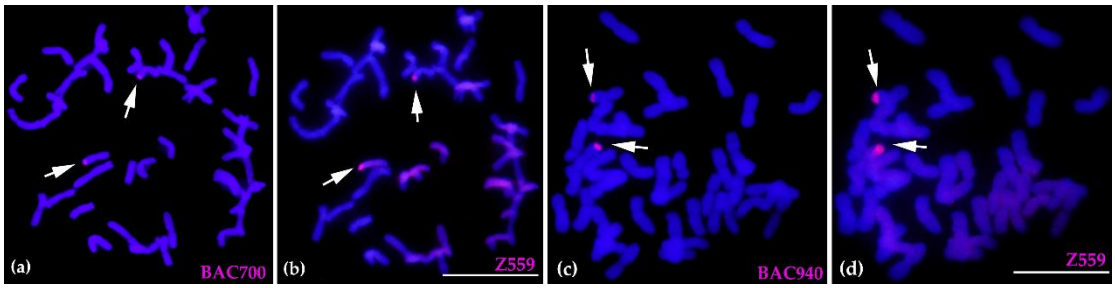

Figure S1. FISH and GISH patterns of the translocation line WAT655 using the DNA of *A. cristatum* BAC clones and *A. cristatum* "Z559" as the probes. (a) and (c) The FISH patterns from the probes BAC700 and BAC940, respectively; (b) and (d) The GISH patterns from *A. cristatum* "Z559" as the probes. *A. cristatum* 6P chromosomal segments were in red, while wheat chromosomes were in blue strained by DAPI. (scale bar=10 μm).

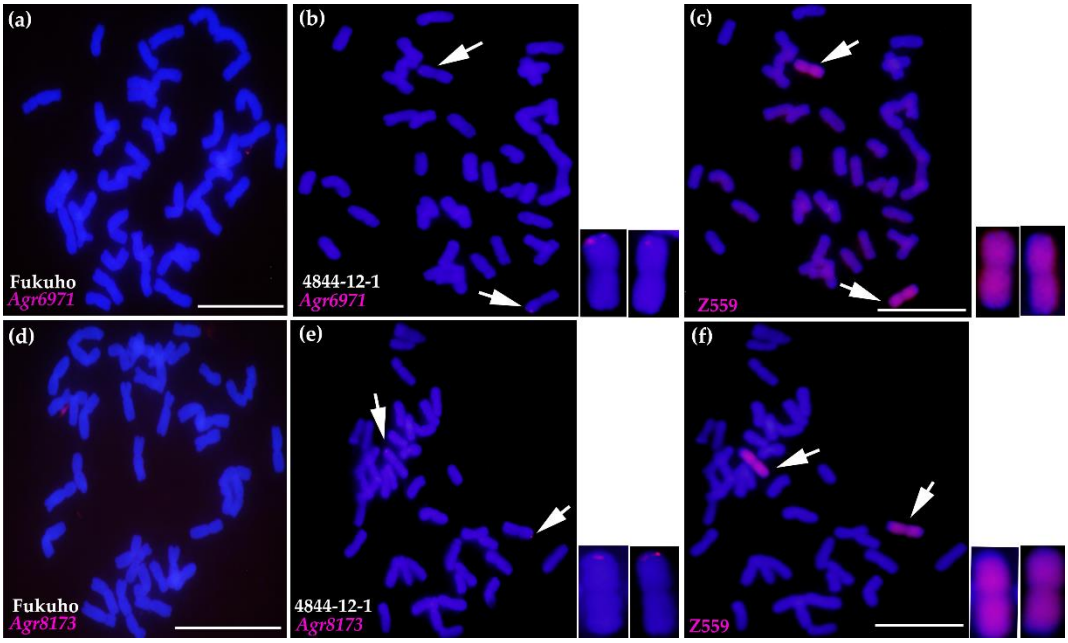

Figure S2. FISH and GISH patterns of the common wheat "Fukuho" and the substitution line 4844-12-1 using the DNA of the disease resistance-related genes and *A. cristatum* "Z559" as probes. (a) and (d) The probes *Agr6971* and *Agr8173* could not hybridize with chromosomal DNA of common wheat "Fukuho". (b) and (e) The DNA of the genes (*Agr6971* and *Agr8173*) was used as the probes for the first round of FISH in the substitution line 4844-12-1; (c) and (f) *A. cristatum* "Z559" DNA was used as the probe for the second round of GISH on the same slides in the substitution line 4844-12-1. *A. cristatum* 6P chromosomal segments were in red, while wheat chromosomes were in blue strained by DAPI. (scale bar = 10 μm).

