

New Insights Into the Introgression Between Agropyron Cristatum P Genome and Wheat Genome

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Research Article

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Abstract

Agropyron cristatum ($2n = 4x = 28$, PPPP) is an important wild relative of common wheat and confers desirable agronomic traits to common wheat. A previous report showed that the wheat-*A. cristatum* 6P translocation line WAT655 carrying *A. cristatum* 6PS (0.81–1.00) exhibited high resistance to prevalent physiological races (CYR32 and CYR33). In this study, three disease resistance-related transcriptomes, which were mapped to *A. cristatum* 6PS (0.81–1.00) through the analysis of specific molecular markers, were searched from among *A. cristatum* full-length transcriptomes. Then, three disease resistance-related gene markers, *A. cristatum* P genome-specific markers, and fluorescence in situ hybridization (FISH)/genomic in situ hybridization (GISH) probes made from the DNA of three bacterial artificial chromosome (BAC) clones, three genes, and *A. cristatum* “Z559” were used to analyze the BC₅F₂ and BC₅F_{2,3} genetic populations of the translocation line WAT655. The results revealed the introgression can spontaneously occur between *A. cristatum* P genome and wheat genome, and indicated the three genes could constitute a gene cluster according to the positions of their FISH signals. Additionally, kompetitive allele-specific PCR (KASP) markers of the three genes were developed to detect and acquire 24 wheat-*A. cristatum* breeding materials, which showed resistance to physiological races (CYR32 and CYR33) and other desirable agronomic traits according to the field investigation. In conclusion, our study not only provides new insights into the introgression between *A. cristatum* P genome and wheat genome, but also provides the desirable breeding materials for breeding practice.

Key Message

The introgression can spontaneously occur between *A. cristatum* P chromatin and wheat chromosomes, and wheat-*A. cristatum* breeding materials were produced by the introgression.

Introduction

Common wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD) is one of the most cultivated cereal crops in the world. The total wheat acreage was approximately 220 million hectares, and the yield was more than 770 million tons globally in 2020 (World Agricultural Production 2021). Wheat is a staple food crop that can provide starch, protein, vitamins, dietary fiber, and phytochemicals for humans, and wheat also provides approximately 20% of the calories consumed by humans (Shewry and Hey 2015). However, wheat often suffers from various biotic and abiotic stresses during production, and the narrow genetic basis of wheat restricts its genetic improvement (Friebe et al. 1996). Wheat stripe rust is a fungal disease caused by the fungus *Puccinia striiformis* f. sp. *tritici* (*Pst*). Approximately 90% of cultivated wheat is susceptible to *Pst*, and more than five million tons of the wheat harvest are lost annually (Wellings 2011; Beddow et al. 2015). With the emergence of new *Pst* races and the variations in existing *Pst* races, many wheat varieties have lost resistance to stripe rust (Wan et al. 2004; Chen et al. 2009). Therefore, providing new wheat germplasms with resistance to *Pst* infection is necessary for advancing wheat breeding.

Wild relatives of wheat are crucial gene resources for broadening the genetic basis of wheat and facilitating wheat breeding. Wild relatives of wheat carry numerous disease resistance genes (Dewey 1984; Dong et al. 1992). Moreover, with the increasing number of successful distant hybridizations, an increasing number of exogenous genes from wild relatives, particularly, disease resistance genes, have been transferred into common wheat. The powdery mildew resistance gene *Pm21* from *Haynaldia villosa* has been transferred into common wheat and widely applied in breeding practice (Cao et al. 2011; Chen et al. 2013; Xing et al. 2018). Multiple stem rust resistance genes from *Triticum monococcum*, such as *Sr21*, *Sr22*, *Sr35*, and *Sr60*, have been cloned in common wheat (Gerechter-Amitai et al. 1971; McIntosh et al. 1984; Saintenac et al. 2013; Steuernagel et al. 2016; Chen et al. 2018). Stripe rust resistance genes, such as *YrAS2388*, *Yr28*, and *YrY201* from *Aegilops tauschii* (Singh et al. 2000; Zhang et al. 2008; Huang et al. 2011; Liu et al. 2013; Zhang et al. 2018), and *Yr15* from wild emmer wheat (Klymiuk et al. 2018), have been cloned from common wheat. Hence, wild relatives of wheat are indeed an abundant gene pool for providing disease resistance genes.

Agropyron cristatum ($2n = 4x = 28$), an important wild relative of wheat, carries several desirable agronomic traits that can be applied in wheat breeding. With the achievement of the distant hybridization of wheat and *A. cristatum*, a series of wheat-*A. cristatum* derived lines were generated (Limin and Fowler 1990; Li et al. 1995, 1997; Wu et al. 2006; Lu et al. 2017; Li et al. 2017; Said et al. 2019). Many desirable genes from *A. cristatum* have been transferred into common wheat in the form of translocation lines. For example, *A. cristatum* P chromosomal segments carrying the powdery mildew resistance gene, the high-thousand grain weight (TGW) gene, and the high-grain number per spike (GNS) gene have been translocated into wheat chromosomes (Li et al. 2017; Lu et al. 2017; Zhang et al. 2019a). According to recent reports, the leaf rust resistance gene and the powdery mildew resistance gene from *A. cristatum* 2P chromosome have been localized on the same segment of *A. cristatum* 2PL (0.66–0.86) (Jiang et al. 2018), and both the stripe rust resistance gene and the leaf rust resistance gene were mapped to *A. cristatum* 6PS (0.81–1.00) (Zhang et al. 2017). Although wheat-*A. cristatum* translocation lines carry many desirable genes, they remain difficult to directly apply in wheat breeding because of the genetic drag caused by the exogenous chromosomal segments. Therefore, the identification of the introgression lines carrying smaller exogenous components is necessary for improving wheat breeding.

At present, over 80 wheat stripe rust resistance genes (*Yr1*–*Yr81*) have been named (Gessese et al. 2019), however, a small number of stripe rust resistance (*Yr*) genes have been cloned. Of the *Yr* genes cloned so far, *Yr5*, *Yr7*, *YrSP*, and *YrAS2388R* encode nucleotide-binding domain and leucine-rich repeat domain (Marchal et al. 2018; Zhang et al. 2019b); *Yr36* and *Yr15* encode kinase domain (Fu et al. 2009; Klymiuk et al. 2018). Moreover, in addition to stripe rust resistance genes, most of the cloned other disease resistance genes in wheat also encode nucleotide-binding

domain, leucine-rich repeat domain, or kinase domain (Krattinger and Keller 2016). For example, powdery mildew resistance genes (*Stpk-V*, *Pm21*, *Pm41*, and *Pm60*) (Cao et al. 2011; Zou et al. 2017; Xing et al. 2018; Li et al. 2020) and stem rust resistance genes (*Sr22*, *Sr35*, *Sr45*, and *Sr60*) (Saintenac et al. 2013; Steuernagel et al. 2016; Chen et al. 2018) encode nucleotide-binding domain, leucine-rich repeat domain, or kinase domain. Therefore, nucleotide-binding domain, leucine-rich repeat domain, and kinase domain can be considered as typical domains to help the clone of disease resistance genes.

In this study, specific molecular markers and fluorescence in situ hybridization (FISH)/genomic in situ hybridization (GISH) probes made from the DNA of three bacterial artificial chromosome (BAC) clones, three disease resistance-related *A. cristatum* genes, and *A. cristatum* “Z559” were used to analyze the genetic populations of the wheat-*A. cristatum* 6P translocation line WAT655. The results revealed the spontaneous introgression between *A. cristatum* P genome and wheat genome. In addition, a few wheat-*A. cristatum* breeding materials, which can be directly applied in breeding practice, were acquired by using kompetitive allele-specific PCR (KASP) markers. This study provides new insights into the introgression between *A. cristatum* P genome and wheat genome, and provides new wheat-*A. cristatum* breeding materials for breeding practice.

