



## Article

# Development and Characterization of Wheat-*Agropyron cristatum* Introgression Lines Induced by Gametocidal Genes and Wheat *ph1b* Mutant

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**Abstract:** The P genome of *Agropyron cristatum* Gaertn. contains many desirable genes that can be utilized as genetic resources to improve wheat. In this research, we used both the gametocidal chromosome 2C<sup>c</sup> and the pairing homologous gene (*Ph1b*) mutant to induce structural aberrations and translocations between wheat and the 4P, 5P, and 6P genome chromosomes. By using the two approaches, a total of 19 wheat-*A. cristatum* translocations have been identified, in which 13 were induced by the *Triticum aestivum* cv. Chinese Spring (CS) *ph1b* mutant (CS *ph1b*) and six were induced by gametocidal chromosome 2C<sup>c</sup> from *Aegilops cylindrica* Host. The wheat-4P, -5P and -6P *A. cristatum* translocations were characterized by in situ hybridization and by a set of conserved orthologous set (COS) molecular markers. The aberrations included centromeric translocations, terminal translocations, dicentric translocations, and deletions. The average induction frequency of chromosome structural aberrations was 10.9% using gametocidal 2C<sup>c</sup> chromosome and 8.8% using *ph1b* mutant. The highest frequency obtained was for chromosome 4P using both approaches. All the wheat-*A. cristatum* translocation lines obtained were valuable for identifying *A. cristatum* chromosome 4P, 5P, and 6P related genes. In addition, these lines provided genetic resources and new germplasm accessions for the genetic improvement of wheat.

**Keywords:** *Agropyron cristatum*; gametocidal chromosome 2C<sup>c</sup>; *Ph1b*; wheat-*A. cristatum* translocations



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## 1. Introduction

*Agropyron cristatum* L. Gaertn (genome P) is a species in the Triticeae tribe, which has diploid ( $2n = 2X = 14$ ), tetraploid ( $2n = 4X = 28$ ), and hexaploid ( $2n = 6X = 42$ ) forms [1]. *A. cristatum* is a valuable donor of important agronomic traits for wheat improvement such as resistance to leaf rust [2], resistance to stripe rust [3], resistance to powdery mildew [4,5], enhanced-grain number per spike and spike length [6], high salt-tolerance [7], and drought stress tolerance [8]. With the objective to introgress these agronomic traits into wheat, hybrids and fertile amphiploids between *Triticum* and *A. cristatum* have been successfully obtained [9–13], allowing the development of both durum and common wheat-*A. cristatum* introgressions [2,6,14–16].

Recombination between cultivated and alien chromosomes is essential for incorporating beneficial alleles into crop plants from their wild relatives. To enhance recombination between donor chromosomes and the wheat genome to produce wheat-alien chromosome translocations, different strategies were designed, such as ionizing irradiation, induction by *Ph1* mutant, and gametocidal chromosomes. Since Sears' [17] pioneering work transferring leaf rust resistance from *Aegilops umbellulata* to wheat, ionizing irradiation has been widely used to induce translocations between wheat and different Triticeae species such as rye (*Secale cereale* L.) [18], *A. intermedium* [19], and *Aegilops* spp. [20]. The pairing homoeologous (*Ph1b*) gene mapped on chromosome 5BL regulates chromosome pairing

and recombination in common wheat [21,22]). In the homozygous *ph1b* locus deletion mutant, the alien chromosomes can pair and recombine with wheat chromosomes at a low frequency [23]. Resistances or tolerances to biotic and abiotic stress into common wheat have been successfully introgressed using the *Ph1*-deficient genetic stocks including nullisomic for chromosome 5B and mutation of the *Ph1b* gene [24–29]. Gametocidal chromosomes (*Gc*) from certain wild species in the genus *Aegilops* introduced into common wheat under monosomic conditions induces chromosome rearrangements in the gametes lacking *Gc* chromosomes [30]. The *Gc* system proved to be effective in inducing structural rearrangements in barley [31], rye [32], *Haynaldia villosa* L. [33], *Leymus racemosus* (Lam.) Tzvelev [34]), and *H. chilense* [35–37] chromosomes added to common wheat.

Both ionizing irradiation and gametocidal chromosome 2C methods have been previously used to produce wheat-*A. cristatum* translocations [2,15,16,38–40]. However, as far as we know no reports have been published about using a *ph1b* deletion to obtain wheat-*A. cristatum* chromosome translocations. In this work we present the development and characterization of structural rearrangements between wheat and chromosomes 4P, 5P, and 6P from *A. cristatum* using both the action of gametocidal 2C<sup>c</sup> genes and *ph1b* deletion.

## 2. Materials and Methods

### 2.1. Plant Material

The plant materials used in this study included the common wheat (*Triticum aestivum* L.) cultivar ‘Chinese Spring’ (CS), *A. cristatum* accession PI222957 (2n = 4x = 28; PPPP) provided by USDA (United States Department of Agriculture), the CS/*A. cristatum* disomic addition lines 4P, 5P, and 6P, and the ditelosomic 4PS, 5PL, 6PS, and 6PL addition lines [10].

