prevented by the *Ph* system. The experiments presented here clearly demonstrate that the KL system from KL wheat is capable of overriding the controls set by the *Ph* system. It is not clear if KL operates as a suppressor of the *Ph* system, or in some fashion promotes homoeologous pairing, but homoeologous recombination does take place. It occurs in a specific and interesting fashion: in haploids, with only wheat genomes present, there was a clear preference for A–D associations, and less frequent for A–B and B–D. This pattern parallels that observed in a hybrid of *Ae. variabilis* with wheat in the presence of *Ph1* [54]. It reflects the levels of genome affinity in wheat [40] and the pattern of homoeologous pairing with the absence of *Ph1* [41]. Genomes A and D are the most closely related, and paired

more than twice as frequently as A–B and B–D combined in wide hybrids of hexaploid wheat [41].

The KL system was further tested on three pairs of more distantly related homoeologous pairs with different genomic affinities (here referred to as the time from divergence): $2S^v$ from *Ae. variabilis* against 2B and 2D of wheat, and 2RL of rye against 2BL of wheat. Chromosome $2S^v$ is a progenitor of 2B; therefore, the two are closely related, and it is more distantly related to 2D. Rye and wheat, diverged around 8 million years ago [55], are members of different genera, and are considered substantially diverged. However, in the entire rye genome, 2RL shows the highest pairing affinity to wheat [41] which is the reason that it was chosen for this study. With these three pairs of chromosomes tested, the KL system induced a decent recombination level (around 2%) between $2S^v$ and 2B, far less recombination between $2S^v$ and 2D (0.17%), and no recombination events were detected between 2RL and 2BL among 1000 progeny (ca. 2000 progeny chromosomes), even though a few undisputed pairing events were observed among 1246 PMCs.

4.2. Comparison of the characteristics of KL and Ph1 systems in inducing homoeologous recombination

It has been known for quite a long time that, in the absence of *Ph1*, some level of homoeologous pairing and consequent recombination in wheat does take place [56]. Naturally, comparison of the two systems, *Ph1* and KL, is inevitable. At the haploid level, the mean number of chiasmata in KL (3.15) is about half that of nullisomic 5B of CS (6.9) [6] or *ph1b* (8.55; this number may also include homologous pairing of rearranged genomes) [57]. Clearly, pairing frequency of wheat homoeologues induced by the KL system is about half that compared with the absence of *Ph1*.

Homoeologous recombination rates induced by the two systems appeared to follow the genome affinity, with more closely related genomes showing higher rates of recombination. The two systems, however, performed differently with different homoeologous pairs. In the KL system, the level of recombination of the closely related chromosomes $2S^v$-2B was reasonably high, 10 times higher than that of a more distantly related pair, $2S^v$-2D. Apart from a single exception, there was no pairing, and no recombination was detected between two distantly related chromosome arms, 2BL-2RL. In *ph1b*, these two arms showed the highest pairing affinity [41]. The 2BL-2RL recombination rate in a sample of the same population that was previously analysed was 14.65% [58]. This indicates that, although the KL system relaxes the *Ph1*-imposed criteria for crossing over, the barrier is still present, and applied selectively. It appears to be related more to the general level of species (genome) divergence than to the actual ability of two arms to pair and recombine.

There may be two plausible explanations for this difference. First, the general mechanism of homology recognition in the KL system and *Ph1* is the same, but the KL system is less effective; therefore, much larger populations would be required to detect recombinants. Second, the mechanisms of homology recognition in the KL system and *Ph1* are different; for example, there is a difference between the levels of the DNA sequence divergence in the region targeted for crossing over versus the general DNA landscape of the targeted chromosome arms. No recombination was detected in 2BL-2RL in KL even though the two arms frequently recombine in *ph1b* (14.65%) [58] and more frequently than 2S-2B in *ph1b* (11.3%) [59]; this indicates that the general DNA landscape/chromatin conformation of the homoeologues may play different roles in the KL and *Ph1* systems.