Materials And Methods

Plant materials

The plant materials utilized in this study included the following: *A. cristatum* accession “Z559” ($2n = 4x = 28$, PPPP, from Xinjiang, China), a wheat-*A. cristatum* 6P disomic addition line 4844-12 ($2n = 44$) (Wu et al. 2006), a wheat-*A. cristatum* 6P disomic substitution line 4844-12-1 ($2n = 42$) (Table 1 and Fig. S1), a wheat-*A. cristatum* 6P terminal translocation line WAT655, a 6PS whole-arm translocation line WAT638a, a 6PL whole-arm translocation line WAT638b (Song et al. 2016a; Zhang et al. 2017) (Table 1), *Triticum aestivum* cv. Fukuhokomugi (Fukuho) ($2n = 6x = 42$, AABBDD), wheat varieties (Zhoumai18, Jimai22, Shi4185, Luyuan502, Gaocheng8901, and Xinong979), and 500 wheat-*A. cristatum* breeding materials. The wheat-*A. cristatum* 6P disomic addition line 4844-12 was produced via distant hybridization of *A. cristatum* accession “Z559” and common wheat “Fukuho” (Wu et al. 2006). The wheat-*A. cristatum* 6P disomic substitution line 4844-12-1 was produced from the progenies of wheat-*A. cristatum* 6P disomic addition line 4844-12. Wheat-*A. cristatum* 6P translocation lines WAT655, WAT638a, and WAT638b were produced by radiating hybrid plants of wheat-*A. cristatum* 6P disomic addition line 4844-12, which were acquired via strict backcrossing with common wheat “Fukuho” as the recurrent parent and self-pollination. A total of 500 wheat-*A. cristatum* breeding materials were selected from wheat-*A. cristatum* introgression lines according to the agronomic traits of *A. cristatum*. All of the plant materials were preserved at the Center of Crop Germplasm Resources Research at the Institute of Crop Science, Chinese Academy of Agricultural Sciences (Beijing, China).

Molecular cytogenetic analysis

A. cristatum P genome-specific markers were designed from P genome-specific repeat sequences (Table S1) (Han et al. 2017). P genome-specific markers were also used to detect *A. cristatum* 6P chromatin in the genetic populations of the translocation line WAT655.

The *A. cristatum* full-length transcriptomes were analyzed by using TransDecoder (version v5.5.0) (Grabherr et al. 2011) to determine the CDSs and protein sequences, the software eggNOG-mapper (Huerta-Cepas et al. 2017) and BLASTx from the National Center of Biotechnology Information (NCBI) website were used to annotate these sequences. The disease resistance-related genes were searched according to the annotation file, and specific molecular markers were developed for the analysis of wheat-*A. cristatum* 6P translocation line WAT655. Additionally, the specific sequences of three genes were used to design KASP markers to trace *A. cristatum* P chromatin in wheat-*A. cristatum* breeding materials (Fig. S2 and Table S2). HiGeno 2x Probe Mix (JasonGen, Beijing, China) was used for the KASP reaction, and PCR products were detected with a PHERAstarplus SNP genotyping instrument (LGC Science Shanghai Ltd., China). The software KlusterCallerTM was used for genotyping assays.

FISH and GISH were performed in root tip cells, as described by Cuadrado et al. (2000). The DNA of three BAC clones, three disease resistance-related genes, and *A. cristatum* “Z559” were used as FISH/GISH probes, and the genomic DNA of common wheat “Fukuho” was used as the blocker. The FISH/GISH probes and the blocker were used at a 1:40 ratio. Oligo-pTa535-1 (red) and Oligo-pSc119.2-1 (green) (Tang et al. 2014) (Table S1) were used to analyze the wheat chromosomes of the substitution line 4844-12-1. The QIAGEN Large-Construct Kit (Qiagen, Germany) was used to extract the DNA of the three BAC clones. The DNA of three disease resistance-related genes was acquired from the three *A. cristatum* “Z559” genomic BAC clones, respectively. The DNA of *A. cristatum* “Z559”, the three BAC clones (BAC1013, BAC700, and BAC940), the three genes (*Agr6971*, *Agr4080*, and *Agr8173*), and pAs1 (Pedersen and Langridge 1997) was labeled with Texas Red®-5-dCTP (red) (PerkinElmer, Waltham, America). The DNA of *A. cristatum* “Z559”, the gene *Agr4080*, and pHvG39 (Pedersen and Langridge 1997) was labeled with Fluorescein-12-dUTP (green) (PerkinElmer, Waltham, America). VECTASHIELD® Antifade Mounting Medium with DAPI (Vector Laboratories, Burlingame, America) was used for the FISH/GISH experiments. Signals were captured using an OLYMPUS AX80 (Olympus Corporation, Tokyo, Japan) fluorescence microscope with a CCD camera (Diagnostic Institute, Inc., Sterling Height, MI, USA) and processed with Photoshop CS 3.0.

Evaluation of the agronomic traits of wheat-*A. cristatum* breeding materials in the field

To evaluate stripe rust resistance, the wheat-*A. cristatum* 6P disomic addition line 4844-12, the wheat-*A. cristatum* 6P disomic substitution line 4844-12-1, the wheat-*A. cristatum* 6P translocation line WAT655, wheat varieties (Zhoumai18, Jimai22, Shi4185, Luyuan502, Gaocheng8901, and

Xinong979), 500 wheat-*A. cristatum* breeding materials, and the common wheat cultivar “Fukuho” were planted in a randomized complete block design with three replicates in the field in Beijing (39°54'20" N, 116°25'29" E, China). Twenty grains of each material were planted in 2.0 m rows that were spaced 0.3 m apart. The common wheat “Fukuho” was used as the control. The prevalent physiological races CYR32 and CYR33 were used to inoculate the plant materials at the elongation stage in the field at Beijing. The infection type (IT) score ranged from 0 to 9 (0: no visible symptoms; 1–2: necrotic flecks or necrotic areas without sporulation; 3–4: trace or light sporulation; 5–6: intermediate or moderate sporulation; 7–8: abundant sporulation; 9: no necrosis or chlorosis and abundant sporulation) (Line and Qayoum 1992). Plants with IT scores of 0–2 were considered as highly resistant type; plants with IT scores of 3–4 were considered as moderately resistant type; plants with IT scores of 5–6 were moderately susceptible type; plants with IT scores of 5–9 were considered as highly susceptible type.

To evaluate agronomic traits, wheat varieties (Zhoumai18, Jimai22, Shi4185, Luyuan502, Gaocheng8901, and Xinong979) and 500 wheat-*A. cristatum* breeding materials were planted in 1.2 × 6 m² plots with four replicates in the field in Xinxiang (35°18'13" N, 113°55'15" E, Henan Province, China). The plant height, plot yield, TGW, and germination rate of each material were measured.

Results

The capture of disease resistance-related *A. cristatum* genes of the translocation line WAT655

In previous reports, the wheat-*A. cristatum* 6P translocation line WAT655 carrying *A. cristatum* 6PS (0.81–1.00) exhibited high resistance to stripe rust (CYR32 and CYR33) and leaf rust in the adult stage (Song et al. 2016a; Zhang et al. 2017). To further explore the disease resistance genes of *A. cristatum* 6PS (0.81–1.00), the disease resistance-related transcriptomes were searched from among the *A. cristatum* full-length transcriptomes (Zhou et al. 2019). The software TransDecoder (version v5.5.0) (Grabherr et al. 2011) was used to determine protein sequences, and the software eggNOG-mapper (Huerta-Cepas et al. 2017) and BLASTx from the NCBI website were used for the functional annotation. Then, according to the sequences of disease resistance-related transcriptomes, molecular markers were developed to trace the *A. cristatum* 6P chromatin of the translocation line WAT655. Three disease resistance-related transcriptomes were mapped to *A. cristatum* 6PS (0.81–1.00) of the translocation line WAT655 through the analysis of molecular markers (Fig. 1 and Table 2). These results provided the basis for further exploring the disease resistance genes of *A. cristatum* 6PS (0.81–1.00).

