Introgression of the *Aegilops speltoides Su1-Ph1* Suppressor into Wheat

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Meiotic pairing between homoeologous chromosomes in polyploid wheat is inhibited by the *Ph1* locus on the long arm of chromosome 5 in the B genome. *Aegilops speltoides* (genomes SS), the closest relative of the progenitor of the wheat B genome, is polymorphic for genetic suppression of *Ph1*. Using this polymorphism, two major suppressor loci, *Su1-Ph1* and *Su2-Ph1*, have been mapped in *Ae. speltoides*. *Su1-Ph1* is located in the distal, high-recombination region of the long arm of the *Ae. speltoides* chromosome 3S. Its location and tight linkage to marker *Xpsr1205-3S* makes *Su1-Ph1* a suitable target for introgression into wheat. Here, *Xpsr1205-3S* was introgressed into hexaploid bread wheat cv. Chinese Spring (CS) and from there into tetraploid durum wheat cv. Langdon (LDN). Sequential fluorescence *in situ* hybridization and genomic *in situ* hybridization showed that an *Ae. speltoides* segment with *Xpsr1205* replaced the distal end of the long arm of chromosome 3A. In the CS genetic background, the chromosome induced homoeologous chromosome pairing in interspecific hybrids with *Ae. peregrina* but not in progenies from crosses involving alien disomic substitution lines. In the LDN genetic background, the chromosome induced homoeologous chromosome pairing in both interspecific hybrids and progenies from crosses involving alien disomic substitution lines. We conclude that the recombined chromosome harbors *Su1-Ph1* but its expression requires expression of complementary gene that is present in LDN but absent in CS. We suggest that it is unlikely that *Su1-Ph1* and *ZIP4-1*, a paralog of *Ph1* located on wheat chromosomes 3A and 3B and *Ae. tauschii* chromosome 3D, are equivalent. The utility of *Su1-Ph1* for induction of recombination between homoeologous chromosomes in wheat is illustrated.

**Keywords:** homoeologous chromosome pairing, *in situ* hybridization, MAS, *Ph1*, *ZIP4*

**INTRODUCTION**

In most allopolyploid plants, only homologous chromosomes pair in meiosis and only bivalents are present at metaphase I (MI); pairing between homoeologous chromosomes (heterogenetic chromosome pairing) is excluded (Jenczewski and Alix, 2004). The best-known example of genetic exclusion of heterogenetic chromosome pairing is in tetraploid and hexaploid wheat (Okamoto, 1957; Riley and Chapman, 1958; Sears and Okamoto, 1958).

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The chromosome complement of tetraploid wheat (Triticum turgidum L., 2n = 4x = 28) consists of the A genome, which was contributed by T. urartu Thum., and the B genome, which was contributed by an extinct or undiscovered species closely related to Aegilops speltoides Tausch (genomes SS≈BB) (Dvorak and Zhang, 1990; Dvorak et al., 1993). The chromosome complement of hexaploid wheat (T. aestivum L., 2n = 6x = 42) consists of the A and B genomes of tetraploid wheat and the D genome, which was contributed by Ae. tauschii Coss. (Kihara, 1944; McFadden and Sears, 1946; Wang et al., 2013). While the chromosomes of these diploid species extensively pair together in hybrids among them, virtually no chromosome pairing takes place in haploids derived from polyploid wheat (Kimber and Riley, 1963; McGuire and Dvořák, 1982; Jauhar et al., 1991, 1999). This paradox is caused by the expression of the Ph1 locus on chromosome 5B of tetraploid and hexaploid wheat, which prevents pairing between homoeologous chromosomes (Okamoto, 1957; Riley and Chapman, 1958; Sears and Okamoto, 1958). If Ph1 is absent due to aneuploidy (Riley, 1960) or a deletion, such as ph1b in hexaploid wheat (Sears, 1977) and ph1c in tetraploid wheat (Giorgi, 1978), meiotic pairing of homoeologous chromosomes is restored.

The Ph1 locus on chromosome 5B was initially associated with a cluster of cyclin-dependent kinase 2 (CdK2)-like protein genes including a DNA fragment translocated from chromosome 3A (Griffiths et al., 2006). The translocated fragment was shown to contain TaZIP4-B2, a highly expressed paralog of TaZIP4-1, which is located on wheat chromosomes 3A (TaZIP4-A1), 3B (TaZIP4-B1), and 3D (TaZIP4-D1) (Rey et al., 2017).

Genes suppressing Ph1 and promoting homoeologous chromosome pairing have been reported in several wheat relatives. Examples are chromosome 5U of Ae. umbellulata Zhuk. (Riley et al., 1973), chromosomes 3E, 4E, and 5E of Lophopyrum elongatum (Host) Á. Löve (Dvořák, 1987), and chromosome 5M of Ae. geniculata Roth (Koo et al., 2016). Polymorphism for the suppression of Ph1 was observed in Ae. speltoides and Amblyopyrum muticum (Boiss.) Eig (Dover and Riley, 1972; Dvořák, 1972; Kimber and Athwal, 1972). Using this polymorphism, major suppressors of Ph1 were mapped on Ae. speltoides chromosome arms 3SL (Su1-Ph1) and 7SL (Su2-Ph1) (Dvorak et al., 2006b).

The first use of Ph1 suppression to introgress a gene from a wheat relative into wheat employed hybridization of a wheat cytogenetic stock with Ae. speltoides (Riley et al., 1973). The presence of the Ae. speltoides genome induced recombination between the alien and wheat homoeologs. A logical extension of this idea is to introgress one of the Ae. speltoides suppressors into wheat. Such a wheat genetic stock would greatly simplify introgression of alien genes into wheat. Induction of recombination between homoeologous chromosomes would require nothing more than substituting the alien chromosome for a wheat homoeolog and crossing the substitution line with the Ph1 suppressor line. The F1 would be doubly monosomic for the homoeologous chromosomes targeted for recombination and the expression of Ph1 would be simultaneously suppressed by heterozygosity for the Ph1 suppressor, provided that the suppressor is dominant. The first attempt to produce such a genetic stock resulted in the introgression of an Ae. speltoides suppressor named Ph11 (Chen et al., 1994). The suppression of Ph1 in this line is relatively weak (Chen et al., 1994). It is also not known what Ae. speltoides gene was introgressed and where Ph11 is located in the wheat genome (Li et al., 2011). This situation has precluded the use of marker assisted selection (MAS) in genetic manipulations with this stock.