Only in the 2RL-2BL combination screening there was a change of phase along the entire lengths of the arms: telomeric C-band on one end and the centromere (as in the centric translocation) on the other. A change in phase would indicate a crossover event anywhere along the entire arm. No double exchanges were anticipated. However, screening for recombinants in $2S^v$-2B and $2S^v$-2D was done by molecular markers. None of the selected markers were exactly at the distal ends of chromosomes or arms; therefore, there is a chance that very distal exchanges might have remained undetected, and most observed pairing events were end-to-end associations. Consequently, the actual recombination rates in $2S^v$-2B and $2S^v$-2D populations may have been underestimated.

There were some KASP-identified exchanges that could not be confirmed by other means. Confirmation by GISH was seriously limited by a close affinity of the genomes; thus, there was low resolution in painting with genome-specific probes [60]. It is plausible that short translocated segments escaped detection by GISH. Additionally, in induced homoeologous recombination, the alien chromosome may pair with other wheat chromosome arms than the targeted ones, and these would likely be undetected by the chromosome arm-specific DNA markers.

The recombination rates were significantly higher in homozygotes for *QPh.sicau-3A*, the main locus associated with the KL effect, than in heterozygotes. This is another similarity with the *Ph1* system. Although *Ph1* is generally considered dominant (no homoeologous pairing in *Ph1ph1b*), it only acts as dominant with sufficiently diverged chromosomes. *Ph1* effectively controls the crossover rates of homologous chromosome segments, drastically reducing them for sufficiently diverged pairs; in *ph1b*, these rates are restored to the levels of identical pairs [8]. *Ph1ph1b* heterozygotes showed crossover rates midway between *Ph1Ph1* and *ph1bph1b*.

The effect of the KL system appears to be plastic and modulated by external factors. Under elevated temperature, the level of homoeologous pairing decreased relative to the control, which was very similar to CS and CS-rye F_1 hybrids in the absence of *Ph1* [61]. Studies have shown that high temperature may cause a proximal shift of recombination in barley [62] and wheat [63]. This was not observed in this study (the difference was not statistically significant) but it was observed with a higher concentration of magnesium ions (2 mmol L^{-1}). Chiasmata positions in a KL haploid grown at higher Mg^{2+} levels were visibly more proximal, and the frequency of homoeologous pairing remained the same. In contrast, the *Tazip4-B2* (candidate of *Ph1*) mutant had a decreased level of homoeologous pairing under this treatment and no crossover shifts were reported [15]. In the study of yeast, magnesium lowers the specificity of MLH proteins binding to Holliday junctions and favours crossover formation [64]. However, its effect on the distribution of crossover requires more studies.

4.3. Potential of the KL system in chromosome engineering of wheat for breeding purposes

As a tool for chromosome engineering in wheat, the efficiency of the KL system is much lower than that of *ph1b*. Frequencies of homoeologous crossovers were either significantly lower, or altogether absent, relative to *ph1b*. However, this may also be viewed as an advantage. In *ph1b*, the frequencies of crossovers among wheat homoeologues are high, and in most reported cases much higher (at times by an order of magnitude or higher) than the crossover rate of the targeted alien chromosome. Consequently, *ph1b* lines of wheat quickly accumulate genomic rearrangements [32] that create chaos in the genomes, reduce fertility, and can make wheat strains more sensitive to it than CS useless for any practical use in 3–4 generations of self-pollination.

When such *ph1b* lines are crossed to regular wheat in any chromosome engineering effort, chromosome rearrangements are transferred to hybrids and their progeny, and new rearrangements are created in every generation. For this reason, every chromosome

engineering effort must be followed by several backcrosses to *Ph1* wheat to restore normal genome and chromosome structure, which severely slows the pace of introduction of alien introgressions into practical agriculture. Although the KL system is less effective in promoting homoeologous recombination of closely related chromosomes and is ineffective for more distantly related chromosomes, it does not appear to accumulate chromosome rearrangements as the line appears stable and uniform from generation to generation. Clearly, the chaos is avoided. Larger efforts to generate recombinants will be compensated by much less clean-up, and quicker development of commercially acceptable products.