Tracing *A. cristatum* P chromatin in the BC₅F₂ and BC₅F_{2:3} genetic populations of the translocation line WAT655 using the molecular markers

To trace *A. cristatum* P chromatin in the BC₅F₂ population (2019–2020) of the translocation line WAT655, *A. cristatum* P genome-specific markers and the gene markers from three disease resistance-related transcriptomes were utilized in this study. The P genome-specific markers were designed by using P genome-specific repeat sequences (Han et al. 2017), and the gene markers were designed from the sequences of the three *A. cristatum* transcriptomes in this study. Therefore, all of these molecular markers can accurately trace *A. cristatum* P chromatin in common wheat. However, the detection results of the gene markers were different from the results of the P genome-specific markers in some plants of the BC₅F₂ population of the translocation line WAT655 (Fig. 2). Preliminarily speculating, some of *A. cristatum* 6PS (0.81–1.00) chromatin in the translocation line WAT655 might have been spontaneously transferred into common wheat background, which resulted in the differences between the two kinds of molecular markers in some plants.

To confirm whether the introgression between *A. cristatum* P chromatin and wheat chromosomes could occur in the BC₅F_{2:3} population (2020–2021), progenies from the BC₅F₂ population (2019–2020) of the translocation line WAT655 were planted in 2020–2021. The two kinds of molecular markers were utilized to detect P genomic components in the BC₅F_{2:3} population, and the results revealed that some plants still showed differences between the gene markers and P genome-specific markers (Fig. 2). These results confirmed the potential for the introgression between *A. cristatum* 6P chromatin of the translocation line WAT655 and wheat chromosomes.

The identification of the introgression between *A. cristatum* P genome and wheat genome in translocation line WAT655

To acquire more sequence information of three disease resistance-related transcriptomes, the gene markers from the three transcriptomes were used to search *A. cristatum* “Z559” P genomic BAC bank, moreover, three BAC clones (BAC1013, BAC700, and BAC940) of three genes were acquired (Table 2). Pacific Biosciences technology was used to sequence the three BAC clones, and the software Hifiasm (Cheng et al. 2021) was used to assemble the sequence data. This work was performed by BioMarker Company (Beijing, China).

To further analyze the introgression between *A. cristatum* P genome and wheat genome, the DNA of the three BAC clones, three disease resistance-related genes, and *A. cristatum* “Z559” was utilized as the probes to perform FISH and GISH in common wheat “Fukuho”, the translocation line WAT655, and the substitution line 4844-12-1 (Fig. 3, Fig. 4, Fig. 5, Fig. 6, and Fig. S3). The FISH and GISH probes made from the DNA of the three BAC clones, the three genes, and *A. cristatum* “Z559” could not hybridize with the chromosomal DNA of common wheat “Fukuho” (Fig. 3 and Fig. 4). The FISH probes made from the DNA of the BAC clones (BAC1013, BAC700, and BAC940) could be used to trace *A. cristatum* 6P chromatin in the translocation line WAT655 (Fig. 3 and Fig. S3), and the locations of the signals were mapped to *A. cristatum* 6PS (0.81–1.00) of the translocation line WAT655 and the *A. cristatum* 6PS terminal of the substitution line 4844-12-1 (Fig. 3 and Fig. S3). The signal positions of the

probes made from the DNA of the BAC clones were identical to those of the probes made from *A. cristatum* “Z559” P genomic DNA on *A. cristatum* 6PS (0.81–1.00) of the translocation line WAT655 (Fig. 3 and Fig. S3). Additionally, the probe made from the DNA of the BAC clone “BAC1013” traced one *A. cristatum* 6PS segment on the wheat chromosome 3D in addition to the previous translocated wheat chromosome 6D (Fig. 3, Fig. 6, and Fig. S4). Therefore, the results demonstrated that *A. cristatum* P chromatin can spontaneously infiltrate the wheat background. Additionally, the signals from the probes made from the DNA of three genes were mapped to the terminal of the chromosomal segment 6PS (0.81–1.00) in the translocation line WAT655 and the terminal of the chromosome 6PS in the substitution line 4844-12-1 (Fig. 4 and Fig. 5). The probe *Agr8173* could detect *A. cristatum* 6PS chromatin on the wheat chromosome 7D in addition to the previous translocated wheat chromosome 6D (Fig. 5, Fig. 6, and Fig. S4). Overall, the above results indicated that the *A. cristatum* P chromatin in *A. cristatum* 6PS (0.81–1.00) could spontaneously infiltrate the wheat background.

Molecular cytogenetic analysis of the gene cluster constituted by three disease resistance-related genes

As shown in Fig. 4 and Fig. 5, the positions of three disease resistance-related genes were mapped to the terminal of *A. cristatum* 6PS (0.81–1.00), which indicated that these three genes could be considerably close on *A. cristatum* 6PS (0.81–1.00). To explore the positional relationships of the three genes on *A. cristatum* 6PS (0.81–1.00) of the translocation line WAT655, the DNA of *Agr6971* and *Agr8173* was labeled with Texas Red®-5-dCTP (red), and the DNA of *Agr4080* was labeled with Fluorescein-12-dUTP (green). The probes *Agr6971* (red) and *Agr4080* (green) were simultaneously used for one FISH experiment (Fig. 6 and Fig. S5), and the probes *Agr8173* (red) and *Agr4080* (green) were simultaneously used for another (Fig. 6 and Fig. S5). The probe made from *A. cristatum* “Z559” DNA was used for the second round of GISH. The signals of the FISH probes showed that *Agr6971* (red) and *Agr4080* (green) were localized at one overlapping zone of *A. cristatum* 6PS (0.81–1.00); moreover, *Agr8173* (red) and *Agr4080* (green) were also localized at one overlapping zone of *A. cristatum* 6PS (0.81–1.00). These FISH results revealed that the three genes were localized at overlapping positions of *A. cristatum* 6PS (0.81–1.00); therefore, the three genes were preliminarily considered as a disease resistance-related gene cluster on *A. cristatum* 6PS (0.81–1.00) (Fig. 6).

Tracing *A. cristatum* P genomic components of wheat-*A. cristatum* breeding materials

The above results indicated that the introgression can spontaneously occur between *A. cristatum* P genome and wheat genome in the translocation line WAT655. In our laboratory, a large number of wheat-*A. cristatum* breeding materials were selected from the progenies of wheat-*A. cristatum* introgression lines according to the agronomic traits of *A. cristatum*. The wheat-*A. cristatum* breeding materials exhibited desirable breeding agronomic traits that can be directly applied in breeding practice. To detect the *A. cristatum* P genomic components of wheat-*A. cristatum* breeding materials, the specific sequences of the three genes, which were acquired according to results of the alignment between the sequences of three genes and Chinese Spring genome sequences RefSeq v1.0 (International Wheat Genome Sequencing Consortium 2018), were used to develop KASP molecular markers (Fig. S2 and Table S2). The KASP molecular markers were used to trace the *A. cristatum* 6P components in the BC₅F_{2:3} population of the translocation line WAT655. The results were completely coincident with those of standard PCR detection of the gene markers relying on agarose gel electrophoresis (Fig. S6), therefore, the results indicated that the KASP molecular markers could be used to accurately trace *A. cristatum* P genomic components in common wheat. Then, the KASP molecular markers were used to trace *A. cristatum* P genomic components of wheat-*A. cristatum* breeding materials, and 24 breeding materials were acquired from among 500 wheat-*A. cristatum* breeding materials (Fig. 7 and Table 3). Moreover, these breeding materials exhibited resistance or moderate resistance to stripe rust (CYR32 and CYR33) according to the investigation in the field (Fig. 7 and Table 3). Therefore, three disease resistance-related genes were closely associated with the resistance to stripe rust. In addition, these breeding materials also exhibited other desirable agronomic traits in the field (Fig. 7 and Table 3). In conclusion, these breeding materials from wheat-*A. cristatum* introgression lines are valuable for breeding practice, and the results provided new insights for the formation of introgression lines.