#### 2.1.1. Gametocidal 2C<sup>c</sup> Chromosome

The disomic addition lines were used as female parents in crosses with the wheat disomic addition line carrying the gametocidal chromosome 2C<sup>c</sup> from *Aegilops cylindrica* Host. The F<sub>1</sub> plants monosomic for both the *A. cristatum* chromosome (4P, 5P, or 6P) and the gametocidal chromosome 2C<sup>c</sup> were selfed to obtain F<sub>2</sub> plants.

#### 2.1.2. *Ph1b* Mutant

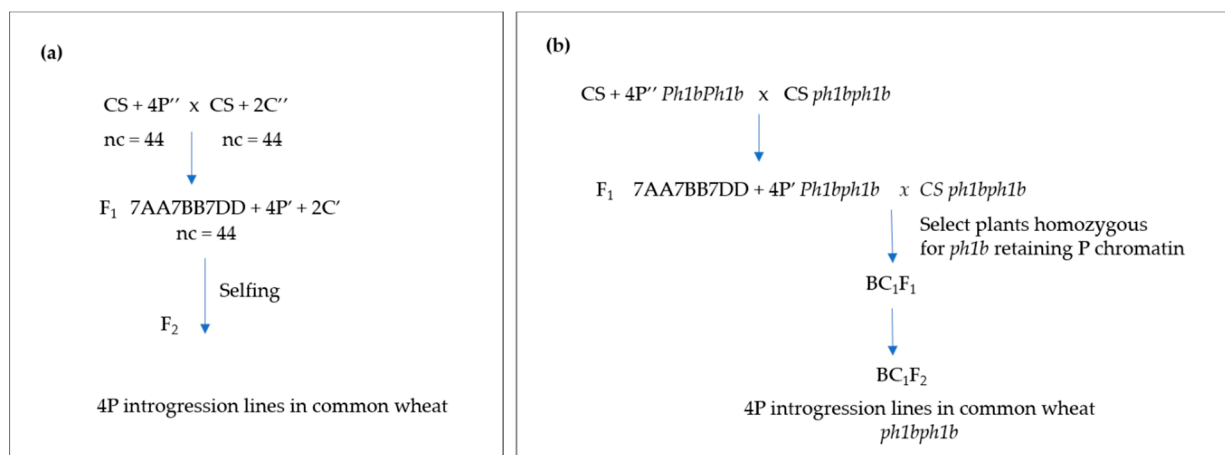
The disomic addition lines were crossed to the homozygous CS *ph1b* mutant. The F<sub>1</sub> plants monosomic for the *A. cristatum* chromosome (4P, 5P, or 6P) and heterozygous for the *Ph1b* gene were backcrossed to CS *ph1b* and the derived BC<sub>1</sub>F<sub>1</sub> individuals were screened using molecular markers to identify individuals with the genotype *ph1bph1b* and P chromatin in their genetic background. The individuals that were homozygous for *ph1b* and heterozygous for the *A. cristatum* chromosome were selfed to obtain the BC<sub>1</sub>F<sub>2</sub> generation. The BC<sub>1</sub>F<sub>2</sub> progeny were characterized by cytological and molecular markers analysis.

Figure 1 shows the breeding procedures used to obtain structural changes in *A. cristatum* chromosomes in a common wheat background.

### 2.2. Molecular Marker Characterization

Total genomic DNA was isolated from young leaf tissue using the CTAB method [41]. Samples were stored at –20 °C until PCR amplification was carried out. The concentration of each sample was estimated using a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

A specific molecular marker (AcOPX11-817) from *A. cristatum* developed by Wu et al. [42] was used to detect P genome introgressions in the wheat background. PCR conditions for the AcOPX11-817 marker were as follows: 5 min at 95 °C, followed by 34 cycles of 1 min at 94 °C, 1 min at 53 °C, and 90 s at 72 °C. Amplification products were analyzed on 2% agarose gels in 1 X TBE buffer and visualized by ethidium bromide staining.



**Figure 1.** The breeding procedures used to obtain structural changes in *A. cristatum* chromosomes in wheat background (i.e., 4P introgressions). (a) Crosses between the *A. cristatum* disomic addition lines in CS background and the gametocidal 2C<sup>c</sup> disomic addition line in CS; (b) Crosses between the *A. cristatum* disomic addition line in CS and the *ph1b* mutant were developed and backcrosses to the *ph1b* mutant to develop *A. cristatum* introgression lines in common wheat in the *ph1b* mutant background.