4.4. New insights into crossover establishment between related chromosomes

Evidence shows that the rate of homoeologous recombination induced by the KL and *Ph1* systems is associated with genome affinity; therefore, homoeologous recombination rate reflects genome affinity. Both B and D genomes of wheat originated from *Aegilops* species. *Aegilops tauschii* is the donor of the D genome [65,66]; the origin of the B genome is less clear. Since the 1950s, it has been hypothesised that the B genome originated from a diploid *Aegilops* species of section Sitopsis. *Aegilops variabilis* (UUS^vS^v), a tetraploid species in the Sitopsis section, originated from a cross of *Ae. umbellulata* (UU) with *Ae. longissima* (S^lS^l). Therefore, its S^v genome originated from *Ae. longissima* (S^lS^l) and it shows higher homology with the B genome. However, recent sequencing of the five species in the section Sitopsis showed that *Ae. longissima* (S^lS^l) is closely related to the D genome in terms of DNA sequence similarity [67,68]. This could be an indication that the S^v genome may also have higher DNA sequence similarity with the D than B genome.

In this study, the recombination rate of $2S^v$-2B was much higher than $2S^v$-2D. However, this may only reflect differences in the definition of homology in cytology and DNA sequencing. It is plausible that, at the chromosome level, DNA sequence similarity is only one criterion involved in the crossover (chiasmata) establishment. The general DNA sequence landscape and structural differences may also play an important role.

5. Conclusions

We report on the KL system promoting homoeologous recombination in hexaploid and haploid wheat. The filter for crossing over in this system appears to be set higher than that in the absence of *Ph1*, and only homoeologues with high affinity were capable of crossing over. Although the recovery rate of recombinants was lower, the system offered one clear benefit: less chaos was generated by recombination among wheat homoeologues. Consequently, fewer backcrosses would be required to clean the genetic background in a practical breeding exercise. Gene features and the mechanism by which this system induces homoeologous recombination require additional research.

Data availability statement

The PacBio IsoSeq sequencing data of $2S^v$ (2B) substitution line can be accessed from the following link: https://bigd.big.ac.cn/gsa/browse/CRA009466.

CRediT authorship contribution statement

Chaolan Fan: Investigation, Visualization, Writing – original draft. **Jiangtao Luo:** Investigation. **Jiaojiao Sun:** Investigation. **Hong Chen:** Investigation. **Liqiong Li:** Investigation. **Lanyue Zhang:** Investigation. **Xue Chen:** Formal analysis. **Yazhou Li:** Investigation. **Shunzong Ning:** Project administration, Writing – review & editing. **Zhongwei Yuan:** Project administration, Writing – review & editing. **Bo Jiang:** Project administration, Writing – review & editing. **Lianquan Zhang:** Project administration, Writing – review & editing. **Xuejiao Chen:** Project administration, Writing – review & editing. **Adam J. Lukaszewski:** Conceptualization, Supervision, Funding acquisition, Writing – review & editing. **Dengcai Liu:** Conceptualization, Supervision, Funding acquisition, Writing – review & editing. **Ming Hao:** Conceptualization, Supervision, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data for this article can be found online at https://doi.org/10.1016/j.cj.2023.01.003.