Discussion

A. cristatum is one of the most important wild relatives of common wheat and carries many desirable agronomic traits (Dewey 1984; Dong et al. 1992). In recent years, many desirable genes from *A. cristatum* have been transferred into common wheat, such as the stripe rust resistance, the leaf rust resistance, the powdery mildew, the high-GNS, and the high-TGW genes (Ochoa et al. 2015; Song et al. 2016a, b; Zhang et al. 2016; Copete and Cabrera 2017; Lu et al. 2017; Li et al. 2017; Zhang et al. 2017; Jiang et al. 2018; Zhang et al. 2019a). These genes from *A. cristatum* are usually on relatively large *A. cristatum* chromosomal segments of translocation lines, which are a common type among the progenies from the hybridization of wheat and wild relatives. However, translocation lines are not easy to apply in breeding practice because of the genetic drag caused by the translocated segments, so many breeders desire the introgression lines carrying smaller exogenous genetic components for breeding. In our study, gene markers and FISH/GISH probes were used to analyze the wheat-*A. cristatum* 6P translocation line WAT655, and the evidence of the introgression between *A. cristatum* 6P chromatin and wheat chromosomes was observed (Fig. 3, Fig. 5, Fig. 6, and Fig. S4). Additionally, KASP markers of the three genes were developed to trace *A. cristatum* P genomic components of wheat-*A. cristatum* breeding materials (Fig. 7), moreover, 24 breeding materials carrying *A. cristatum* P genomic components were acquired from 500 wheat-*A. cristatum* breeding materials. These breeding materials exhibited resistance to stripe rust (CYR32 and CYR33) according to the investigation in the field, which indicated that three disease resistance-related genes were closely associated with the resistance to stripe rust. These materials exhibited

other favorable breeding agronomic traits (Fig. 7 and Table 3) that can be directly applied in breeding practice. In addition, to further explore the function of three disease resistance-related genes, transgenic work involving coding sequences (CDSs) and full-length sequences of the three disease resistance-related genes is already underway. Therefore, our study not only provides new insights into the introgression between *A. cristatum* P genome and wheat genome, but also provides a new approach for acquiring valuable breeding materials for breeding practice.

Distant hybridization is an efficient and important method for broadening the genetic base of common wheat. Particularly, distant hybridization has contributed much to disease resistance breeding of common wheat. For example, the powdery mildew gene *Pm21* of *Haynaldiavillosa* 6VS has been widely applied in wheat breeding (Chen et al. 2013; Xing et al. 2018); the 1BL·1RS translocation line carried many disease resistance genes, such as the stripe rust resistance gene *Yr9*, the powdery mildew resistance gene *Pm8*, the stem rust resistance gene *Sr31*, and the leaf rust resistance gene *Lr26* (Zeller 1973; Singh et al. 1990; Friebe et al. 1996; Mago et al. 2002). In this study, the wheat-*A. cristatum* translocation line WAT655 and the wheat-*A. cristatum* breeding materials that exhibited resistance to stripe rust can be the new germplasms and contribute to disease resistance breeding of common wheat. Moreover, the molecular markers designed from the sequences of three disease resistance-related genes can improve the efficiency of wheat breeding. Therefore, our study not only provides valuable breeding materials, but also promotes the application of marker-assisted selection of distant hybridization between wheat and *A. cristatum* in wheat breeding.

Some disease resistance genes have been shown to be distributed in the genome in the form of a gene cluster. For example, the powdery mildew resistance genes *Pm21* and *Stpk-V* were mapped to the same locus of *Haynaldia villosa* 6VS bin (FL 0.45–0.58) (Cao et al. 2011; Xing et al. 2018); the stripe rust resistance genes *Yr5*, *Yr7*, and *YrSP* were on a gene cluster (Marchal et al. 2018). Both the powdery mildew resistance gene and the leaf rust resistance gene of *A. cristatum* 2P chromosome were mapped to *A. cristatum* 2PL (0.66–0.86). In a previous report about the wheat-*A. cristatum* 6P translocation line WAT655, the stripe rust resistance gene and the leaf rust resistance gene were localized on *A. cristatum* 6PS (0.81–1.00) of the translocation line WAT655. In this study, the DNA of three disease resistance-related genes was used as FISH probes to identify the positions of the genes on *A. cristatum* 6PS (0.81–1.00). The results revealed that the FISH signals of the probes were located at the overlapping positions of *A. cristatum* 6PS (0.81–1.00) (Fig. 6 and Fig. S5), so we speculated that one disease resistance gene cluster existed on *A. cristatum* 6PS (0.81–1.00).

A. cristatum P genome and wheat genome were revealed to present a homoeologous relationship by using a wheat 660K SNP array, and apparent chromosomal rearrangements and introgression spread throughout the P genome were observed (Zhou et al. 2018). In addition, genetic rearrangements of *A. cristatum* 6P chromosomes could usually occur at the terminal of chromosomes through analyzing the different wheat-*A. cristatum* 6P addition lines (Han et al. 2014). In this study, the results of FISH using the DNA of the BAC clones and three disease resistance-related genes as probes indicated that genetic exchange can spontaneously occur between *A. cristatum* P chromatin and wheat chromosomes (3, Fig. 5, and Fig. 6). The mechanism of the introgression may involve chromosomal rearrangements between *A. cristatum* P genome and wheat genome resulting from their homoeologous relationship; moreover, the introgression observed in this study also occurred in the terminal of the chromosomes (Fig. 3, Fig. 5, Fig. 6, and Fig. S4). Therefore, the homoeologous relationship and chromosomal rearrangements might explain the introgression between *A. cristatum* P genome and wheat genome.

In summary, gene markers, *A. cristatum* P genome-specific markers, and FISH/GISH probes (the DNA of *A. cristatum* BAC clones, three disease resistance-related genes, and *A. cristatum* “Z559”) were used to analyze wheat-*A. cristatum* 6P translocation line WAT655. The results revealed that the introgression spontaneously occurred between *A. cristatum* P genome and wheat genome. Additionally, KASP markers of the genes were developed to detect and acquire a few wheat-*A. cristatum* breeding materials that can be directly applied in breeding practice. Therefore, our study not only provides new insights into the introgression between wild relatives and wheat, but also provides new wheat-*A. cristatum* breeding materials for wheat breeding.

Abbreviations

FISH	Fluorescence in situ hybridization
GISH	Genomic in situ hybridization
BAC	Bacterial artificial chromosome
KASP	Kompetitive allele-specific PCR
TGW	Thousand grain weight
GNS	Grain number per spike
NCBI	National Center of Biotechnology Information

Declarations

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Conflicts of interest

The authors declare that they have no conflict of interest.

Author contributions

LHL conceived and designed the experiments. ZZ performed the experiments, collected data, and wrote the paper. ZZ and SHZ analyzed data. WHL and LQS produced the plant materials. JPZ, HMM, XMY, YDL, and XQL contributed reagents/materials/analysis tools. All authors commented on and approved the final version.