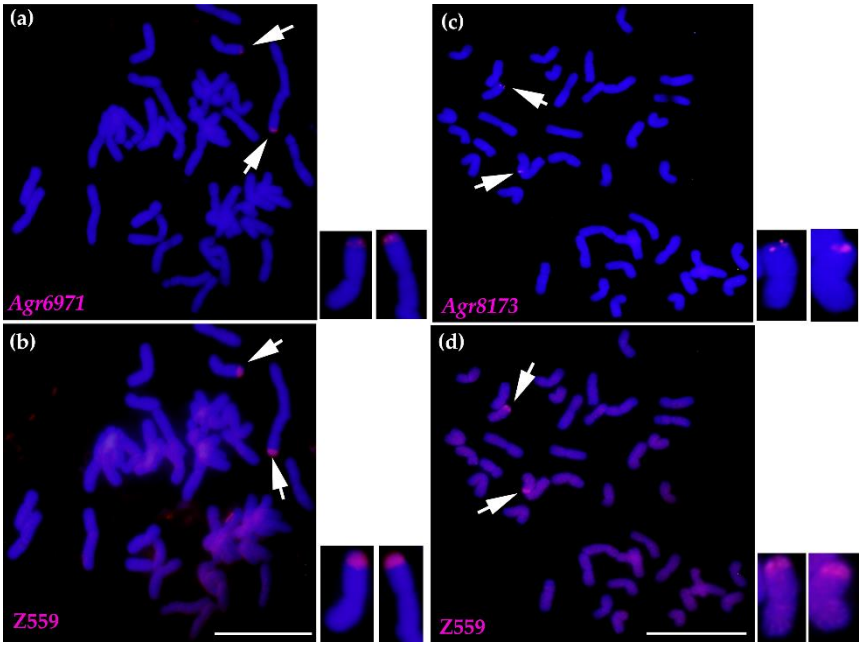

Figure S3. FISH and GISH patterns of the translocation line WAT655 using the DNA of the disease resistance-related genes and *A. cristatum* "Z559" as the probes. (a) and (c) The DNA of the genes (*Agr6971* and *Agr8173*) was used as the probes for the first round of FISH; (b) and (d) The DNA of *A. cristatum* "Z559" was used as the probes for the second round of GISH on the same slides. *A. cristatum* 6P chromosomal segments were in red, while wheat chromosomes were in blue strained by DAPI. (scale bar = 10 μm).

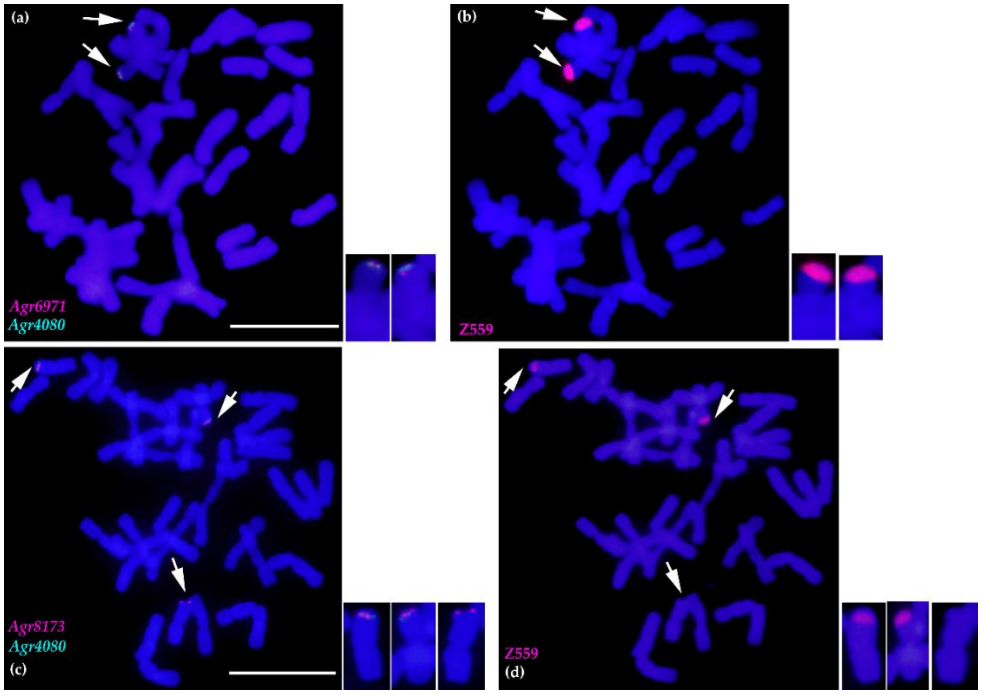

Figure S4. FISH and GISH patterns of the translocation line WAT655 using the DNA of three genes and *A. cristatum* "Z559" as the probes. (a) and (b) The signals of the probes *Agr6971* (red) and *Agr4080* (green) were simultaneously mapped to the overlapping zones of *A. cristatum* 6PS (0.81–1.00) of the translocation line WAT655, moreover, (c) and (d) The signals of the probes *Agr8173* (red) and *Agr4080* (green) also were mapped to the overlapping zones. (scale bar=10 μm).