Another introgression of an Ae. speltoides suppressor into wheat occurred inadvertently as a by product of introgression of the leaf rust resistance gene Lr66 located on chromosome 3S in Ae. speltoides (Marais et al., 2010). The introgressed gene could have been Su1-Ph1. The introgression was accompanied by sterility and has not been practically exploited.

Here, we report introgression of Su1-Ph1 into the genetic background of hexaploid wheat (T. aestivum) cv. Chinese Spring (CS) and from there into the genetic background of tetraploid durum wheat (T. turgidum ssp. durum) cv. Langdon (LDN) utilizing MAS with Xpsr1205-3S. The marker is 0.2 to 0.4 cM distal to Su1-Ph1. MAS was aided by the development of an Ae. speltoides-specific assay for the Xpsr1205-3S haplotype (Dvorak et al., 2006b). The expression of the introgressed Su1-Ph1 in the CS and LDN genetic backgrounds is evaluated and used to test the hypothesis that MAS for Xpsr1205-3S resulted in the introgression of Su1-Ph1. The locations of the Su1-Ph1 relative to the ZIP4-1 loci in the genomic sequences of chromosomes 3A and 3B of wild emmer (Avni et al., 2017) and that of chromosome 3D of Ae. tauschii (Luo et al., 2017) are used to test the hypothesis that Su1-Ph1 is equivalent to ZIP4-1. A strategy for using the introgressed Su1-Ph1 for introgression of alien genes in wheat is suggested.

### MATERIALS AND METHODS

#### Genetic Stocks

The initial material for introgression of Su1-Ph1 into CS and LDN was Ae. speltoides F2 family #134. The family was derived from an F2 plant #134 in a population used for Ph1 suppressor mapping (Dvorak et al., 2006b). The plant was homozygous for an active allele at Su1-Ph1 and an inactive allele at Su2-Ph1. Family #134 inherited chromosome 3S with Su1-Ph1 from Ae. speltoides accession PI 369609. The family was crossed with CS (accession DV148) to produce a CS × Ae. speltoides hybrid. To produce an octoploid amphiploid (2n = 8x = 56), the CS × Ae. speltoides #134 F1 hybrid was removed from its pot and the crown was immersed into 0.6% aqueous solution of colchicine overnight. The disomic substitution (DS) line of L. elongatum chromosome 3E for CS chromosome 3B, designated as DS3E(CS3B) (Tuleen and Hart, 1988) was employed in the initial stages of Su1-Ph1 interorgression into CS. In addition, the DS lines of Ae. searsii chromosomes 1Sse, 5Sse, and 6Sse for their CS homoeologs, designated DS1Sse(CS1A), DS1Sse(CS1B), DS1Sse(CS1D), DS3Sse(CS5A), DS6Sse(CS6A),...
and DS65<sup>a</sup>(CS6D) (Friebe et al., 1995) were provided by N. E. Tuleen, Texas A&M University, College Station, TX, United States. The DS line of <i>L. elongatum</i> chromosome 1E in LDN, DS1E(LDN1A) and DS1E(LDN1B) (Jauhar and Peterson, 2011) were supplied by P. Jauhar, University of North Dakota, Fargo.

### Molecular Markers

A genome-specific PCR assay for Xpsr1205-3S (Dvorak et al., 2006b) was used in MAS for Su1-Ph1 during backcrossing in the CS and LDN genetic backgrounds. Sequence information for wheat ESTs BE426080 and CD454867 and conserved primers of BE426080 (Forward: TGCACTTGCAATCAAAGC; Reverse: CGATCTTGACCACCTCTTCC) and CD454867 (Forward: AGCTCCAGCAATCCTCTCAA; Reverse: GATGGTCGGCTATGCTCTTC) were obtained from the wheat single-nucleotide polymorphism (SNP) database<sup>1</sup> (Akhunov et al., 2010). Using these conserved primers, amplicons were amplified from genomic DNAs of <i>Ae. searsii</i> accession TE10, DSS5<sup>a</sup>(CS5B), CS, and LDN and were treated with ExoSAP-IT (USB) and Sanger sequenced according to the manufacturers protocol (Applied Biosystems, Foster City, CA, United States). A homology search was then performed using BLAST at the bread wheat chromosome-based survey sequence site with these sequences. Alignments and comparisons of these sequences were used to discover SNPs at which the <i>Ae. searsii</i> nucleotide sequence differed from the wheat A-, B-, and D-genome nucleotide sequences. These polymorphisms were used to design <i>Ae. searsii</i> genome-specific primers. Their specificity was tested by PCR of DNAs of <i>Ae. searsii</i> accession TE10, DSS5<sup>a</sup>(CS5B), CS, and LDN. Two <i>Ae. searsii</i> chromosome 55<sup>a</sup>-specific SNP markers, BE426080-55<sup>a</sup> (Forward: TTCTAGTAGAAGCTATTTCATGAG; Reverse: TAACGTGTAAGCTATTTCATGAG) and CD454867-55<sup>a</sup> (Forward: AGCTCCAGCAATCCTCTCAA; Reverse: GAAAGGAGTTCAATGTGCTTCG) were developed and used for selection of plants with the 55<sup>a</sup> chromosome and for study of recombination between 55<sup>a</sup> and 5B.

Markers for studying recombination between LDN chromosome 1A and <i>L. elongatum</i> chromosome 1E were developed as follows. Markers were selected based upon their position on the <i>Ae. tauschii</i> SNP genetic map (Luo et al., 2013). Conserved primers were designed as described previously (Akhunov et al., 2010) and used to generate amplicons from <i>Ae. tauschii</i> accession AL8/78, <i>Ae. speltoides</i> line 134, T. urartu accession G1812, and <i>L. elongatum</i>. The amplicons were sequenced according to the protocol above. Amplicon sequences were aligned and differences between the sequences leveraged to design genome specific primers. E-genome specific primers (Supplementary Table S1) were tested for PCR amplification in genomic DNA of <i>L. elongatum</i>, <i>T. aestivum</i> cv. Chinese Spring, and DS1E(LDN1A) and DS1E(LDN1B).