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Tables

Table 1 The information of the plant materials

Materials	Zygosity	Progeny	Type	6P segment size	Chromosome constitution	Reference
4844-12-1	Homozygous		disomic substitution line	6P	42 (6P/6D)	
4844-12	Homozygous		disomic addition line	6P	44	Wu et al. (2006)
WAT638b	Homozygous	BC ₂ F ₆	6AS•6PL	6PL arm	42	Song et al. (2016a); Zhang et al. (2017)
WAT638a	Homozygous	BC ₂ F ₆	6PS•6AL	6PS arm	42	
WAT655	Homozygous	BC ₂ F ₆	6DS•6DL-6PS	6PS (0.81–1.00)	42	
	Heterozygous	BC ₅ F ₂ , BC ₅ F _{2:3}				

6P segments of wheat-*A. cristatum* 6P translocation lines were compared with intact 6P arms. The position of the centromere was considered as 0, while the terminal end of the 6PS/6PL arm was considered as 1 (Song et al. 2016a, b)

Table 2 Sequences of molecular markers designed according to *A. cristatum* transcriptome sequences

Transcriptome id	Gene name	BAC clone	Primer name	Left primer (5'–3')	Right primer (5'–3')	Annealing temperature
transcript/6971	<i>Agr6971</i>	BAC1013	Agr6971-3	TCACCAAAGATCGAGCTCCT	ACCCGTCTGCAGATTGTACC	60°C
			Agr6971vgs-2	GACCAGCTAGACAACCAGGT	TGCTGTTTGGGACCATCTCT	60°C
transcript/4080	<i>Agr4080</i>	BAC700	Agr4080-3	GGGCGGTTTTACTTCACAAA	ACTTGCAGCTGTCAATGTGC	60°C
			Agr4080-4	TGAAACTGGATGGACGATGA	GTGCTGCTGTGGTGTGACT	60°C
			Agr4080vgs-1	CCGAGGTGGCGAATGAACTTGT	AGAGATGGAGGCTGTGGTGACT	60°C
			Agr4080cds-6	TTGTCGGACTAATAATAGCGC	TGCCGAGATAATGGGGTT	60°C
transcript/8173	<i>Agr8173</i>	BAC940	Agr8173-2	GGTCCCATACCTCCCAGTTT	TCGATGAAAGGTCCAGTTCC	60°C
			Agr8173-9	ATCGTTGGCCAATTCGATAG	CAGCAGAAGGAGCATGTTGA	60°C
			Agr8173-6	TGATCATTGTGGAGACCGGA	CACCCTCTTTTGGCAACCTT	60°C

PCR Program: 95°C 5mins; 95°C 45s, Annealing temperature °C 45s, 72°C 45s, 35cycles; 72°C 5mins

Table 3 The agronomic traits of wheat-*A. cristatum* breeding materials in 2019–2020

Materials	Plant height (cm)	The plot yields (kg)	Thousand grain weight (g)	Germination rate (%)	Stripe rust response
WAg20	77	6.09	\	99	R
WAg37	80	7.16	46.75	94	R
WAg38	80	6.59	\	100	R
WAg60	90	8.40	43.10	98	MR
WAg115	83	6.77	\	97	MR
WAg130	85	8.04	42.23	95	MR
WAg165	80	7.49	\	91	R
WAg220	75	7.63	\	97	R
WAg270	76	7.35	\	98	R
WAg274	82	6.46	\	98	R
WAg301	85	8.37	40.84	99	MR
WAg308	85	7.07	\	99	MR
WAg314	80	7.09	\	99	MR
WAg317	85	7.25	\	99	R
WAg322	87	8.32	39.73	96	MR
WAg330	82	8.11	37.89	100	MR
WAg335	80	7.52	38.96	99	R
WAg337	83	8.22	41.08	100	MR
WAg339	80	7.13	38.66	96	R
WAg409	81	7.08	39.32	98	MR
WAg457	82	7.84	43.80	98	R
WAg466	75	7.45	38.58	98	MR
WAg475	85	8.24	45.94	98	R
WAg480	85	7.96	41.84	99	R

The letters MR and R in “Stripe rust response” column indicated plants were moderately resistant type and resistant type, respectively

Figures

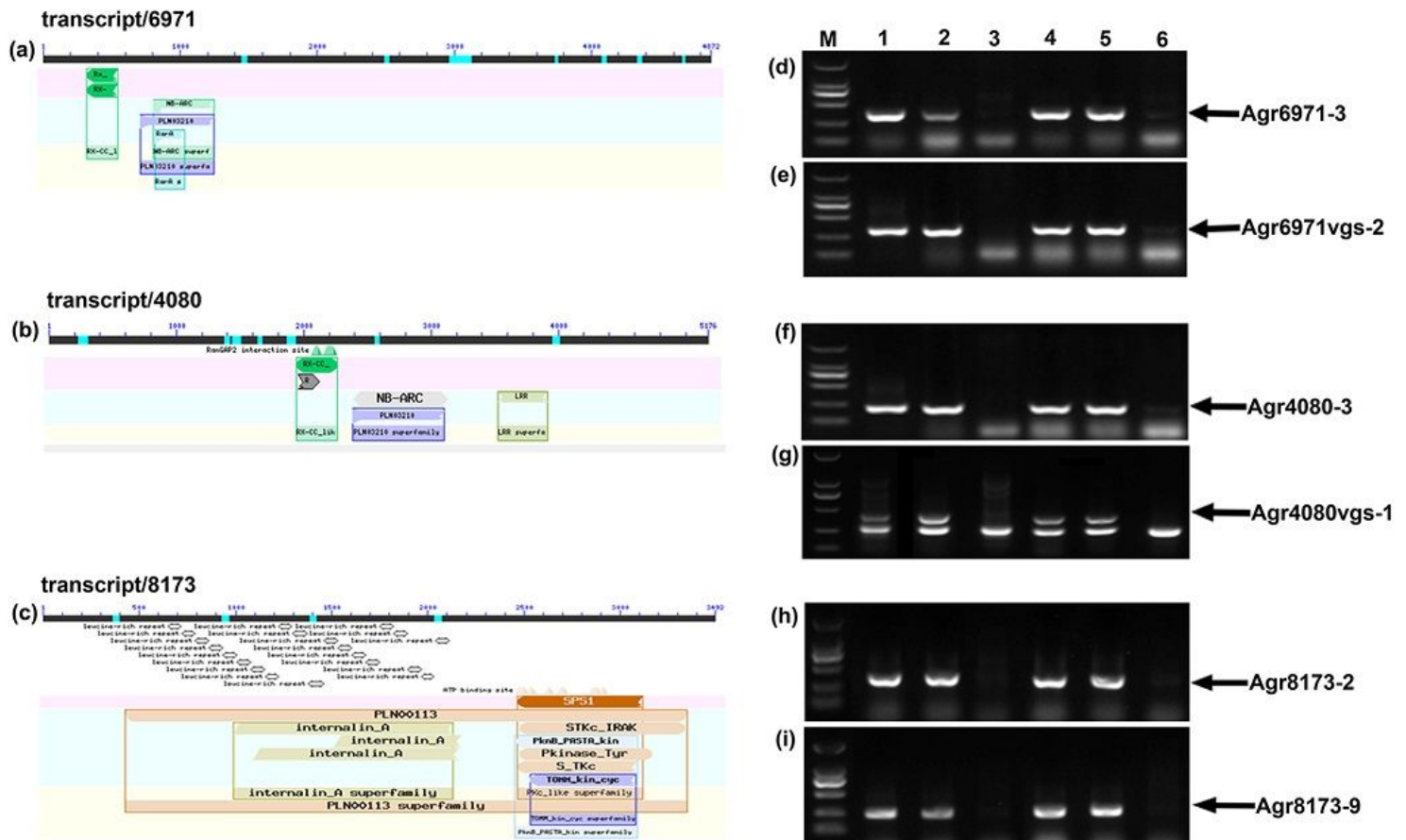


Figure 1

Functional annotations and molecular marker detection results for three *A. cristatum* full-length transcriptomes in wheat-*A. cristatum* 6P translocation line WAT655. (a), (b), and (c) showed the functional annotations of three full-length transcriptomes from the BLASTx on the NCBI website. (d), (e), (f), (g), (h), and (i) showed that the gene specific molecular markers designed from the sequences of three full-length transcriptomes were mapped to *A. cristatum* 6PS (0.81–1.00) of the translocation line WAT655. Lanes: M DL2000 DNA marker; 1 Z559; 2 4844-12; 3 Fukuho; 4 WAT655; 5 WAT638a; 6 WAT638b