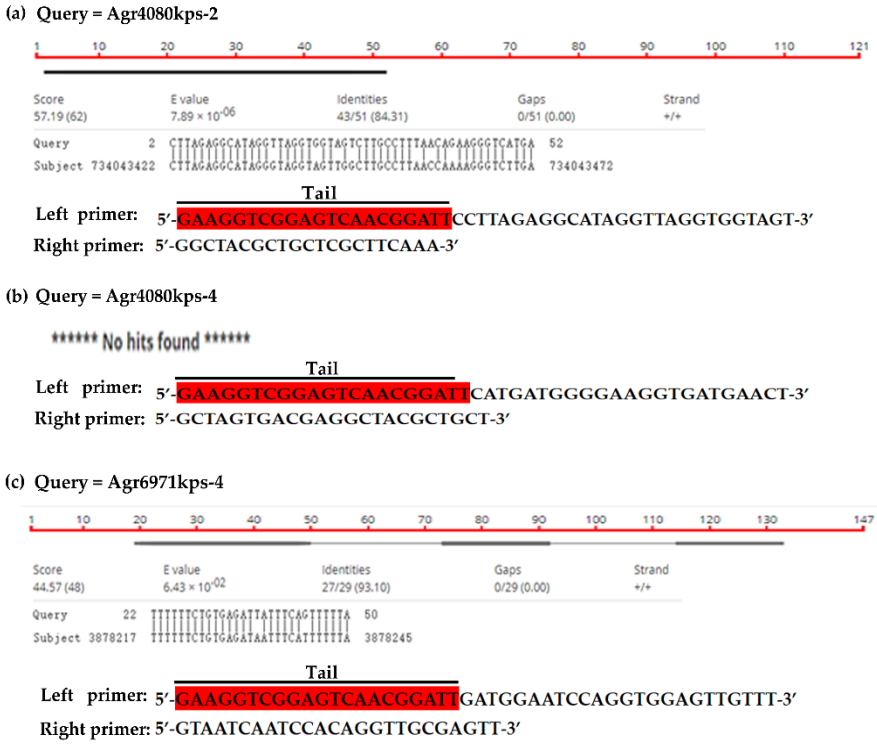

Figure S5. The alignment results of the sequences amplified by the KASP markers (Agr4080kps-2, Agr4080kps-4, and Agr6971kps-4) and Chinese Spring genome sequences RefSeq v1.0 and the sequences of the KAPS markers.