The PCR reaction contained 1 unit of Taq DNA polymerase, 3 mM MgCl<sub>2</sub>, 50 pmol of forward and reverse primers, and 50 ng of template. The reaction conditions were as follows. DNA was denatured at 98°C for 5 min, which was followed by 40 cycles consisting of 96°C for 30 s, 30 s at annealing temperature, and extension at 72°C for 2 min. The 40 cycles were terminated by maintaining 72°C for 5 min. The amplicons were visualized by electrophoresis in a 2% agarose gel and stained with ethidium bromide.

### Cytological Observations

To verify that <i>Su1-Ph1</i> was introgressed into CS, BC<sub>4</sub>F<sub>2</sub> plants heterozygous for Xpsr1205-3S were crossed with accessions G634, G637, G666, G1326, and G4984 of <i>Ae. peregrina</i> (Hack. in J. Fraser) Mair & Weiller (2n = 4x = 28) provided originally under the synonym <i>Ae. variabilis</i> by B. L. Johnson, University of California, Riverside, CA, United States. The presence of Xpsr1205-3S and the level of chromosome pairing was determined in each hybrid. <i>Ae. peregrina</i> accessions G1326 and G666 were also crossed with CS and the ph1b deletion mutant (Sears, 1977), respectively. To determine whether <i>Su1-Ph1</i> was active in the CS genetic background, B<sub>5</sub>F<sub>2</sub> introgression plants heterozygous for Xpsr1205-3S were crossed with DS lines for <i>Ae. searsii</i> chromosomes 15<sup>6</sup> and 65<sup>a</sup> (Friebe et al., 1995) (provided by N. E. Tuleen, Texas A&M University, College Station, TX, United States). Hybrids were genotyped with Xpsr1205-3S and chromosome pairing was examined.

For chromosome pairing studies, immature spikes were collected from greenhouse-grown plants, fixed in freshly prepared Carnoy’s 6:3:1 (ethanol/acetic-acid/chloroform) fixative for 24 h at room temperature, and then stored in 70% ethanol. Squashes of pollen mother cells (PMCs) were stained with 2% aceticarmine. Meiotic chromosome pairing was scored in 20–30 meiocytes per plant. In the meiotic configurations, univalents, bivalents, trivalents, quadriavlents, and quinquevalents are indicated by I, II, III, IV, and V, respectively. The number of such configurations are indicated by an Arabic numeral preceding the symbol.

Mitotic chromosome spreads of tetraploid introgression lines were subjected to sequential fluorescence in situ hybridization (FISH) and genomic in situ hybridization (GISH). Chromosome spread preparation, probe labeling, and in situ hybridization were carried out according to the methods previously described by Li et al. (2014). In GISH procedures, genomic DNA of <i>Ae. speltoides</i> PI 369609 was labeled with ChromaTide Alexa Fluor-488-5-dUTP (Thermo Fisher Scientific, Waltham, MA, United States) as probe, and genomic DNA of LDN was used as blocking DNA. In FISH procedures, oligonucleotide probes Oligo-pTa535 and Oligo-pSc119.2 were used. Oligo-pTa535 and Oligo-pSc119.2 were reported to be equivalent to repetitive sequence probes in distinguishing wheat A-, B-, and D-genome chromosomes (Tang et al., 2014). Oligonucleotide probes labeled with 6-carboxyfluorescein (6-FAM) or 6-carboxytetramethylrhodamine (Tamra) at the 5′ end, were synthesized by Thermo Fisher Scientific. Photographs were captured with cellSens Standard 1.8 software (Olympus Corporation, Tokyo, Japan) on Olympus BX53 fluorescence microscope with a DP80 microscope digital camera (Olympus Corporation, Tokyo, Japan), and then processed with Adobe

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1. https://wheat.pw.usda.gov/SNP/new/index.shtml
2. https://urgi.versailles.inra.fr/blast/blast.php
RESULTS

Introgression of the *Ae. speltoides* Ph1 Suppressor (*Su1-Ph1*) into Hexaploid and Tetraploid Wheat

In our effort to introgress the entire *Ae. speltoides* chromosome 3S into wheat, the 56-chromosome amphiploid CS × *Ae. speltoides* was crossed with DS3E(CS3B) (Figure 1A). Ten $F_1$ plants positive for the *Ae. speltoides* Xpsr1205-3S haplotype were obtained. The plants were male-sterile and were backcrossed once as females to DS3E(CS3B). The $BC_1F_1$ plants were again male sterile and were backcrossed as females to CS. Five male-sterile $BC_2F_1$ plants positive for Xpsr1205-3S were obtained and backcrossed once more as the female and male-fertile progeny was three times backcrossed as males to CS. Plants bearing Xpsr1205-3S were selected in each generation. The family of hexaploid introgression lines with Xpsr1205-3S in the CS genetic background was designated as CS-Su1-Ph1.

To introgress *Su1-Ph1* from CS-Su1-Ph1 into *T. turgidum* ssp. *durum* cv. LDN, a heterozygous $BC_5F_2$ plant (37*95*4*14-8) was crossed with LDN and six Xpsr1205-3S-positive $F_1$ plants were grown. The pentaploid hybrid was male sterile. It was backcrossed as the female parent to LDN four times, always selecting for the Xpsr1205-3S haplotype in progeny. Fertile plants positive for Xpsr1205-3S were ultimately obtained. They were designated as LDN-Su1-Ph1.

The CS-Su1-Ph1 lines were similar to CS in their morphology except for a pyramidal spike shape (Figure 1B). All CS-Su1-Ph1 plants, irrespective of the presence or absence of Xpsr1205-3S, had significantly lower seed set than CS but there was no difference in seed set between siblings with and without Xpsr1205-3S (Table 1) (one-way ANOVA, $\alpha = 0.05$, LSD). Except for a single plant with a translocation, CS-Su1-Ph1 plants did not show multivalent chromosome pairing (Table 2).
TABLE 2 | Mean meiotic chromosome pairing in CS-Su1-Ph1 and LDN-Su1-Ph1 with the Xpsr1205-3S marker.

| Stock          | Generation | I       | II      | III      | IV      |
|----------------|------------|---------|---------|----------|---------|
| CS-Su1-Ph1     | BC3 F2     | 6       | 0.2     | 19.0     | 0.2     | 0.8     |
|                | BC3 F4     | 10      | 0.2     | 20.9     | 0.0     | 0.0     |
|                | BC3 F5     | 11      | 0.6     | 20.7     | 0.0     | 0.0     |
|                | BC3 F6     | 19      | 0.0     | 21.0     | 0.0     | 0.0     |
| LDN-Su1-Ph1    | BC4 F2     | 19      | 0.4     | 13.4     | <0.1    | 0.2     |
|                | BC4 F4     | 19      | 0.5     | 13.6     | 0.0     | <0.1    |
|                | BC4 F5     | 32      | 0.7     | 13.6     | <0.1    | 0.0     |

*I* = univalent; **II** = bivalent; **III** = trivalent; **IV** = quadrivalent.