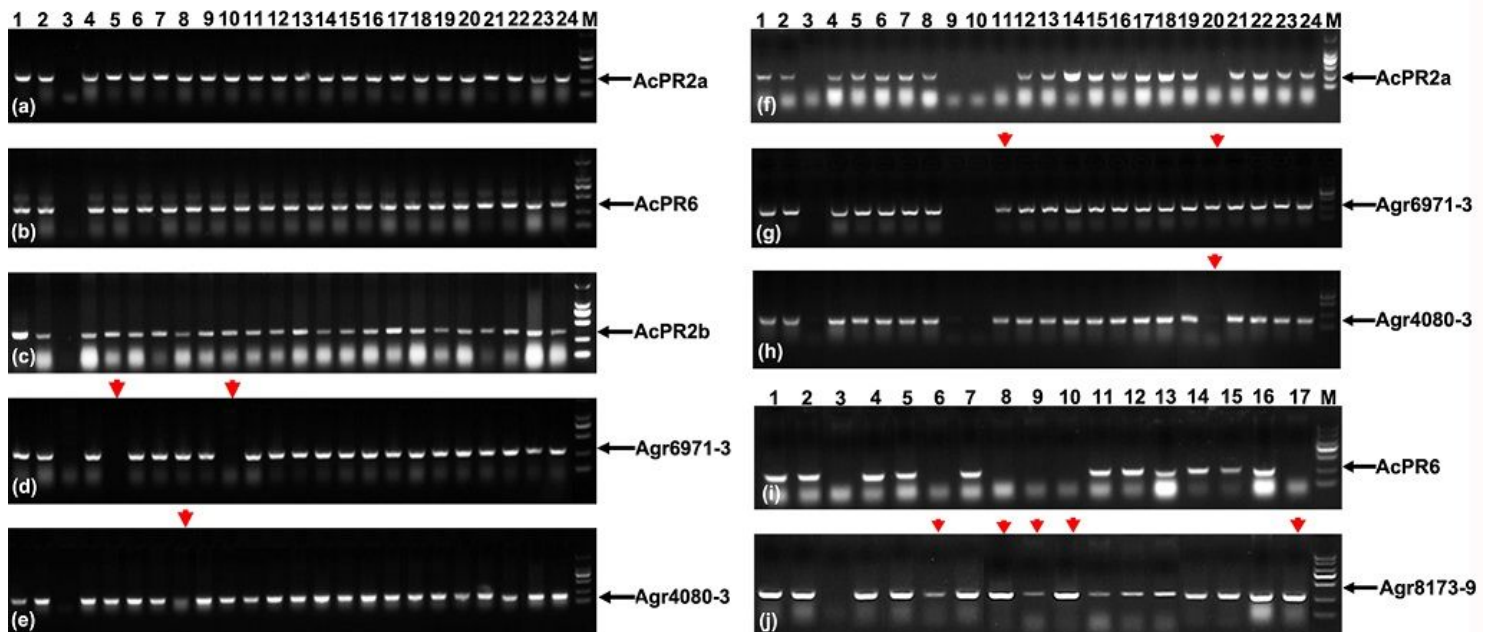


Figure 2

Detection results of *A. cristatum* P genome-specific markers and gene makers. (a), (b), (c), (d), and (e) showed the detection results for the BC5F2 population of the translocation line WAT655; (f), (g), (h), (i), and (j) showed the detection results for the BC5F2:3 population of the translocation line WAT655. The red arrows indicated the differences between P genome-specific markers and the gene makers in these plants. Lanes: M DL2000 DNA marker; 1 Z559; 2 4844-12; 3 Fukuho; 4–24 of (a), (b), (c), (d), and (e) the partial plants of the BC5F2 population; 4–24 of (f), (g), and (h) the partial plants of the WAT655 BC5F2:3 population; 4–17 of (i) and (j) the partial plants of the BC5F2:3 population

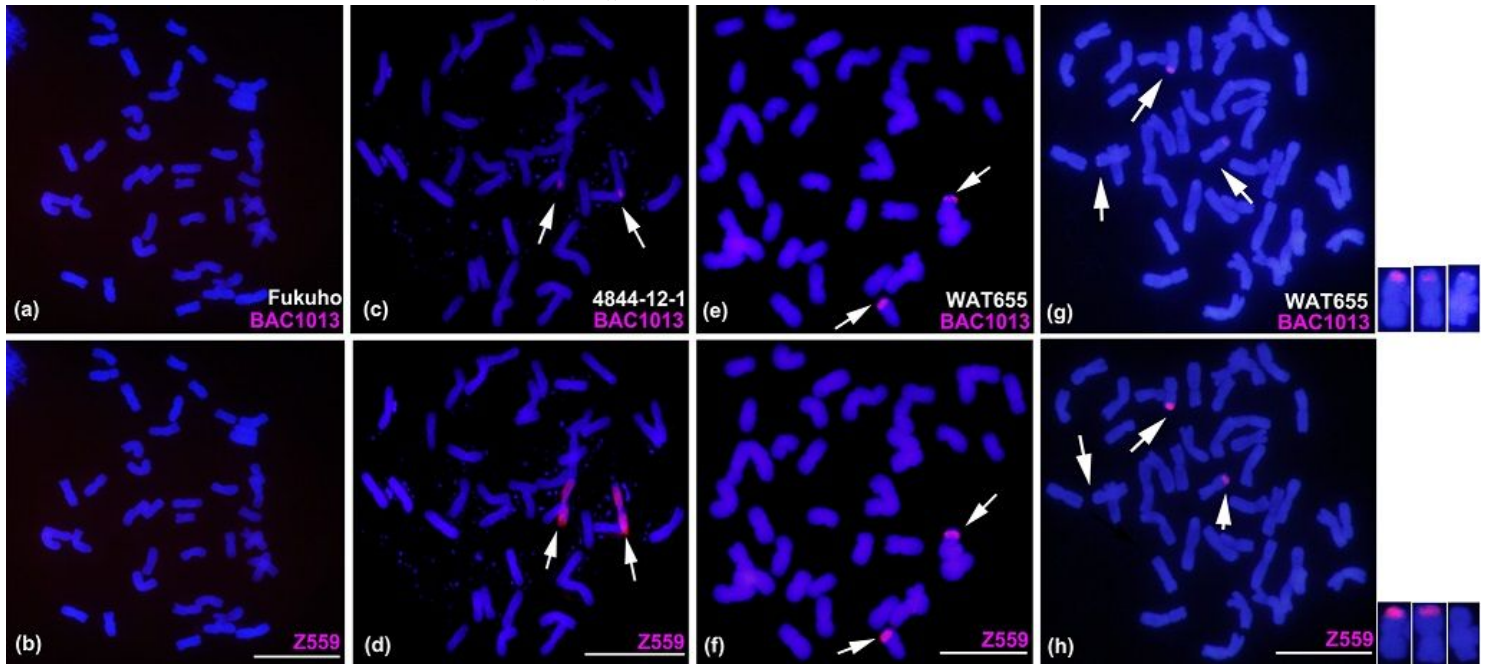


Figure 3

FISH and GISH patterns of common wheat "Fukuho", the substitution line 4844-12-1, and the translocation line WAT655 using the DNA of the BAC clone "BAC1013" and *A. cristatum* "Z559" as the probes. The BAC clone DNA was used as the probe for the first round of FISH; *A. cristatum* "Z559" DNA was used as the probe for the second round of GISH on the same slides. (a) and (b) showed these probes could not hybridize with chromosomal DNA of common wheat "Fukuho". (c) and (d) showed the FISH and GISH patterns of the substitution line 4844-12-1 using the two probes. (e), (f), (g), and (h) showed FISH and GISH patterns of the translocation line WAT655 using the two probes in the two different plants. (scale bar = 10 μm)