Conserved orthologous set (COS) markers [43] were used to identify chromosome arms involved in the translocations. Primer sequences for these markers and the annealing temperature (Ta) are given in Supplementary Table S1. These COS markers were selected for being polymorphic between *A. cristatum* and wheat CS and having previously transferred and determined arm locations on *A. cristatum* chromosomes [4,44]. PCR was performed with 40 ng of template DNA in a 25 µL reaction mixture containing 5 µL of 1× PCR buffer, 0.5 µM of each primer, 1.5 mM MgCl<sub>2</sub>, 0.3 mM dNTPs, and 0.625 U of Taq DNA polymerase (Promega, Madison, WI, USA). PCR conditions for the COS markers were as follows: 4 min at 94 °C, followed by 35 cycles of 45 s at 94 °C, 50 s at 58 °C, and 50 s at 72 °C. The PCR products were analyzed on polyacrylamide gels (10% w/v, C: 2.67%) stained with ethidium bromide.

A PCR assay described by Wang et al. [45] was used to verify the presence of *Ph1*. Each 30 µL PCR reaction contained 20 ng template DNA, 1× PCR buffer with MgCl<sub>2</sub>, 0.25 mM dNTPs, 0.17 µM primers, and 0.02 U/µL of Taq DNA polymerase (Promega, Madison, WI, USA). The reaction was first denatured at 94 °C for 5 min, and then subjected to 35 cycles of 94 °C for 60 s, 51 °C for 60 s, and 72 °C for 60 s, followed by a final extension at 72 °C for 7 min). The PCR products were electrophoretically separated through a 1% agarose gel and visualized by ethidium bromide staining.

### 2.3. Fluorescence In Situ Hybridization (FISH)

Chromosome spreads were prepared from root tip cells. Seeds were germinated on wet filter paper in the dark for 3 days at 4 °C, followed by a period of 24 h at 25 °C. Root tips from germinating seeds were excised and pretreated in ice water for 24 h and then fixed in freshly prepared ethanol-acetic acid (3:1 v/v) and stored at 4 °C for at least 1 month.

The plants were grown at greenhouse with a 16 h photoperiod and a day/night temperature of 25 °C/20 °C. The in situ hybridization protocol was that described by Cabrera et al. [46]. Both pAs1 and GAA-satellite probes were used to identify wheat chromosomes. The pAs1 probe hybridized primarily on the D-genome chromosomes and the GAA satellite sequence hybridized on B-genome chromosomes from wheat. Genomic DNA from *A. cristatum* was used as a probe to identify P genome introgressions. Total DNA of *A. cristatum* and GAA-satellite sequences were labeled with biotin-16-dUTP or digoxigenin-11-dUTP (Roche) using nick translation. The pAs1 probe was labeled with biotin-16-dUTP (Roche). Biotin- and digoxigenin-labeled probes were detected with streptavidin-Cy3 conjugates (Sigma, St. Louis, MO, USA) and anti-digoxigenin-FITC antibodies (Roche), respectively.

Chromosome preparations were hybridized with *A. cristatum* genomic DNA probe or simultaneously with both pAs1 and *A. cristatum* genomic DNA probes. After examination of metaphases, preparations were re-probed with the GAA-satellite sequence. The chromosomes were counterstained with 4',6-diamidino-2-phenylindole (DAPI) and mounted in Vectashield (Vector Laboratories, Inc., Burlingame, CA, USA). Hybridization signals were visualized using a Leica DMRB epifluorescence microscope and the images were captured with a Leica DFC7000T camera equipped with an exposimeter Spot Leica WildMPS52 and were processed with LEICA application suite v4.0 software (Leica, Germany).

### 3. Results

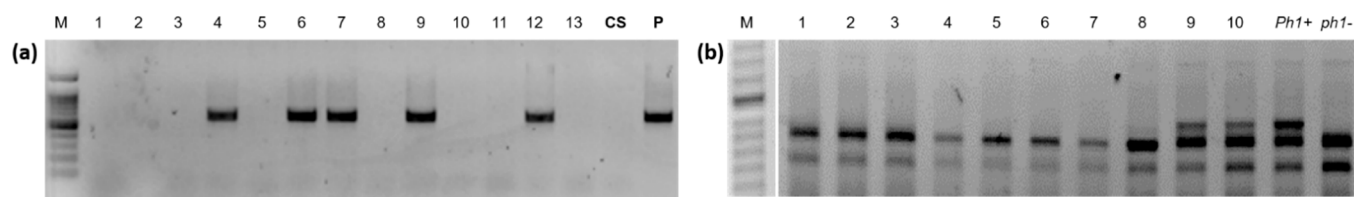
#### 3.1. Induction of Wheat-*A. cristatum* Introgressions by Gametocidal 2C<sup>c</sup> Chromosome