LDN-Su1-Ph1 lines were similar to LDN in spike shape, seed shape, and seed size. LDN-Su1-Ph1 seeds had darker pericarp than those of LDN (Figure 1C). LDN-Su1-Ph1 were less fertile than LDN (*P* = 0.001, *t*-test, *N* = 12 and 2, Table 1). All of the LDN-Su1-Ph1 plants showed a multivalent at MI in some PMCs (Table 2).

To identify the wheat chromosome harboring the introgressed chromosome segment, sequential FISH and GISH were performed on LDN-Su1-Ph1. FISH with the oligo-pTa535 and oligo-pScl119.2 probes paints all wheat chromosomes and facilitates their identification (Tang et al., 2014). GISH with labeled genomic DNA of *Ae. speltoides* PI 369609 facilitated identification of the wheat chromosome harboring *Ae. speltoides* segments. Sequential FISH and GISH karyotyping of plants heterozygous for *Xpsr1205-3S* revealed the presence of a single recombined chromosome 3A with an *Ae. speltoides* segment.
TABLE 3 | Mean numbers of chiasmata per PMC in hybrids between allotetraploid Aeglops peregrina and Chinese Spring, CS $ph1b$, and CS-Su1-Ph1 in the presence and absence of $Xpsr1205$-$3S$.

| Accession | Female parent | Xpsr1205-3S | No. hybrids | Mean chiasmata/PMC* |
|-----------|---------------|-------------|-------------|---------------------|
| G1326     | CS            | –           | 3           | 3.7                 |
| G1326     | CS-Su1-Ph1    | –           | 1           | 3.7                 |
| G1326     | CS-Su1-Ph1    | +           | 1           | 16.8                |
| G666      | CS-Su1-Ph1    | –           | 1           | 1.1                 |
| G637      | CS-Su1-Ph1    | –           | 1           | 12.7                |
| G637      | CS-Su1-Ph1    | +           | 3           | 3.1                 |
| G637      | CS-Su1-Ph1    | +           | 1           | 1.6                 |
| G637      | CS-Su1-Ph1    | –           | 1           | 16.8                |
| G634      | CS-Su1-Ph1    | +           | 1           | 16.1                |
| G636      | ph1b          | –           | 1           | 1.5                 |

*Mean number of chiasmata in hybrids CS-Su1-Ph1 × Ae. peregrina with and without Xpsr1205-3S significantly differed (P = 0.001, two-tailed t-test, N = 5 and 11).

replacing the distal portion of the long arm (Figures 2A,B). Sequential FISH and GISH karyotyping of plants homozygous for $Xpsr1205$-$3S$, showed two such chromosomes (Figures 2C,D). Thus, FISH and GISH karyotyping revealed that a single chromosome segment of $Ae. speltoides$ was introgressed into LDN. The agreement between heterozygosity and homozygosity of the $Xpsr1205$-$3S$ marker and the number of 3A chromosomes with the $Ae. speltoides$ segments indicated that $Xpsr1205$-$3S$ was located on that introgressed segment in LDN 3A.

**Chromosome Pairing in Hybrids Involving CS-Su1-Ph1**

Previous studies suggested that the $Ae. peregrina$ genome does not modify $Ph1$ expression in wheat × $Ae. peregrina$ hybrids (McGuire and Dvořák, 1982). If $Ph1$ is active in the wheat parent, there would be little chromosome pairing in wheat × $Ae. peregrina$ hybrids but if it is inactive, extensive pairing would take place.

Seven hybrids without $Xpsr1205$-$3S$ were analyzed. One hybrid without $Xpsr1205$-$3S$ differed from the remaining six by having an intermediate level of chromosome pairing. The remaining six hybrids without $Xpsr1205$-$3S$ had an average of 2.7 chiasmata/PMC, which was comparable to the mean chiasma number in hybrids of CS × $Ae. peregrina$ (Table 3). In contrast, the four hybrids having $Xpsr1205$-$3S$ had an average of 16.6 chiasmata per PMC ($P = 0.004$, t-test, N = 11 and 4). We excluded one hybrid with an exceptionally low level of chromosome pairing, although it was positive for $Xpsr1205$-$3S$ (Table 3). Two hybrids from the cross $ph1b$ × $Ae. peregrina$ had 18.6 chiasmata/PMC, which was still higher ($P = 0.012$, t-test, N = 3 and 2) than the mean chiasma frequency of hybrids with $Xpsr1205$-$3S$. Overall, $Ae. peregrina$ hybrids seemed to confirm that MAS for $Xpsr1205$-$3S$ resulted in introgression of Su1-Ph1 into CS.

CS-Su1-Ph1 heterozygous for $Xpsr1205$-$3S$ was crossed with $Ae. searsii$ DS5S$^{se}$(CS5A) and chromosome pairing was analyzed in progeny with and without $Xpsr1205$-$3S$. No PMC with all chromosomes paired was observed in a plant without $Xpsr1205$-$3S$ (Table 4). Surprisingly, only 6.8% of PMCs showed complete pairing (a measure of pairing of the $Ae. searsii$ 5S$^{se}$ with the wheat homoeolog) in three progeny plants with $Xpsr1205$-$3S$ (Table 4). This level of pairing between closely related homoeologous chromosomes was below what was expected for $Ph1$ expression being suppressed.

To validate this result, CS-Su1-Ph1 heterozygous for $Xpsr1205$-$3S$ was crossed with DS1S$^{se}$(CS1A), DS1S$^{se}$(CS1B), DS1S$^{se}$(CS1D), DS6S$^{se}$(CS6A), and DS6S$^{se}$(CS6D). These DS lines were selected because chromosomes in these two homoeologous groups have not been reported to harbor genes affecting homoeologous chromosome pairing. DS lines for $Ae. searsii$ chromosomes 1S$^{se}$ and 6S$^{se}$ were also crossed with CS as a control. PMCs that had complete chromosome pairing were rare irrespective of the presence or absence of $Xpsr1205$-$3S$ (Table 4), suggesting that only minor or no suppression of $Ph1$ took place in the presence of $Xpsr1205$-$3S$. This finding contradicted the conclusion made on the basis of chromosome pairing in hybrids involving $Ae. peregrina$ and indicated that $Ae. peregrina$ genome may not be entirely neutral with respect to suppression of $Ph1$.