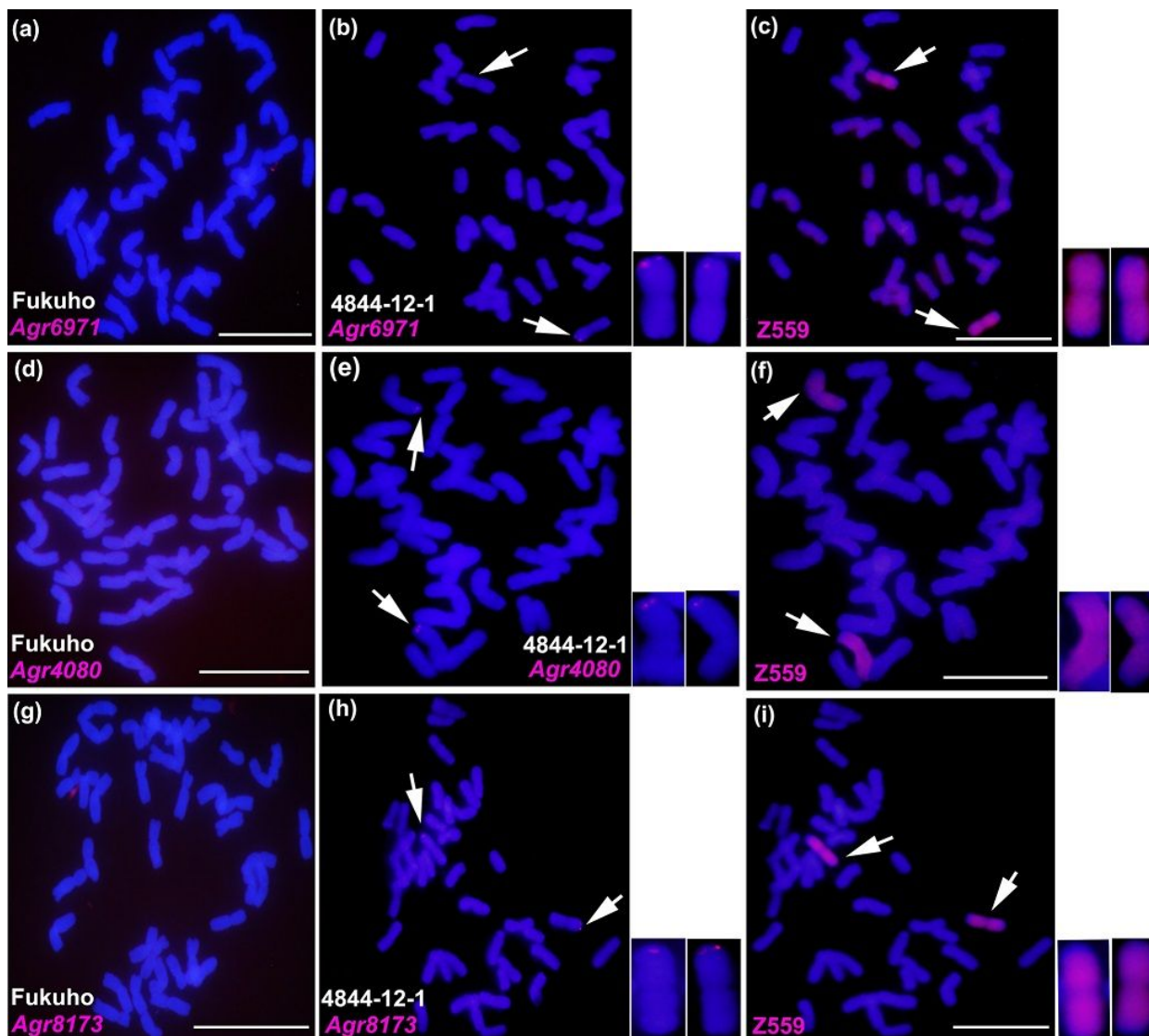


Figure 4

FISH and GISH patterns of the common wheat “Fukuho” and the substitution line 4844-12-1 using the DNA of three disease resistance-related genes and *A. cristatum* “Z559”. The DNA of three genes was used as the probes for the first round of FISH; *A. cristatum* “Z559” DNA was used as the probe for the second round of GISH on the same slides. (a), (d), and (g) showed three probes Agr6971, Agr4080, and Agr8173 could not hybridize with chromosomal DNA of common wheat “Fukuho”. (b), (e), and (h) showed that the signals of the three probes were mapped to the terminal of *A. cristatum* 6PS in the substitution line 4844-12-1. *A. cristatum* “Z559” DNA as the probe was used for the second round of GISH on the same slides in (c), (f), and (i). (scale bar = 10 μ m)

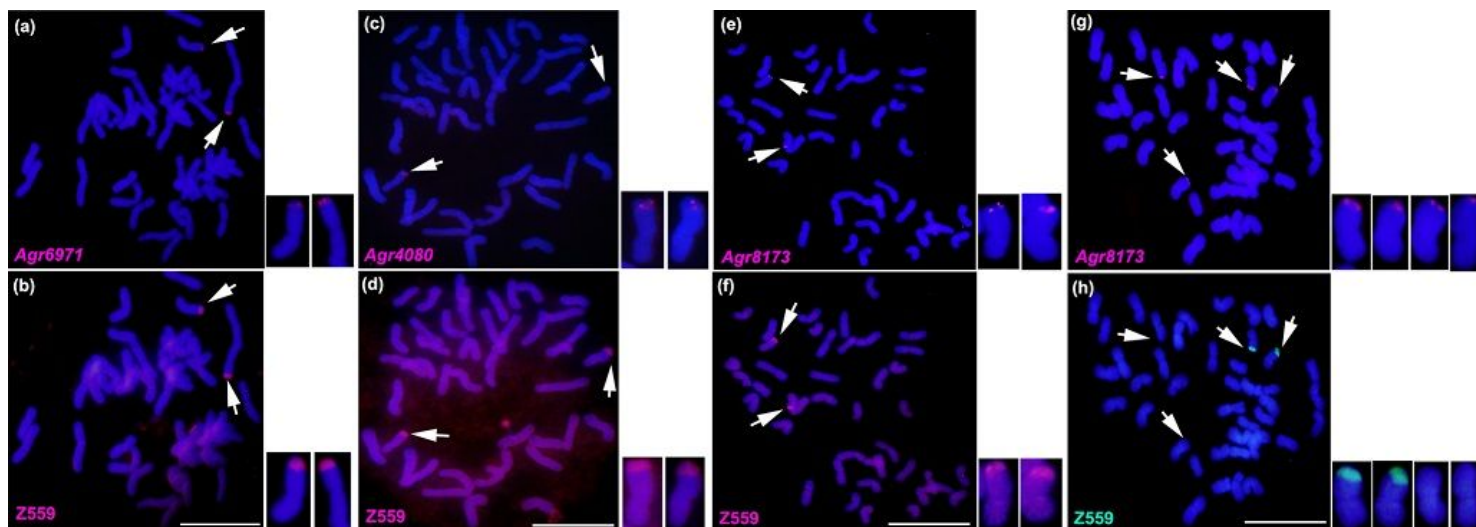


Figure 5

FISH and GISH patterns of the translocation line WAT655 using the DNA of three disease resistance-related genes and *A. cristatum* "Z559" as the probes. The DNA of three genes was used as the probes for the first round of FISH; the DNA of *A. cristatum* "Z559" was used as the probes for the second round of GISH on the same slides. (a), (b), (c), and (d) showed FISH and GISH patterns using the probes Agr6971, Agr4080, and *A. cristatum* "Z559" DNA. (e), (f), (g), and (h) showed FISH and GISH patterns using the DNA of the gene Agr8173 and *A. cristatum* "Z559" as the probes in two different plants. (scale bar = 10 μ m)