References

[1] J. Masterson, Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms, Science 264 (1994) 421–424.
[2] K.W. Hilu, Polyploidy and the evolution of domesticated plants, Am. J. Bot. 80 (1993) 1494–1499.
[3] G.G. Doyle, The allotetraploidization of maize: 4. cytological and genetic evidence indicative of substantial progress, Theor. Appl. Genet. 71 (1986) 585–594.
[4] E. Jenczewski, K. Alix, From diploids to alloploids: the emergence of efficient pairing control genes in plants, Crit. Rev. Plant Sci. 23 (2004) 21–45.
[5] E.R. Sears, M. Okamoto, Intergenomic chromosome relationships in hexaploid wheat, Proc. Int. Congress Genet. 2 (1958) 258–259.
[6] R. Riley, V. Chapman, Genetic control of the cytologically diploid behaviour of hexaploid wheat, Nature 13 (1958) 713–715.
[7] J. Dvorak, P. McGuire, Nonstructural chromosome differentiation among wheat cultivars with special reference to differentiation of chromosomes in related species, Genetics 97 (1981) 391–414.
[8] C. Fan, M. Hao, Z. Jia, C. Neri, X. Chen, W. Chen, D. Liu, A.J. Lukaszewski, Some characteristics of crossing over in induced recombination between chromosomes of wheat and rye, Plant J. 105 (2021) 1665–1676.
[9] S. Griffiths, R. Sharp, T.N. Foote, I. Bertin, M. Wanous, S. Reader, I. Colas, G. Moore, Molecular characterization of *Ph1* as a major chromosome pairing locus in polyploid wheat, Nature 439 (2006) 749–752.
[10] N. Al-Kaff, E. Knight, I. Bertin, T. Foote, N. Hart, S. Griffiths, G. Moore, Detailed dissection of the chromosomal region containing the *Ph1* locus in wheat *Triticum aestivum*: with deletion mutants and expression profiling, Ann. Bot. 101 (2008) 863–872.
[11] F.K. Yousafzai, N. Al-Kaff, G. Moore, Structural and functional relationship between the *Ph1* locus protein 5B2 in wheat and *CDK2* in mammals, Funct. Integr. Genomics 10 (2010) 157–166.
[12] R. Bhullar, R. Nagarajan, H. Bennypaul, G.K. Sidhu, G. Sidhu, S. Rustigi, V.D. Wettstein, K.S. Gill, Silencing of a metaphase I-specific gene results in a phenotype similar to that of the Pairing homoeologous (*Ph1*) gene mutations, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 14187–14192.
[13] K.S. Rawale, M.A. Khan, K.S. Gill, The novel function of the *Ph1* gene to differentiate homologs from homoeologs evolved in *Triticum turgidum* ssp. *dicoccoides* via a dramatic meiosis-specific increase in the expression of the 5B copy of the *C-Ph1* gene, Chromosoma 128 (2019) 561–570.
[14] M.D. Rey, A.C. Martín, J. Higgins, D. Swarbreck, C. Uauy, P. Shaw, G. Moore, Exploiting the *ZIP4* homologue within the wheat *Ph1* locus has identified two