**Chromosome Pairing and Recombination in the LDN Genetic Background**

Because of concerns that the $Ae. peregrina$ genome may be obscuring the true effects of the introgressed chromosome segment on the expression of $Ph1$, we used the CS genome in the assessment of the status of $Ph1$ expression in the presence of the introgressed $Xpsr1205$-$3S$. LDN-Su1-Ph1 heterozygous for $Xpsr1205$-$3S$ was crossed with CS and chromosome pairing was studied in progeny. If the chromosome segment harboring $Xpsr1205$-$3S$ did not also harbor Su1-Ph1, the seven D-genome monosomes would not pair with their A- and B-genome homoeologs in the pentaploid hybrids ($2n = 5x = 35$) and 14I + 7I would be expected at MI in most PMCs. If, on the other hand, introgression of $Xpsr1205$-$3S$ did result in the introgression of Su1-Ph1 into LDN and Su1-Ph1 suppressed $Ph1$, the D-genome chromosomes would pair with their A and B genome homoeologues and trivalents accompanied by fewer than 7I would be expected in PMCs of the pentaploid hybrids.
TABLE 4 | Percentages of PMCs with all 42 chromosomes paired indicating pairing between wheat and Ae. searsii homoeologous chromosomes in F1 progenies from crosses of Ae. searsii DS lines (parent 1) × CS-Su1-Ph1 or CS (parent 2).

| Parent 1          | Parent 2          | Plants | PMCs | % PMCs with complete chromosome pairing |
|-------------------|-------------------|--------|------|----------------------------------------|
| DS1S\(^{se}\)(CS1A) | CS-Su1-Ph1        | –      | 1    | 28                                      |
|                   |                   | +      | 3    | 98                                      |
| DS1S\(^{se}\)(CS1B) | CS-Su1-Ph1        | –      | 2    | 60                                      |
|                   |                   | +      | 1    | 32                                      |
| DS1S\(^{se}\)(CS1D) | CS-Su1-Ph1        | –      | 1    | 30                                      |
|                   |                   | +      | 1    | 11                                      |
| DS5S\(^{se}\)(CS5A) | CS-Su1-Ph1        | –      | 1    | 32                                      |
|                   |                   | +      | 3    | 87                                      |
| DS6S\(^{se}\)(CS6A) | CS-Su1-Ph1        | –      | 1    | 27                                      |
|                   |                   | +      | 2    | 63                                      |
| DS6S\(^{se}\)(CS6D) | CS-Su1-Ph1        | –      | 1    | 24                                      |
|                   |                   | +      | 4    | 125                                     |
| Mean CS            |                   | –      | 0    |                                         |
| Mean\(^*\) CS-Su1-Ph1 |               | –      |     | 1.3                                     |
| Mean\(^*\) CS-Su1-Ph1 |               | +      |     | 3.9                                     |

*The means do not statistically differ from each other (P = 0.16, two-tailed t-test, N = 6).

TABLE 5 | Meiotic pairing in pentaploid (2n = 35) progeny from the cross LDN-Su1-Ph1 × Chinese Spring.

| Plant     | Xpsr1205-3S | Pairing configuration** | No. of cells | I | II | III | Range of III | IV |
|-----------|-------------|-------------------------|--------------|---|----|-----|--------------|----|
| GH47583   | +           |                         | 21           | 6.7 | 12.9 | 0.8 | 0–2           | <0.1 |
| GH47584   | +           |                         | 20           | 5.9 | 13.1 | 0.9 | 0–3           | <0.1 |
| GH47585   | +           |                         | 27           | 5.4 | 13.0 | 1.1 | 0–3           | <0.1 |
| GH47588   | +           |                         | 25           | 5.6 | 13.2 | 1.0 | 0–3           | 0.0  |
| GH47589   | +           |                         | 30           | 5.4 | 12.5 | 1.6 | 0–3           | 0.0  |
| GH47590   | +           |                         | 24           | 5.7 | 12.7 | 1.3 | 0–3           | 0.0  |
| GH47591   | +           |                         | 30           | 5.7 | 13.1 | 1.0 | 0–3           | 0.0  |
| GH47596   | –           |                         | 32           | 7.7 | 13.7 | 0.0 | 0             | 0.0  |
| GH47592   | –           |                         | 44           | 8.0 | 13.1 | 0.1 | 0–1           | 0.1  |
| GH47593   | –           |                         | 32           | 8.7 | 13.2 | 0.0 | 0             | 0.0  |
| GH47594   | –           |                         | 32           | 7.7 | 13.7 | 0.0 | 0             | 0.0  |
| Mean\(^*\) | +           |                         | 5.8a         | 12.9a| 1.1a | <0.1a | <0.1a        |
| Mean\(^*\) | –           |                         | 8.0b         | 13.4a| <0.1b| <0.1a |

*Means in columns followed by the same letter are not significantly different at the 5% significance level.
**I = univalent; II = bivalent; III = trivalent; IV = quadrivalent.

with Xpsr1205-3S. In the four hybrids without Xpsr1205-3S, an average of 8.0 univalents and 13.4 bivalents were observed (Table 5). In one of these hybrids, an occasional trivalent and quadivalent was also observed; none were observed in the other three hybrids. Chromosome pairing indicated that Ph1 was active in all four hybrids. In the 11 hybrids with Xpsr1205-3S, the mean number of univalents per PMC was 5.8 (Table 5), which was significantly lower (P = 0.0002, two-tailed t = test with unequal variance) than the mean of 8.0 univalents per PMC in hybrids without Xpsr1205-3S. Concomitantly, the mean number of trivalents significantly increased to an average of 1.1 per PMC (P < 0.0001, two-tailed t = test with unequal variance). Up to four trivalents accompanied by three univalents were observed in a single PMC (Figure 3). These data were consistent with the hypothesis that Ph1 was suppressed in the pentaploid hybrids with Xpsr1205-3S and indicated that introgression of Xpsr1205-3S to LDN was accompanied by introgression of an active Su1-Ph1 allele.