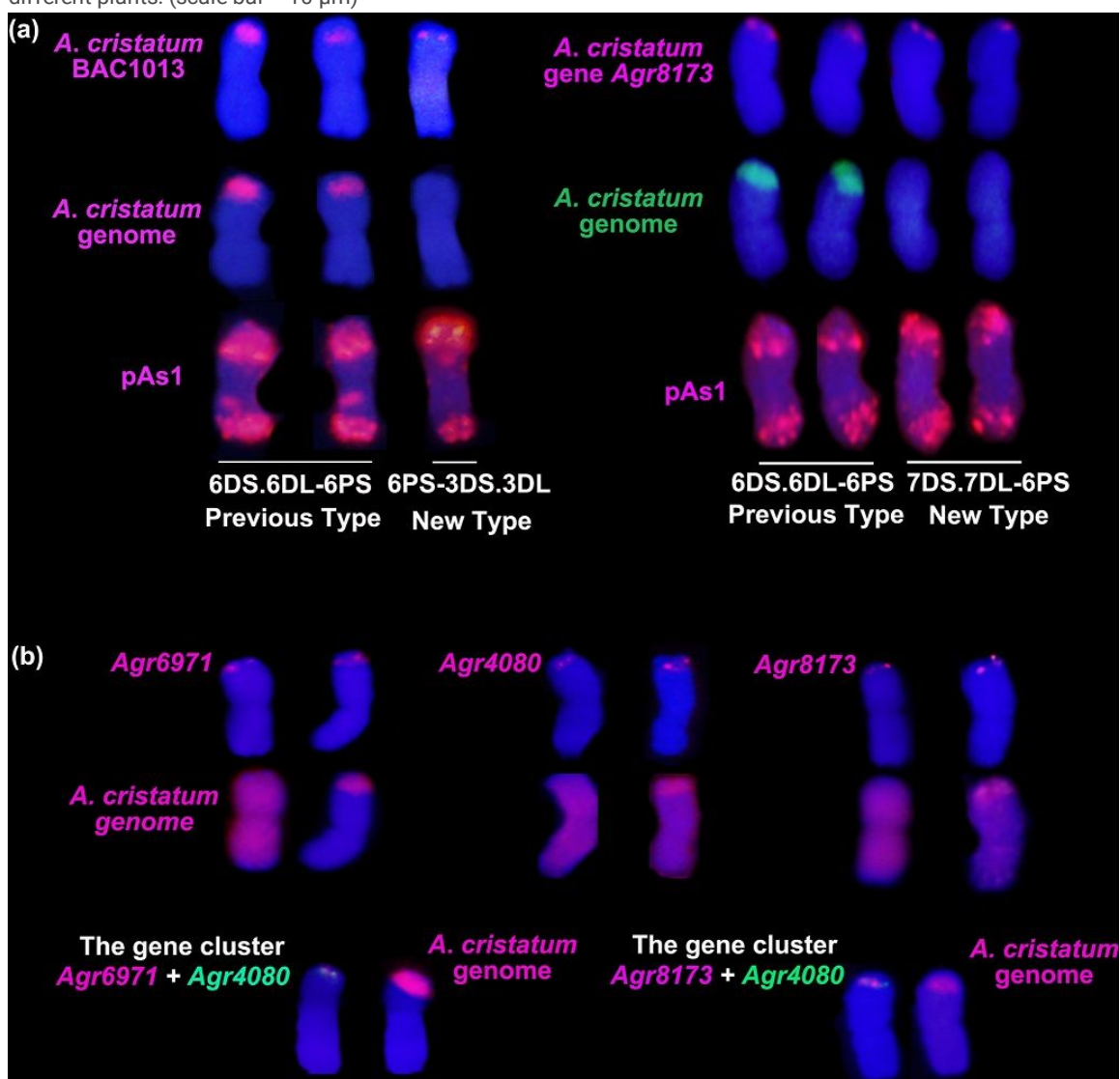


Figure 6

FISH and GISH patterns of the introgression and the gene cluster in the translocation line WAT655. (a) showed the new translocated types were produced through the introgression between *A. cristatum* P genome and wheat genome. (b) showed the three genes *Agr6971*, *Agr4080*, and *Agr8173* could constitute a gene cluster according to the positions of their FISH signals

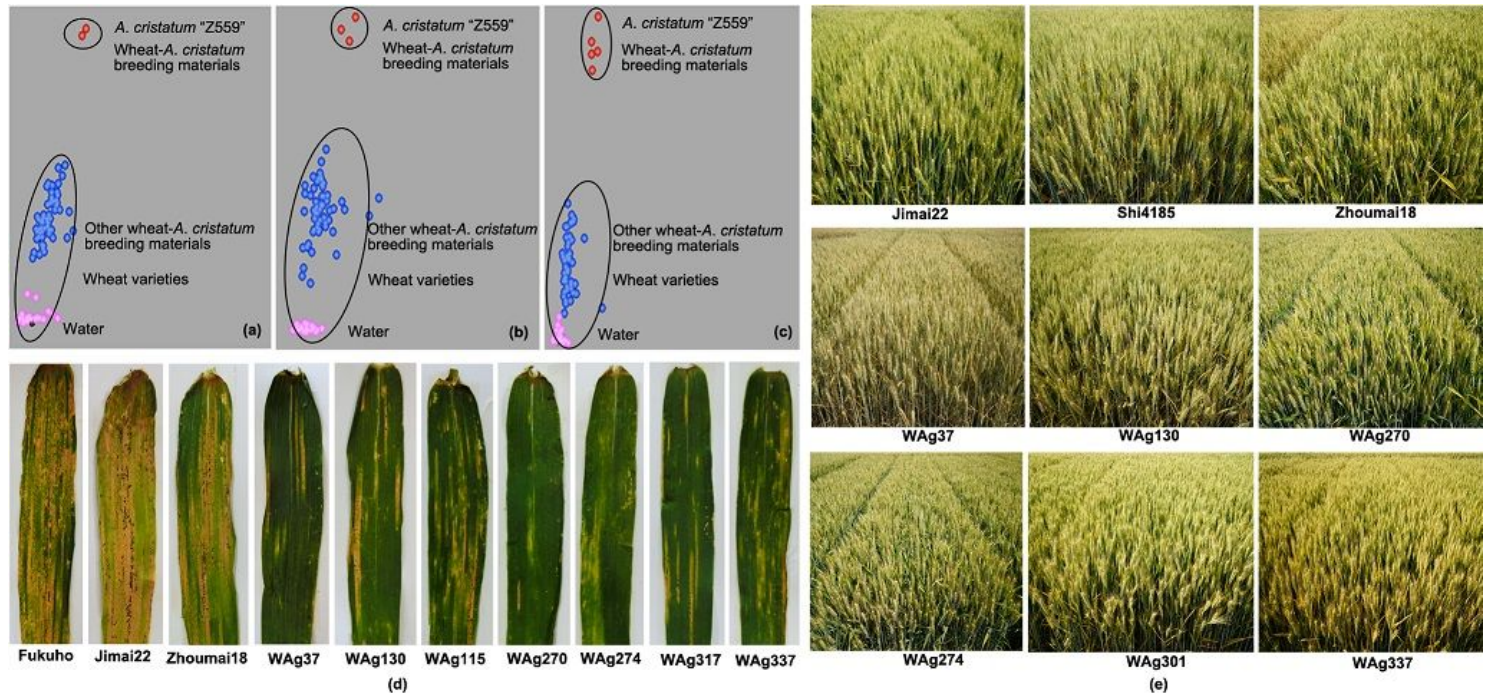


Figure 7

The detection results of KASP markers and the investigation of agronomic traits of the wheat-A. cristatum breeding materials. (a), (b), and (c) showed the detection results of *Agr4080kps-2*, *Agr4080kps-4*, and *Agr6971kps-4*, respectively. (d) and (e) showed the agronomic traits of partial wheat-A. cristatum breeding materials including resistance to stripe rust and other agronomic traits in the field

Supplementary Files

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