lines exhibiting homoeologous crossover in wheat-wild relative hybrids, Mol. Breed. 37 (2017) 95.
[15] M.D. Rey, A.C. Martín, M. Smedley, S. Hayta, W. Harwood, P. Shaw, G. Moore, Magnesium increases homoeologous crossover frequency during meiosis in *ZIP4* (*Ph1* gene) mutant wheat-wild relative hybrids, Front. Plant Sci. 20 (2018) 509.
[16] S. Ortega, I. Prieto, J. Odajima, A. Martin, P. Dubus, R. Sotillo, J.L. Barbero, M. Malumbres, M. Barbacid, Cyclin-dependent kinase 2 is essential for meiosis but not for mitotic cell division in mice, Nat. Genet. 35 (2003) 25–31.
[17] A. Viera, J.S. Rufas, I. Martínez, J.L. Barbero, S. Ortega, J.A. Suja, *CDK2* is required for proper homologous pairing, recombination and sex-body formation during male mouse meiosis, J. Cell Sci. 122 (2009) 2149–2159.
[18] C. Lambing, F.C. Franklin, C.J. Wang, Understanding and manipulating meiotic recombination in plants, Plant Physiol. 173 (2017) 1530–1542.
[19] T. Mello-Sampayo, Genetic regulation of meiotic chromosome pairing by chromosome 3D of *Triticum aestivum*, Nat. New Biol. 230 (1971) 22–23.
[20] C.J. Driscoll, Genetic suppression of homoeologous chromosome pairing in hexaploid wheat, Can. J. Genet. Cytol. 14 (1972) 39–42.
[21] T. Sutton, R. Whitford, U. Baumann, C. Dong, J.A. Able, P. Langridge, The *Ph2* pairing homoeologous locus of wheat (*Triticum aestivum*): identification of candidate meiotic genes using a comparative genetics approach, Plant J. 36 (2003) 443–456.
[22] H. Serra, R. Svačina, U. Baumann, R. Whitford, T. Sutton, J. Bartoš, P. Sourdille, *Ph2* encodes the mismatch repair protein *MSH7-3D* that inhibits wheat homoeologous recombination, Nat. Commun. 12 (2021) 803.
[23] S.M. Tam, J.B. Hays, R.T. Chetelat, Effects of suppressing the DNA mismatch repair system on homeologous recombination in tomato, Theor. Appl. Genet. 123 (2011) 1445–1458.
[24] L. Li, M. Jean, F. Belzile, The impact of sequence divergence and DNA mismatch repair on homeologous recombination in *Arabidopsis*, Plant J. 45 (2006) 908–916.
[25] C.J. Driscoll, Minor genes affecting homoeologous pairing in hybrids between wheat and related genera, Genetics 74 (1973).
[26] E.R. Sears, The Aneuploids of Common Wheat, Research Bulletin No. 572, Agricultural Experiment Station, University of Missouri, Columbia, MO, USA, 1954.
[27] M. Feldman, The effect of chromosomes 5B, 5D, and 5A on chromosomal pairing in *Triticum aestivum*, Proc. Natl. Acad. Sci. U. S. A. 55 (1966) 1447–1453.
[28] R. Riley, V. Chapman, Effect of 5BS in suppressing the expression of altered dosage of 5BL on meiotic chromosome pairing in *Triticum aestivum*, Nature 216 (1967) 60–62.
[29] Y. Gyawali, W. Zhang, S. Chao, S. Xu, X. Cai, Delimitation of wheat *ph1b* deletion and development of *ph1b*-specific DNA markers, Theor. Appl. Genet. 132 (2019) 195–204.
[30] M. Hao, L. Zhang, S. Ning, L. Huang, Z. Yuan, B. Wu, Z. Yan, S. Dai, B. Jiang, Y. Zheng, D. Liu, The resurgence of introgression breeding, as exemplified in wheat improvement, Front. Plant Sci. 11 (2020) 252.
[31] E. Sánchez-Morán, E. Benavente, J. Orellana, Analysis of karyotypic stability of homoeologous-pairing (*ph*) mutants in allopolyploid wheats, Chromosoma 110 (2001) 371–377.
[32] A.C. Martín, P. Borrill, J. Higgins, A. Alabdullah, R.H. Ramírez-González, D. Swarbreck, C. Uauy, P. Shaw, G. Moore, Genome-wide transcription during early wheat meiosis is independent of synapsis, ploidy level, and the *Ph1* locus, Front. Plant Sci. 9 (2018) 1791.