A total of 86, 90, and 217 F<sub>2</sub> plants were established from the crosses between the CS/*A. cristatum* disomic addition line for chromosomes 4P, 5P, and 6P, respectively and the CS/*Ae. cylindrica* disomic addition line for the 2C<sup>c</sup> gametocidal chromosome (Table 1). All 393 F<sub>2</sub> plants were screened for the presence of P genome chromatin using specific AcOPX11-817 molecular marker from *A. cristatum* (Figure 2a). Approximately 30% of these F<sub>2</sub> plants retained *A. cristatum* chromatin. The number of F<sub>2</sub> plants containing P genome introgressions varied in the offspring of the three different crosses, namely, 21 (24.4%), 32 (35.6%), and 66 (30.4%) F<sub>2</sub> plants were found with chromosome 4P, 5P, and 6P, respectively (Table 1). A cytogenetic analysis using FISH was carried out to identify the P genome introgression of the 119 wheat F<sub>2</sub> plants that were positive for the AcOPX11-817 molecular marker. Overall, approximately 4% were disomic and 80% were monosomic. Telosomic chromosomes, which most likely resulted from univalent misdivisions, were observed in six plants affecting chromosomes 4P, 5P, and 6P (Table 2). A total of 13 (10.9%) plants contained wheat-*A. cristatum* translocations: four involving chromosome 4P (19.0%), four involving chromosome 5P (12.5%), and five (7.6%) involving chromosome 6P (Table 2). Most of these wheat-*A. cristatum* rearrangements were whole-arm translocations involving A genome chromosomes from wheat, as demonstrated by the absence of both pAs1 and GAA hybridization signals on wheat chromosome arms involved in the translocations (Figure 3b,d,e,l,m). A dicentric chromosome involving chromosome 5P (Figure 3h), as well as wheat-*A. cristatum* chromosomes carrying small translocated fragments from 4PS and 5PS chromosome arms were also observed (Figure 3i,j). A deletion in chromosome 4P was also found (Figure 3k). The *A. cristatum* chromosome arm involved in the wheat-*A. cristatum* translocations was identified using chromosome specific COS molecular markers transferred from wheat to *A. cristatum* (Figure 4).

**Table 1.** Progeny retaining *A. cristatum* chromatin. (a) F<sub>2</sub> plants from crosses between the disomic addition line for chromosomes 4P, 5P and 6P, respectively, and the CS disomic addition line for gametocidal 2C<sup>c</sup> chromosome; (b) BC<sub>1</sub>F<sub>1</sub> and BC<sub>1</sub>F<sub>2</sub> plants from crosses between the disomic addition line for chromosomes 4P, 5P and 6P, respectively, and CS *ph1* mutant.

Cross	No. of Plants	No. of Plants Retaining P Chromatin	No. of BC <sub>1</sub> F <sub>1</sub> Plants Retaining P Chromatin and <i>ph1ph1</i>	No. of BC <sub>1</sub> F <sub>2</sub> Plants Evaluated	No. of BC <sub>1</sub> F <sub>2</sub> Plants Retaining P Chromatin
(a) 2C <sup>c</sup> Gametocidal					
CS + 4P × CS + 2C <sup>c</sup>	86 <sup>1</sup>	21 (24.4%) <sup>1</sup>	-	-	-
CS + 5P × CS + 2C <sup>c</sup>	90 <sup>1</sup>	32 (35.6%) <sup>1</sup>	-	-	-
CS + 6P × CS + 2C <sup>c</sup>	217 <sup>1</sup>	66 (30.4%) <sup>1</sup>	-	-	-
Total	393 <sup>1</sup>	119 (30.3%) <sup>1</sup>	-	-	-
(b) CS <i>ph1b</i> mutant					
CS + 4P × CS <i>ph1</i>	97 <sup>2</sup>	36 (37.1%) <sup>2</sup>	17 (47.2%)	28	19 (67.8%)
CS + 5P × CS <i>ph1</i>	94 <sup>2</sup>	45 (47.8%) <sup>2</sup>	24 (53.3%)	56	24 (42.8%)
CS + 6P × CS <i>ph1</i>	140 <sup>2</sup>	57 (38.0%) <sup>2</sup>	22 (38.6%)	72	25 (34.7%)
Total	331 <sup>2</sup>	138 (41.4%) <sup>2</sup>	63 (45.6%)	156	68 (43.6%)

<sup>1</sup> F<sub>2</sub> plants; <sup>2</sup> BC<sub>1</sub>F<sub>1</sub> plants.



**Figure 2.** Genotypic assays for the presence of *A. cristatum* introgressions and *Ph1*. (a) The presence of P genome introgression from *A. cristatum* (lanes 4, 6, 7, 9 and 12) is marked by the successful amplification of the AcOPx11-817 fragment. (b) The absence of *Ph1* is marked by the ABC920 SCAR marker (individuals 1-8). M: size marker; CS: *Triticum aestivum* cv Chinese Spring; P: *Agropyron cristatum*; *Ph1+*: wild type wheat CS; *ph1-*: the parental *ph1b* mutant.