Additional evidence confirming suppression of Ph1 by Su1-Ph1 in the LDN genetic background was provided by meiotic pairing of Ae. searsii chromosome 5S\(^{se}\) with LDN chromosome 5B. A double monosomic for Ae. searsii chromosome 5S\(^{se}\)
and LDN chromosome 5B in the LDN genetic background (2n = 28) was developed as described in Figure 4. Plants from BC1 and BC2 generation were genotyped with BE426080-5Sse and CD454867-5Sse to select plants with chromosome 5Sse and crossed with LDN-Su1-Ph1 heterozygous for Xpsr1205-3S. In F1 plants with Xpsr1205-3S, the mean numbers of PMCs with complete chromosome pairing, indicating pairing of 5Sse with 5B, was 58.3%, and was significantly higher than mean of 7.9% of PMCs in F1 lacking Xpsr1205-3S (P < 0.01, two-tailed t-test with unequal variance) (Table 6).

The F1 plants were selfed and also backcrossed as females to LDN-Su1-Ph1 to study recombination. Genotypes at BE426080-5Sse and CD454867-5Sse were determined in 35 F2 and 28 BC1F1 progeny. The two markers are proximally located in the Ph1 region of chromosome 5B and cosegregated at 160.6 cM on the genetic map from the cross of wild emmer (T. turgidum ssp. dicoccoides) × LDN (Jorgensen et al., 2017). Two recombined chromosomes, one in an F2 plant (GH45780) and the other in a BC1F1 plant (GH45804), were identified among 98 progeny chromosomes (Figure 5).

Chromosome pairing and recombination was also assessed between distantly related homoeologous chromosomes, the L. elongatum chromosome 1E and wheat chromosome 1A, in progeny from the cross LDN-Su1-Ph1 × DS1E(LDN1A). The L. elongatum chromosome 1E did not pair with wheat chromosome 1A (N = 64) in progeny without Xpsr1205-3S but it paired with it in 6.0% PMCs (N = 83) in progeny with Xpsr1205-3S. Based on the relationship 1% MI pairing = 0.5%
recombination, the length of the 1E/1A linkage group (LG) was 3% recombination. Three recombined chromosomes were identified among 63 F2 progeny (Table 7). All three crossovers were validated by genotyping of 15 to 20 F3 progeny plants. The length of the 1E/1A LG based on recombination was 2.3%, which was comparable to 3% recombination based on MI pairing ($P = 0.74$, $2 \times 2$ contingency table). Two crossovers were located within the long arm and one was located within the short arm (Table 7). Thus, also this study confirmed that Ph1 was suppressed in plants with Xpsr1205-3S in the LDN genetic background.

**DISCUSSION**

**Introgression of Su1-Ph1 into Wheat**

Tight linkage of the Xpsr1205-3S marker to Su1-Ph1 (Dvorak et al., 2006b) was exploited here in introgression of Su1-Ph1 from Ae. speltoides into hexaploid wheat and from hexaploid wheat into tetraploid wheat. MAS was employed rather than selection for the meiotic pairing phenotype. Selection based only on meiotic phenotype would require a testcross with Ae. peregrina, or a similar tester, each backcross generation to ascertain that Ph1 is suppressed. Moreover, it will be difficult to detect the presence of a suppressor allele in the CS background if the Ph1 effect is not completely suppressed. Heterozygosity for translocations resulting from recombination between homeologous chromosomes during backcrossing or from chromosome breakage and non-homologous end-joining due to the activity of gametocidal genes (Tsujimoto and Tsunewaki, 1984; Kota and Dvorak, 1988; Marais et al., 2010) could potentially lead to mistaking such multivalent pairing for homeologous pairing.

The CS $\times$ Ae. speltoides hybrid treated with colchicine had dehiscent anthers and produced two octoploid seeds, indicating that it was male fertile but the two octoploid progeny plants were male sterile. They were therefore backcrossed as females with DS3E(CS3B) for two generations. Since L. elognatum chromosome 3E partially suppresses Ph1 (Dvořák, 1987), it is therefore theoretically possible that the suppressor of Ph1 introgressed in CS-Su1-Ph1 and LDN-Su1-Ph1 was the 3E suppressor rather than Su1-Ph1. This possibility is very unlikely for the following reasons. First, chromosome 3E has a weak suppressor, which is located in the short arm (Dvořák, 1987), as a template, no amplification was obtained. Therefore, using Xpsr1205-3S primers for PCR amplification were Ae. speltoides specific, and when DS3E(CS3B) was used as a template, no amplification was obtained. Therefore, using Xpsr1205-3S in introgression could not have introgressed a 3E segment in place of the targeted Ae. speltoides chromosome segment. Third, in situ hybridization showed that in plants with Xpsr1205-3S, the terminal segment of 3A was replaced by an Ae. speltoides chromosome segment. Finally, the LDN-Su1-Ph1 introgression lines were subjected to nine generations of backcrossing, first to CS and then to LDN. The probability that a recombined chromosome bearing the 3E suppressor was present in the LDN-Su1-Ph1 lines was $<0.002$.

Five approaches were employed to test the hypothesis that Su1-Ph1 was introgressed along with introgression of Xpsr1205-3S with the following results. (1) D-genome chromosomes frequently paired in trivalents with their A- and B-genome