[33] M.C. Luo, Z.L. Yang, C. Yen, J.L. Yang, The cytogenetic investigation on F_1 hybrid of Chinese wheat landrace, in: Z.L. Ren, J.H. Peng (Eds.), Exploration of Crop Breeding, Science and Technology Press of Sichuan, Sichuan, China, 1992, pp. 169–176.
[34] D.C. Liu, C. Yen, J.L. Yang, Evaluation of crosses of bread wheat cv. Kaixianluohanmai with alien species, Acta. Agron. Sin. 25 (1999) 777–781.
[35] Z.G. Xiang, D.C. Liu, Y.L. Zheng, L.Q. Zhang, Z.H. Yan, The effect of *phKL* gene on homoeologous pairing of wheat-alien hybrids is situated between gene mutants of *Ph1* and *Ph2*, Hereditas 27 (2005) 935–940.
[36] C. Fan, J. Luo, S. Zhang, M. Liu, Q. Li, Y. Li, L. Huang, X. Chen, S. Ning, Z. Yuan, L. Zhang, J. Wang, Y. Zheng, D. Liu, M. Hao, Genetic mapping of a major QTL promoting homoeologous chromosome pairing in a wheat landrace, Theor. Appl. Genet. 132 (2019) 2155–2166.
[37] R.A. Fischer, R. Maurer, Crop temperature modification and yield potential in a dwarf spring wheat, Crop Sci. 16 (1976) 855–859.
[38] I. Barclay, High frequencies of haploid production in wheat (*Triticum aestivum*) by chromosome elimination, Nature 256 (1975) 410–411.
[39] L. Zhao, S. Ning, J. Yu, M. Hao, L. Zhang, Z. Yuan, Y. Zheng, D. Liu, Cytological identification of an *Aegilops variabilis* chromosome carrying stripe rust resistance in wheat, Breed. Sci. 16011 (2016).
[40] L.C. Alonso, G. Kimber, The analysis of meiosis in hybrids. II. Triploid hybrids, Can. J. Genet. Cytol. 23 (1981) 221–234.
[41] T. Naranjo, P, Fernández-Rueda, Pairing and recombination between individual chromosomes of wheat and rye in hybrids carrying the *phlb* mutation, Theor. Appl. Genet. 93 (1996) 242–248.
[42] P. Sourdille, S. Singh, T. Cadalen, G.L BrownGuedira, G. Gay, L.L. Qi, B.S. Gill, P. Dufour, A. Murigneux, M. Bernard, Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.), Funct. Integr. Genomics 4 (2004) 12–25.
[43] J.C. Zadoks, T.T. Chang, C.F. Konzak, A decimal code for the growth stages of cereals, Weed. Res. 14 (1974) 415–421.
[44] C. Chen, H. Chen, Y. Zhang, H.R. Thomas, M.H. Frank, Y. He, R. Xia, TBtools: an integrative toolkit developed for interactive analyses of big biological data, Mol. Plant. 13 (2020) 1194–1202.
[45] P. Sarkar, G.L. Stebbins, Morphological evidence concerning the origin of the B genome in wheat, Am. J. Bot. 43 (1956) 297–304.
[46] R. Riley, J. Unrau, V, Chapman, Evidence on the origin of the B genome of wheat, J. Hered. 49 (1958) 91–98.
[47] J. Salse, V. Chagué, S. Bolot, G. Magdelenat, C. Huneau, C. Pont, H. Belcram, A. Couloux, S. Gardais, A. Evrard, B. Segurens, New insights into the origin of the B genome of hexaploid wheat: evolutionary relationships at the SPA genomic region with the S genome of the diploid relative *Aegilops speltoides*, BMC Genomics 9 (2008) 555.
[48] Y. Miki, K. Yoshida, N. Mizuno, S. Nasuda, K. Sato, S. Takumi, Origin of wheat B-genome chromosomes inferred from RNA sequencing analysis of leaf transcripts from section *Sitopsis* species of *Aegilops*, DNA Res. 26 (2019) 171–182.
[49] R. Appels, K. Eversole, N. Stein, C. Feuillet, B. Keller, J. Rogers, C.J. Pozniak, F. Choulet, A. Distelfeld, J. Poland, G. Ronen, Shifting the limits in wheat research and breeding using a fully annotated reference genome, Science 361 (2018) eaar7191.
[50] S. Komuro, R. Endo, K. Shikata, A. Kato, Genomic and chromosomal distribution patterns of various repeated DNA sequences in wheat revealed by a fluorescence *in situ* hybridization procedure, Genome 56 (2013) 131–137.
[51] Z. Tang, M. Li, L. Chen, Y.Y. Wang, Z.L. Ren, S.L. Fu, New types of wheat chromosomal structural variations in derivatives of wheat-rye hybrids, PLoS ONE 9 (2014).