**Table 2.** Transmission of P-genome chromatin and wheat-*A. cristatum* translocations. (a) F<sub>2</sub> plants from crosses between the disomic addition line for chromosomes 4P, 5P and 6P, respectively, and the CS disomic addition line for gametocidal 2C<sup>c</sup> chromosome; (b) BC<sub>1</sub>F<sub>2</sub> plants from crosses between the disomic addition line for chromosomes 4P, 5P and 6P, respectively and CS *ph1* mutant.

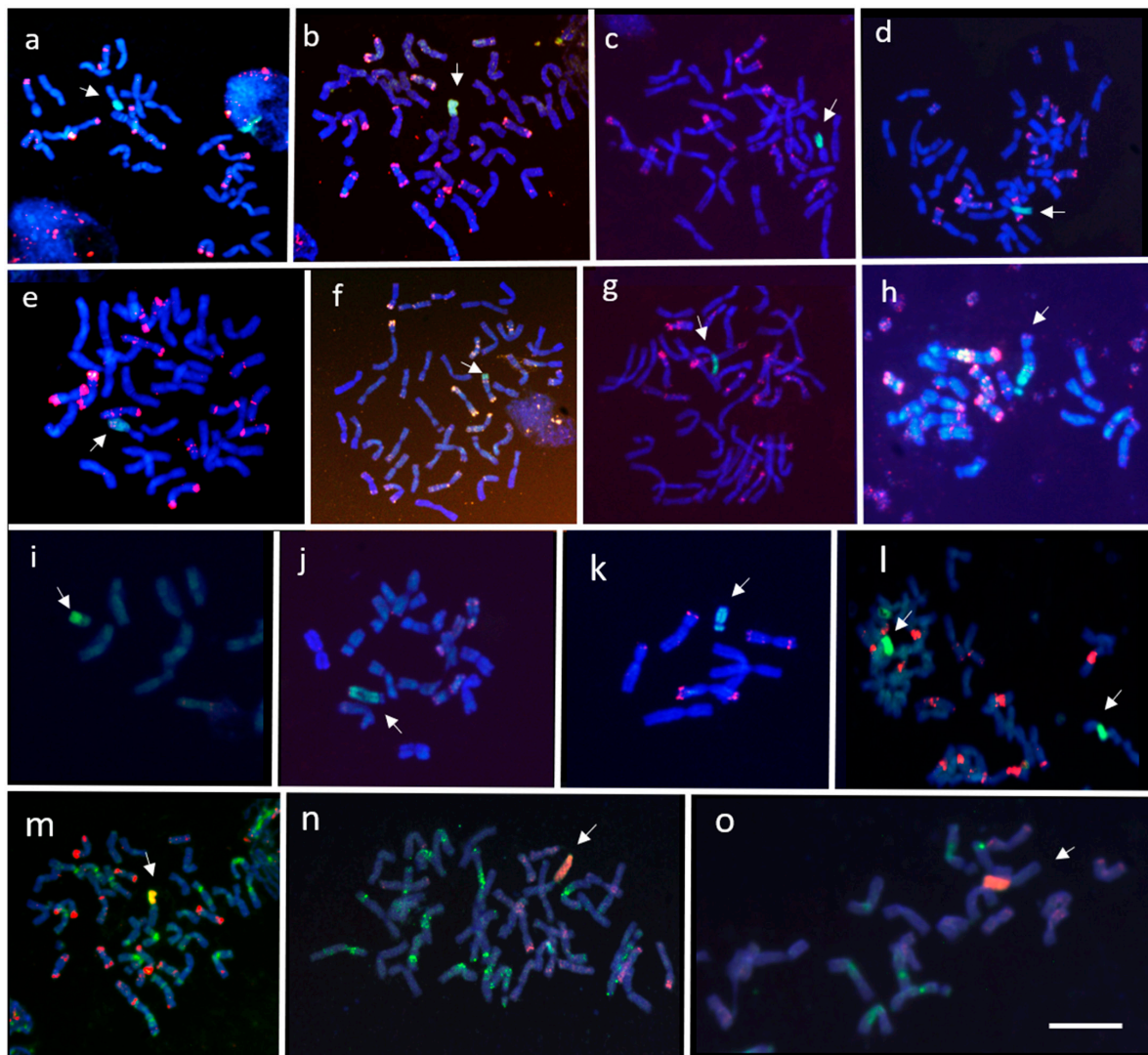
Cross	No. of Plants	Complete Chromosome		Telosomic Chromosome	Ditelosomic Chromosome	Wheat- <i>A. cristatum</i> Translocation
		2 Copies	1 Copy			
<b>(a) 2C<sup>c</sup> Gametocidal</b>						
CS + 4P × CS + 2C <sup>c</sup>	21	2 (9.5%)	13 (61.9%)	2 (9.5%)	0	4 (19.0%)
CS + 5P × CS + 2C <sup>c</sup>	32	1 (3.1%)	25 (78.1%)	2 (6.2%)	0	4 (12.5%)
CS + 6P × CS + 2C <sup>c</sup>	66	2 (3.0%)	57 (86.4%)	1 (1.5%)	1 (1.5%)	5 (7.6%)
Total	119	5 (4.2%)	95 (79.8%)	5 (4.2%)	1 (0.8%)	13 (10.9%)
<b>(b) CS <i>ph1b</i> mutant</b>						
CS + 4P × CS <i>ph1</i>	19	1 (5.3%)	13 (68.4%)	2 (10.5%)	0	3 (15.8%)
CS + 5P × CS <i>ph1</i>	24	4 (16.7%)	16 (66.67%)	2 (8.4%)	1 (4.2%)	1 (4.2%)
CS + 6P × CS <i>ph1</i>	25	4 (16.0%)	15 (60.0%)	2 (8.0%)	2 (8.0%)	2 (8.0%)
Total	68	9 (13.2%)	44 (64.7%)	6 (8.8%)	3 (4.4%)	6 (8.8%)