### Table 6

| Plant          | Generation | Xpsr1205-3S | 5S* marker | Pairing configuration** | % PMCs with complete chromosome pairing |
|----------------|------------|-------------|------------|-------------------------|----------------------------------------|
|                |            |             |            | I | II | III | IV |                                      |
| GH36580        | BC1        | −           | +          | 30 | 1.9 | 13.1 | 0.0 | 0.0 | 6.7                                    |
| GH36585        | BC1        | −           | +          | 29 | 1.8 | 13.1 | 0.0 | 0.0 | 10.3                                   |
| GH45834        | BC2        | −           | +          | 62 | 1.8 | 13.1 | 0.0 | 0.0 | 8.1                                    |
| GH44117        | BC2        | −           | +          | 32 | 1.9 | 13.1 | 0.0 | 0.0 | 6.3                                    |
| GH36589        | BC1        | +           | +          | 51 | 0.7 | 13.7 | 0.0 | 0.0 | 68.6                                   |
| GH36586        | BC1        | +           | +          | 40 | 0.7 | 13.7 | 0.0 | 0.0 | 65.0                                   |
| GH36587        | BC1        | +           | +          | 43 | 0.2 | 13.9 | 0.0 | 0.0 | 90.3                                   |
| GH45830        | BC2        | +           | +          | 35 | 1.4 | 13.2 | <0.1 | 0.0 | 45.7                                   |
| GH45831        | BC2        | +           | +          | 25 | 1.0 | 13.4 | <0.1 | <0.1 | 56.0                                   |
| GH45833        | BC2        | +           | +          | 32 | 0.5 | 13.8 | 0.0 | 0.0 | 75.0                                   |
| GH45835        | BC2        | +           | +          | 50 | 1.0 | 13.5 | 0.0 | 0.0 | 56.0                                   |
| GH45839        | BC2        | +           | +          | 24 | 1.6 | 13.2 | 0.0 | 0.0 | 29.2                                   |
| GH44153        | BC2        | +           | +          | 70 | 1.5 | 13.3 | 0.0 | 0.0 | 38.6                                   |
| Mean           |            | +           | +          |    |    |    |    | 1.9a | 13.1a | 0.0 | 0.0 | 7.9a                                   |
| Mean           |            | +           | +          |    |    | 1.0b | 13.5b | <0.1 | <0.1 | 58.3b |<0.01 |<0.01 |<0.01 |

**Means followed by the same letter are not significantly different at the 5% significance level.**

**I = univalent; II = bivalent; III = trivalent; IV = quadrivalent.**

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**Table 7**

Meiotic chromosome pairing in the F1 plants from the crosses of two generations (BC1 and BC2) of monosomic substitution 5S*°(LDNSB) × LDN-Su1-Ph1.

| Plant          | Generation | Xpsr1205-3S | 5S* marker | Pairing configuration** | % PMCs with complete chromosome pairing |
|----------------|------------|-------------|------------|-------------------------|----------------------------------------|
|                |            |             |            | I | II | III | IV |                                      |
| GH36580        | BC1        | −           | +          | 30 | 1.9 | 13.1 | 0.0 | 0.0 | 6.7                                    |
| GH36585        | BC1        | −           | +          | 29 | 1.8 | 13.1 | 0.0 | 0.0 | 10.3                                   |
| GH45834        | BC2        | −           | +          | 62 | 1.8 | 13.1 | 0.0 | 0.0 | 8.1                                    |
| GH44117        | BC2        | −           | +          | 32 | 1.9 | 13.1 | 0.0 | 0.0 | 6.3                                    |
| GH36589        | BC1        | +           | +          | 51 | 0.7 | 13.7 | 0.0 | 0.0 | 68.6                                   |
| GH36586        | BC1        | +           | +          | 40 | 0.7 | 13.7 | 0.0 | 0.0 | 65.0                                   |
| GH36587        | BC1        | +           | +          | 43 | 0.2 | 13.9 | 0.0 | 0.0 | 90.3                                   |
| GH45830        | BC2        | +           | +          | 35 | 1.4 | 13.2 | <0.1 | 0.0 | 45.7                                   |
| GH45831        | BC2        | +           | +          | 25 | 1.0 | 13.4 | <0.1 | <0.1 | 56.0                                   |
| GH45833        | BC2        | +           | +          | 32 | 0.5 | 13.8 | 0.0 | 0.0 | 75.0                                   |
| GH45835        | BC2        | +           | +          | 50 | 1.0 | 13.5 | 0.0 | 0.0 | 56.0                                   |
| GH45839        | BC2        | +           | +          | 24 | 1.6 | 13.2 | 0.0 | 0.0 | 29.2                                   |
| GH44153        | BC2        | +           | +          | 70 | 1.5 | 13.3 | 0.0 | 0.0 | 38.6                                   |
| Mean           |            | +           | +          |    |    |    |    | 1.9a | 13.1a | 0.0 | 0.0 | 7.9a                                   |
| Mean           |            | +           | +          |    |    | 1.0b | 13.5b | <0.1 | <0.1 | 58.3b |<0.01 |<0.01 |<0.01 |

**Means followed by the same letter are not significantly different at the 5% significance level.**

**I = univalent; II = bivalent; III = trivalent; IV = quadrivalent.**
homoeologs in the pentaploid LDN-Su1-Ph1 × CS hybrids that acquired Xpsr1205-3S but not in those that did not acquire Xpsr1205-3S. (2) Chromosome 5Sse paired with LDN chromosome 5B in 58.3% of PMCs if Xpsr1205-3S was present but only in a few percent of PMCs if Xpsr1205-3S was absent. Recombined 5Sse/5B chromosomes were recovered in progeny of plants with Xpsr1205-3S. (3) L. elongatum chromosome 1E paired in the LDN genetic background with chromosome 1A if Xpsr1205-3S was present but did not pair with it if Xpsr1205-3S was absent. Recombined 1E/1A chromosomes were recovered in progeny. (4) A mean of 16.6 chiasmata per PMC was observed in F1 hybrids CS-Su1-Ph1 × Ae. peregrina if Xpsr1205-3S was present but only 2.7 chiasmata/PMC were observed if Xpsr1205-3S was absent. The last approach (5) provided evidence contradictory to that provided by approaches (1) to (4). Single Ae. searsii chromosomes 1Sse, 5Sse, and 6Sse paired with wheat homoeologs in only a few percent of PMCs in progenies involving CS-Su1-Ph1 in the presence of
Xpsr1205-3S. Thus, the presence of the introgressed Xpsr1205-3S consistently resulted in suppression of Ph1 in the LDN genetic background but the results were inconsistent in the CS genetic background.

Sequential FISH and GISH showed that LDN-Su1-Ph1 plants with Xpsr1205-3S harbored an Ae. speltoides chromosome segment replacing a distal portion of the long arm of chromosome 3A. The correct orientation of the segment in the arm suggests that the translocation originated by recombination between LDN chromosome 3A and Ae. speltoides long arm of chromosome 3A. Does the spatial and functional relationship of the two loci? The locations of these loci on the reference genome sequences of wild emmer (Avni et al., 2017) and Ae. tauschii (Luo et al., 2017) suggest that they are not equivalent. Whether ZIP4-1 and ZIP4-2 loci are on the same pseudomolecules at 712,850,941, 786,992,681, and 591,216,484 bp, respectively (Dvorak et al., 2006b), the orthologous Xpsr1205 loci are on the 3A, 3B, and 3D pseudomolecules at 712,850,941, 786,992,681, and 591,216,484 bp, respectively (Figure 6). The orthologous XBF497740 marker and about 70 Mb proximal to Xpsr1205, which indicates that it is very unlikely that ZIP4-1 and Su1-Ph1 are equivalent.