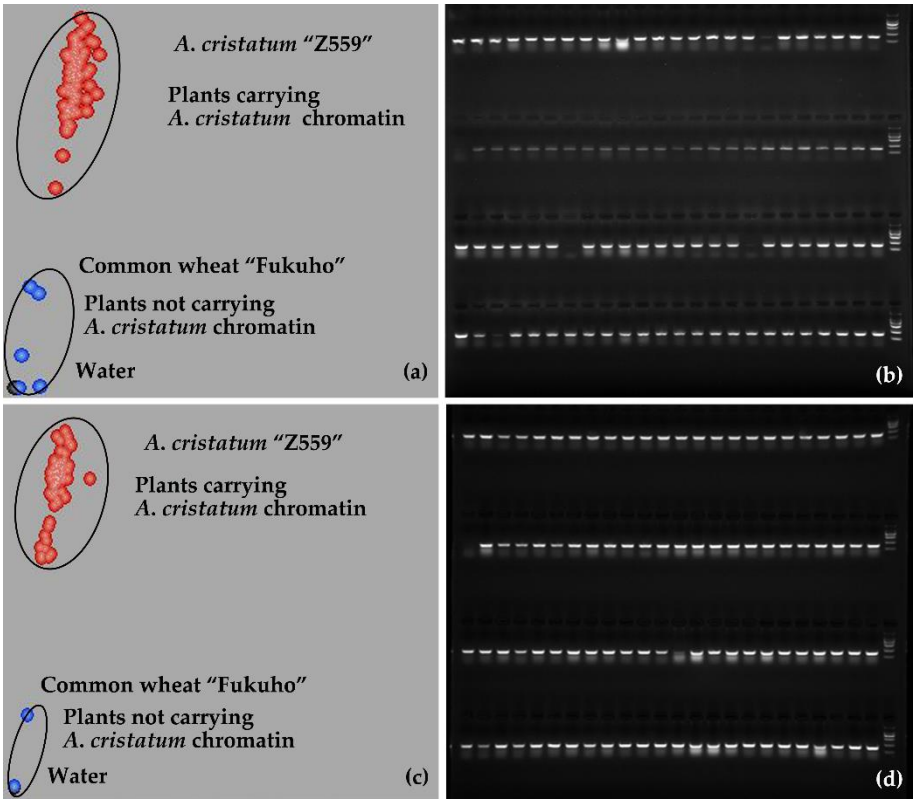

Figure S6. Comparison of the results of the KASP markers and the gene markers relying on agarose gel electrophoresis in the $BC_5F_{2:3}$ population of the translocation line WAT655. (a) and (b) indicated the results for the KASP marker Agr4080kps-2 and the gene marker Agr4080-3, respectively; (c) and (d) indicated the results for the KASP marker Agr6971kps-4 and the gene marker Agr6971-3, respectively. The genotype of wheat was in blue; the genotype of *A. cristatum* "Z559" was in red.

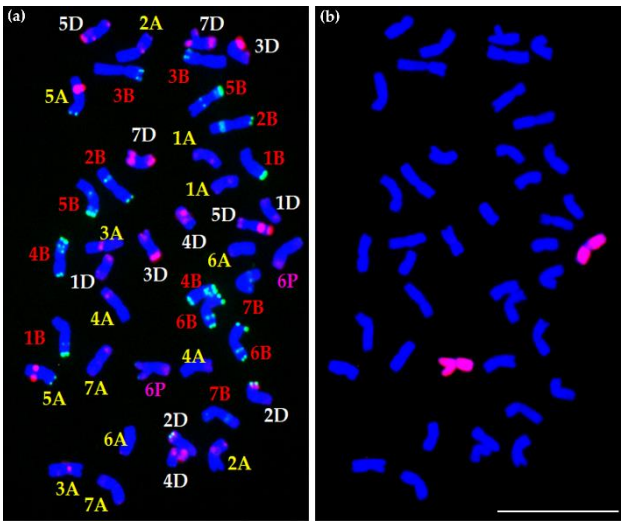

Figure S7. FISH and GISH patterns of the wheat-*A. cristatum* 6P disomic substitution line 4844-12-1. (a) The Oligo-pTa535-1 was in red and the Oligo-pSc119.2-1 was in green, while wheat chromosomes were in blue stained by DAPI. (b) *A. cristatum* 6P chromosomes were in red, while wheat chromosomes were in blue stained by DAPI.

Figure S8. The agronomic traits of wheat-*A. cristatum* 6P disomic addition line 4844-12, common wheat "Fukuho", and wheat-*A. cristatum* 6P translocation line WAT655 (BC_5F_2 and $BC_5F_{2:3}$).

Table S1. Sequences of *A. cristatum* P genome-specific markers and the oligo probes.

Markers	Left primer (5'–3')	Right primer (5'–3')	Annealing temperature (°C)	Reference
Oligo-pSc119.2-1	6-FAM-5′CCGTTTTGTGGACTATTACTCACCGCTTTGGGGTC CCATAGCTAT3′			Tang *et al*. [58]
Oligo-pTa535-1	Tamra-5′AAAAACTTGACGCACGTCACGTACAAATTGGACAAACTCTTTCGGAGTATCAGGGTTTC3′			
AcPR2a	CTCTTTTGTTTTTGCTTTTG	CTTGGAAATCTTCTTTGTTG	52	Han *et al*. [45]
AcPR2b	GCGGCAAGGGAAAAGTAGTC	GGCATTGACCTCAGGGAAGT	59	
AcPR6	TGAGATGACGATGGATGAAG	ACATCCTGACATTTCTGGGC	56	

Table S2. Sequences of the KASP markers of the disease resistance-related genes.

Markers	Left primer (5'–3')	Right primer (5'–3')
Agr4080kps-2	GAAGGTCGGAGTCAACGGATTCCTTAGAGGCATAGGTTAGGTGGTAGT	GGCTACGCTGCTCGCTTCAAA
Agr4080kps-4	GAAGGTCGGAGTCAACGGATTCATGATGGGGAAGGTGATGAACT	GCTAGTGACGAGGCTACGCTGCT
Agr6971kps-4	GAAGGTCGGAGTCAACGGATTGATGGAATCCAGGTGGAGTTGTTT	GTAATCAATCCACAGGTTGCGAGTT
Agr8173kps-2	GAAGGTCGGAGTCAACGGATTGCCGAACCACAAACGAGAGAAGT	CTGCAAACAGTCCCCACTCACC

PCR program: an initial denaturation at 94 °C for 3 min, followed by 6 cycles of touchdown (94 °C for 20 s, touchdown at 56 °C initially and decreasing by 1 °C per cycle for 2 min), and 32–36 (for gel-based size separation) or 39 (for gel-free fluorescence signals) additional cycles of denaturation at 94 °C for 20 s, annealing/extension at 62 °C for 1 min, and a final extension at 62 °C for 2 min.