### 3.2. Induction of Wheat-*A. cristatum* Introgressions by *ph1b* Mutant

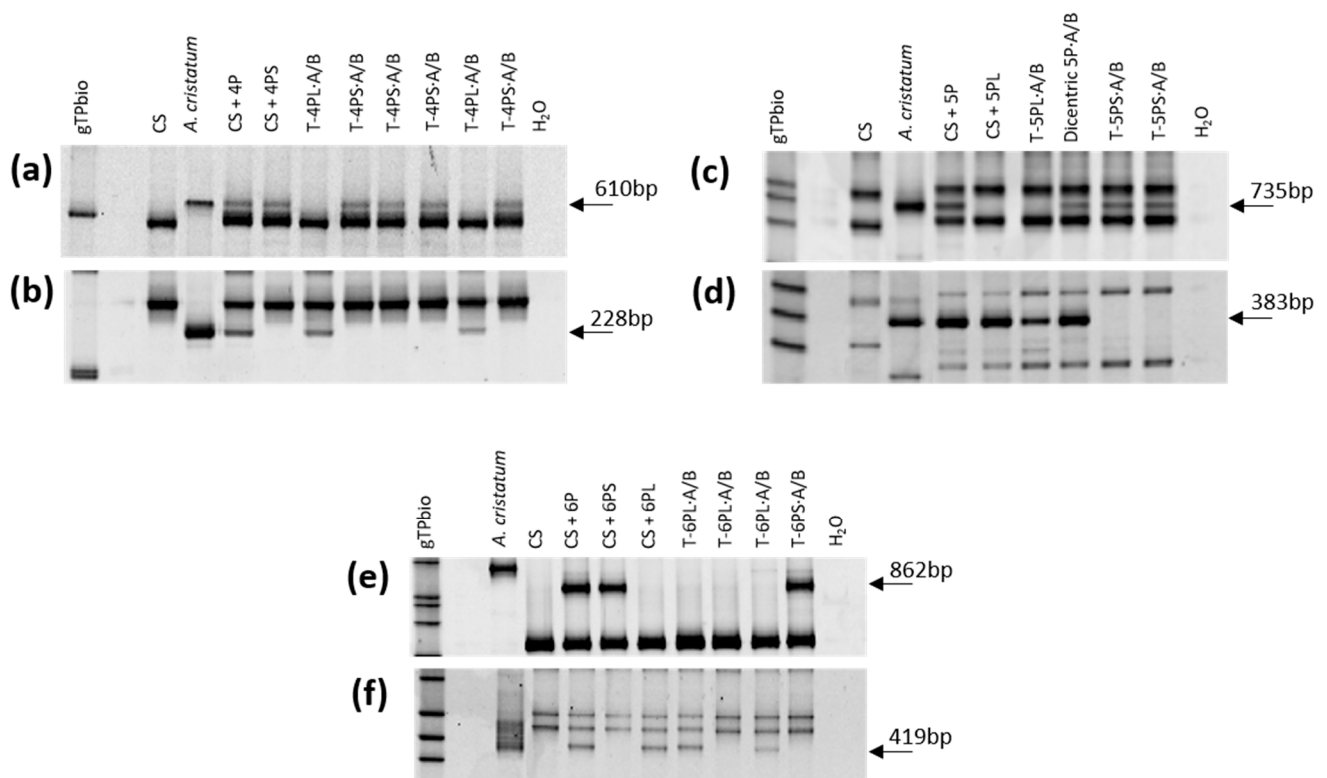
A total of 97, 94, and 140 BC<sub>1</sub>F<sub>1</sub> plants were established from the crosses between the CS/A *cristatum* disomic addition line for chromosomes 4P, 5P, and 6P, with CS *ph1* mutant (Table 1). These 331 BC<sub>1</sub>F<sub>1</sub> plants were genotyped using P-specific AcOPX11-817 molecular marker (Figure 2a). The transmission frequencies of *A. cristatum* chromosomes through female gametes varied in the offspring of the three different crosses: 36 (37.1%), 45 (47.8%), and 57 (38.0%) plants for chromosome 4P, 5P, and 6P, respectively (Table 1). Overall, approximately 41% of the progeny retained *A. cristatum* chromatin. Zygosity at the *Ph1* locus was predicted using a PCR assay (Figure 2b). Based on an analysis using the *ph1b* diagnostic ABC920 SCAR marker, 63 of the 138 BC<sub>1</sub>F<sub>1</sub> plants were identified to be homozygous for *ph1b* and heterozygous for the *A. cristatum* chromosome: 17 (47.2%) for 4P, 24 (53.3%) for 5P, and 22 (38.6%) for 6P, respectively. Among the derived BC<sub>1</sub>F<sub>2</sub> plants, 156 were genotyped using the P-specific AcOPX11-817 molecular marker and 68 retained P chromatin. These BC<sub>1</sub>F<sub>2</sub> plants were analyzed by FISH and specific COS molecular markers to detect and characterize wheat-*A. cristatum* chromosome arrangements in the background of the *ph1b* mutant (Table 2). Overall, approximately 13% were disomic and 65% were monosomic. Mono-telosomic and di-telosomic chromosome arrangements most likely resulted from univalent misdivisions and were observed in nine plants in total. A total of six individuals harbored translocations involving *A. cristatum*: three (15.8%) for chromosome 4P, one for chromosome 5P (4.2%) and two (8.0%) for chromosome 6P. The average induction fre-