Occasional multivalents from heterogenetic chromosome pairing were observed in all LDN-Su1-Ph1 plants. In contrast, no multivalents were observed in the CS-Su1-Ph1 plants.

Since tetraploid and hexaploid wheat share the A and B genomes, and since there has been extensive gene flow between tetraploid and hexaploid wheat (Dvorak et al., 2006a; Akhunov et al., 2010), it is very likely that hexaploid wheat is polymorphic for the complementary gene. A search for the gene in T. aestivum will require the development of a complementation assay that would indicate the presence of homeologous chromosome pairing in hybrids involving Xpsr1205-3S.

**Molecular Nature of Su1-Ph1 and Its Relationship to ZIP4-1 and Zip4-B2**

The Su1-Ph1 locus and ZIP4-1 gene, the likely source of the Ph1 gene (Rey et al., 2017), are both located on the long arm of chromosome 3A. Does the spatial and functional relationship of Su1-Ph1 and ZIP4-1 indicate equivalence of the two loci? The locations of these loci on the reference genome sequences of wild emmer (Avni et al., 2017) and Ae. tauschii (Luo et al., 2017) suggest that they are not equivalent. Su1-Ph1 was mapped between Xpsr1205-3S and EST locus XBF497740 on two independent maps. Su1-Ph1 and XBF497740 were 0.2 and 13 cM proximal to Xpsr1205-3S, respectively (Dvorak et al., 2006b). Orthologous Xpsr1205 loci are on the 3A, 3B, and 3D pseudomolecules at 712,850,941, 786,992,681, and 591,216,484 bp, respectively (Figure 6). The orthologous XBF497740 loci are about 30 Mb proximal to Xpsr1205 in each pseudomolecule (Figure 6) but the ZIP4-A1 and ZIP4-B1 and ZIP4-D1 loci, which are at collinear locations on pseudomolecules 3A, 3B and 3D, are about 30 Mb further proximal to the XBF497740 marker and about 70 Mb proximal to Xpsr1205. It is therefore very unlikely that ZIP4-1 and Su1-Ph1 are equivalent. Whether ZIP4-B1 is actually the complementary locus detected in LDN or whether Su1-Ph1 is equivalent.
Comparison of chromosome pairing in hybrids of frameshift mutants of ZIP4-B2 (Cad1691 and Cad0348) × Ae. peregrina (Rey et al., 2017) with the hybrids of CS-Su1-Ph1 × Ae. peregrina suggests that Ph1 expression was not entirely abolished by the Cad1691 and Cad0348 mutations. The hybrids had an average of 12.2 chiasmata/PMC (Rey et al., 2017) whereas those involving CS-Su1-Ph1 had an average of 16.6 chiasmata/PMC (P < 0.001, t-test, N = 3 and 2). Both sets of hybrids had lower chiasma frequency than the hybrids involving the ph1b deletion mutation (18.6 chiasmata/PMC). The average number of chiasmata in hybrids involving the ZIP4-B22 mutants was similar to the exceptional hybrid involving CS-Su1-Ph1 × Ae. peregrina accession G666, which showed an intermediate level of chromosome pairing (12.7 chiasmata/PMC). The mutants of ZIP4-B2 did not show multivalents at MI in meiosis (Rey et al., 2017), which is consistent with partial activity of Ph1. Evidence that ZIP4-B2 is equivalent to Ph1 hinges on chromosome pairing in the hybrids with Ae. peregrina. As we have learned, the Ae. peregrina genome can obscure the actual effect of genes affecting Ph1, and it would be prudent to provide additional evidence that Ph1 expression was abolished in Cad1691 and Cad0348 ZIP4-B2 mutants.

Practical Utility of the Introgressed Su1-Ph1

Since the presence of heterozygous Xpsr1205-3S in the LDN-Su1-Ph1 × CS F1 hybrids elicited homeologous chromosome pairing, Su1-Ph1 must be dominant. The dominant epistasis of Su1-Ph1 over Ph1 makes it a very flexible means of inducing recombination between homeologs. To achieve recombination between an alien chromosome and a wheat homoeolog, the alien chromosome should be substituted for a wheat homoeolog and the substitution line should be crossed with an introgression line harboring the T(3AL;3SL)Dv1 chromosome. The introgression line supplies both the Ph1 suppressor and the wheat homoeologs for recombination with the alien chromosome. This strategy was illustrated here by targeting recombination between homeologous chromosomes 55P with 5B and 1E with 1A.

There are however two caveats. One is that Su1-Ph1 will suppress Ph1 only if one of the parents contributes the complementary gene to F1 progeny. This requirement is satisfied by the LDN-Su1-Ph1 introgression lines but not by the CS-Su1-Ph1 introgression lines. The alien chromosome substitution lines should therefore be in the LDN genetic background, as was done here to achieve recombination of 55P with 5B and 1E with 1A. It is also possible to use hexaploid alien chromosome substitutions and rely on the induction of recombination between the homeologs in the pentaploid hybrids, but that strategy has not been tested.

The other caveat is that Su1-Ph1 does not suppress Ph1 completely. The chiasma frequency in CS-Su1-Ph1 × Ae. peregrina F1 hybrids was significantly lower than the average chiasma frequency in the ph1b × Ae. peregrina F1 hybrids. Therefore, Su1-Ph1 will be the most effective in the manipulation of closely related homeologous chromosomes, although, as illustrated here, even more distantly related chromosomes, such as L. elongatum and wheat, could be recombined.

AUTHOR CONTRIBUTIONS

JD, HL, and AD conceived and designed the experiments. HL, KD, and JD performed the experimental work. JD, HL, and KD analyzed the data. JD, HL, KD, M-CL, WJ, and AD discussed the findings and interpreted the results. JD and HL wrote the first draft of the paper. All authors have read and approved the final draft.

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SUPPLEMENTARY MATERIAL

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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