[52] J.J. Doyle, J.L. Doyle, A rapid DNA isolation procedure for small quantities of fresh leaf tissue, Photochem. Bull. 19 (1987) 11–15.
[53] T. Zhu, L. Wang, H. Rimbert, J.C. Rodriguez, K.R. Deal, R. de Oliveira, F. Choulet, G. Keeble-Gagnère, J. Tibbits, J. Rogers, K. Eversole, Optical maps refine the bread wheat *Triticum aestivum* cv. Chinese Spring genome assembly, Plant J. 107 (2021) 303–314.
[54] B. Fernandez-Calvin, J. Orellana, Metaphase I bound arms frequency and genome analysis in wheat-*Aegilops* hybrids. 1. *Ae. variabilis*-wheat and *Ae. kotschyi*-wheat hybrids with low and high homoeologous pairing, Theor. Appl. Genet. 83 (1991) 264–272.
[55] C.P. Middleton, N. Senerchia, N. Stein, E.D. Akhunov, B. Keller, T. Wicker, B. Kilian, Sequencing of chloroplast genomes from wheat, barley, rye and their relatives provides a detailed insight into the evolution of the *Triticeae* tribe, PLoS ONE 9 (2014).
[56] T. Naranjo, E. Benavente, The mode and regulation of chromosome pairing in wheat-alien hybrids (*Ph* genes, an updated view), in: M. Molnár-Láng, C. Ceoloni, J. Doležel (Eds.), Alien introgression in Wheat, Springer, Cham, Switzerland, 2015, pp. 133–162.
[57] P.P. Jauhar, O. Riera-Lizarazu, W.G. Dewey, B.S. Gill, C.F. Crane, J.H. Bennett, Chromosome pairing relationships among the A, B and D genomes of bread wheat, Theor. Appl. Genet. 82 (1991) 441–449.
[58] A.J. Lukaszewski, K. Rybka, V. Korzun, S.V. Malyshev, B. Lapinski, R. Whitkus, Genetic and physical mapping of homoeologous recombination points involving wheat chromosome 2B and rye chromosome 2R, Genome 47 (2004) 36–45.
[59] W. Zhang, X. Zhu, M. Zhang, S. Chao, S. Xu, X. Cai, Meiotic homoeologous recombination-based mapping of wheat chromosome 2B and its homoeologues in *Aegilops speltoides* and *Thinopyrum elongatum*, Theor. Appl. Genet. 131 (2018) 2381–2395.
[60] A.J. Lukaszewski, B. Lapinski, K. Rybka, Limitations of *in situ* hybridization with total genomic DNA in routine screening for alien introgressions in wheat, Cytogenet. Genome Res. 109 (2005) 373–377.
[61] A.C. Martín, M.D. Rey, P. Shaw, G. Moore, Dual effect of the wheat *Ph1* locus on chromosome synapsis and crossover, Chromosoma 126 (2017) 669–680.
[62] D. Phillips, G. Jenkins, M. Macaulay, C. Nibau, J. Wnetrzak, D. Fallding, I. Colas, H. Oakey, R. Waugh, L. Ramsay, The effect of temperature on the male and female recombination landscape of barley, New Phytol. 208 (2015) 421–429.
[63] A. Coulton, A.J. Burridge, K.J. Edwards, Examining the effects of temperature on recombination in wheat, Front. Plant Sci. 11 (2020) 230.
[64] L. Ranjha, R. Anand, P. Cejka, The *Saccharomyces cerevisiae Mlh1-Mlh3* heterodimer is an endonuclease that preferentially binds to Holliday junctions, J. Biol. Chem. 289 (2014) 5674–5686.
[65] H. Kihara, Discovery of the DD-analyser, one of the ancestors of *Triticum vulgare*, Agric. Hort. 19 (1944) 13–14.
[66] E.S. McFadden, E.R. Sears, The artificial synthesis of *Triticum spelta*, Rec. Genet. Soc. Am. 13 (1944) 26–27.
[67] L.F. Li, Z.B. Zhang, Z.H. Wang, N. Li, Y. Sha, X.F. Wang, N. Ding, Y. Li, J. Zhao, Y. Wu, L. Gong, F. Mafessoni, A.A. Levy, B. Liu, Genome sequences of the five *Sitopsis* species of *Aegilops* and the origin of polyploid wheat B-subgenome, Mol. Plant 15 (2022) 488–503.
[68] R. Avni, T. Lux, A. Minz-Dub, E. Millet, H. Sela, A. Distelfeld, J. Deek, G. Yu, B. Steuernagel, C. Pozniak, J. Ens, Genome sequences of three *Aegilops* species of the section *Sitopsis* reveal phylogenetic relationships and provide resources for wheat improvement, Plant J. 38 (2022) 307–309.