quency of chromosome structural aberrations was 8.8%. Among these recombinants, most of the observed aberrations were whole arm translocations (Figure 3a,c,g). The absence of both pAs1 and GAA hybridization signals on wheat chromosome arms involved in the centromeric translocations suggest that these chromosome arms were from genome A (Figure 3a,c,g,n,o). One small terminal introgression involving the 6PS chromosome arm and 6D wheat chromosome was obtained (Figure 3f). The P chromosome arm involved in the wheat-*A. cristatum* translocation was identified using chromosome specific COS molecular markers transferred from wheat to *A. cristatum* (Supplementary Table S1). Examples of PCR amplification profiles used for identifying wheat-*A. cristatum* translocations are shown in Figure 4.



**Figure 3.** FISH with *A. cristatum* genomic DNA (green) and pAs1 repetitive (red) probes to mitotic metaphase of wheat-*A. cristatum* introgression lines. (a) wheat-4PS centromeric translocation; (b) wheat-4PL centromeric translocation; (c) wheat-5PS centromeric translocation; (d) wheat-5PL centromeric translocation; (e) wheat-6PS centromeric translocation; (f) terminal wheat-6PS translocation; (g) wheat-6PL centromeric translocation; (h) dicentric wheat-*A. cristatum* involving chromosome 5P (i) and (j) small wheat-*A. cristatum* translocations; (k) deficient 4P chromosome (l) wheat-5PS centromeric translocations hybridized with genomic *A. cristatum* genomic DNA (green) and GAA repetitive sequence (red); (m) the same cell as in (b) re-probed with GAA repetitive sequence (green); (n) and (o) centromeric wheat-6PL and wheat-6PS centromeric translocation, respectively, hybridized with *A. cristatum* genomic DNA (red) and GAA repetitive sequence (green). Chromosome rearrangements were arrowed. The wheat genetic background is ‘Chinese Spring’. Wheat chromosomes are counterstained with DAPI. (a,c,f,g,l,n,o) obtained by *Ph1b* mutant; (b,d,e,h,i,j,k,m) obtained by gametocidal 2C<sup>c</sup> chromosome. Scale bar, 10  $\mu$ m.



**Figure 4.** Molecular characterization of wheat-*A.cristatum* translocation lines using chromosome-specific COS markers. (a) C:757404-4PS; (b) C:739175-4PL; (c) C:728036-5PS; (d) C:762245-5PL; (e) C:741456-6PS; (f) C:755292-6PL. The differences in the size of the amplified COS fragment in the *A. cristatum* genome used as control and the *A. cristatum* chromosome present in the addition lines for some markers are due to genetic differences between the *A. cristatum* accession used in this study and the accession used to obtain the addition lines.

#### 4. Discussion

Chromosome 2C<sup>c</sup> from *Ae. cylindrica* induces chromosome breaks in CS wheat [30] and some of its relatives [31,32,35–37]. In the present study, wheat-*A. cristatum* translocations were successfully obtained utilizing the gametocidal chromosome 2C<sup>c</sup> from *Ae. cylindrica*. Most of the translocation lines obtained by both gametocidal and *Ph1b* mutation approaches were whole-arm translocations, indicating that both the *A. cristatum* and wheat chromosomes were more easily broken at the centromere. In addition, chromosome breakages were also found in the interstitial regions of the chromosome arms as demonstrated by the wheat-*A. cristatum* dicentric translocation detected in the F<sub>2</sub> progeny from the CS + 4P × CS + 2C<sup>c</sup> cross (Figure 3h), as well as the terminal 6P-6D translocation detected in the progeny from the CS + 6P × CS<sup>ph1</sup> cross (Figure 3f). Chromosome breakages in the interstitial regions of the chromosome arms also resulted in deficient chromosomes (Figure 3k).

Chromosome translocations between wheat and the P genome have not been widely reported. Luan et al. [15] used the wheat-*A. cristatum* disomic addition line for chromosome 6P as bridge materials to produce wheat-*A. cristatum* 6P translocation lines induced by gametocidal chromosomes. Luan et al. [15] found that the frequency of the translocation induced by the gametocidal chromosome was 5.08%, which is similar to the frequency found for individuals carrying wheat-6P chromosome translocations generated by the gametocidal chromosome 2C<sup>c</sup> (7.6%) in this work. Liu et al. [38] obtained a translocation frequency of 3.75% between wheat and an *A. cristatum* chromosome 4P induced by gametocidal 2C<sup>c</sup>, which was lower than the 19.0% obtained in the present study.

Attempts to induce translocations between *A. cristatum* and wheat genomes using the *ph1b* mutation have been previously carried out by Jubault et al. [47]. The authors [47] found

that allosyndetic association between P and ABD genomes in F<sub>1</sub> hybrids are very rare and that the low frequency of pairing is likely due to divergence between *Agropyron* and wheat genomes. It has been also suggested that P chromosomes might carry a genetic system that inhibits the *Ph* gene in wheat [48,49]. The *A. cristatum Ph1b* suppressor system appears to be polygenic with all chromosomes, except 2P, promoting homoeologous pairing [49]. However, the genes involved in the *A. cristatum* suppression system are not efficient enough to induce homoeologous pairing between *A. cristatum* and wheat genomes and its effect on the *Ph* genetic system is much weaker than the effect of the *ph1b* deletion [49]. In this work, no wheat-*A. cristatum* translocations were observed in plants with genotype *Ph1bPh1b* (data not shown), which agrees with these findings.

In this study the frequency of *A. cristatum*-wheat translocations induced by the *ph1b* deletion was 15.8% for chromosome 4P, 4.2% for chromosome 5P, and 8.0% for chromosome 6P. In *Hordeum chilense*, a species from the tertiary gene pool of wheat (similar to *A. cristatum*), the frequency of *Hordeum chilense*-wheat translocations induced using *ph1b* mutant ranged from 3.3% to 8% depending of the *H. chilense* chromosome involved in the translocation [50]. These results are similar to the frequency that we observed for the *A. cristatum*-wheat translocation induced by the *ph1b* deletion. Most of the wheat-*A. cristatum* translocations were obtained with wheat genome A chromosomes, as demonstrated by the absence of both pAs1 and GAA hybridization signals in the wheat chromosomes involved in these translocations (see Figure 3). These results suggested that the P genome from *A. cristatum* may be more closely related to the wheat A genome than B or D genome. Zhou et al. [51] suggested that, compared with the wheat A and B genomes, the P and D genomes had a greater variant density and a longer evolutionary distance, suggesting that *A. cristatum* is more distantly related to the wheat D genome. Unfortunately, the absence of GAA hybridization signals on wheat chromosomes arms involved in the translocations hampered the identification of the wheat chromosome involved in the translocations. Further studies should be conducted to identify the wheat chromosome involved in the translocations.

It has been suggested that, among the different strategies developed to produce wheat-alien chromosome translocations, induced homoeologous pairing is the method of choice if gene synteny is conserved [23]. A highly conserved synteny between wheat and *A. cristatum* has been found. For example, a high transferability of COS markers between wheat and *A. cristatum* has been reported [4,44,52]. By sequencing the transcriptome of a tetraploid *A. cristatum* accession, Zhou et al. [53] found that the sequence similarity of most *A. cristatum* and wheat genes were greater than 95%. The synteny in homoeologous group 5 is also conserved between *A. cristatum* and wheat, as shown for agronomically important genes, such as the grain hardness (*Ha*) locus (mapped on the short arm of chromosome 5P) [54] and the vernalization (*VRN-1*) locus (mapped on the long arm of chromosome 5P) [55], indicating that these loci are collinear which those located on the short and long arms, respectively of chromosome 5 in each of the wheat A, B, and D genomes.

In conclusion, the genetic stocks characterized here included new wheat-*A. cristatum* recombinations useful for genetic studies and for transferring agronomics traits from the tertiary gene pool to wheat cultivars.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/2073-4395/11/2/277/s1>, Supplemental Table S1.

**Author Contributions:** A.C. conceived and designed the study; A.C.-P. and C.P. performed the experiments; A.C. and A.C.-P. analyzed the data; A.C. wrote the paper. All authors have read and agreed to the published version of the manuscript.

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