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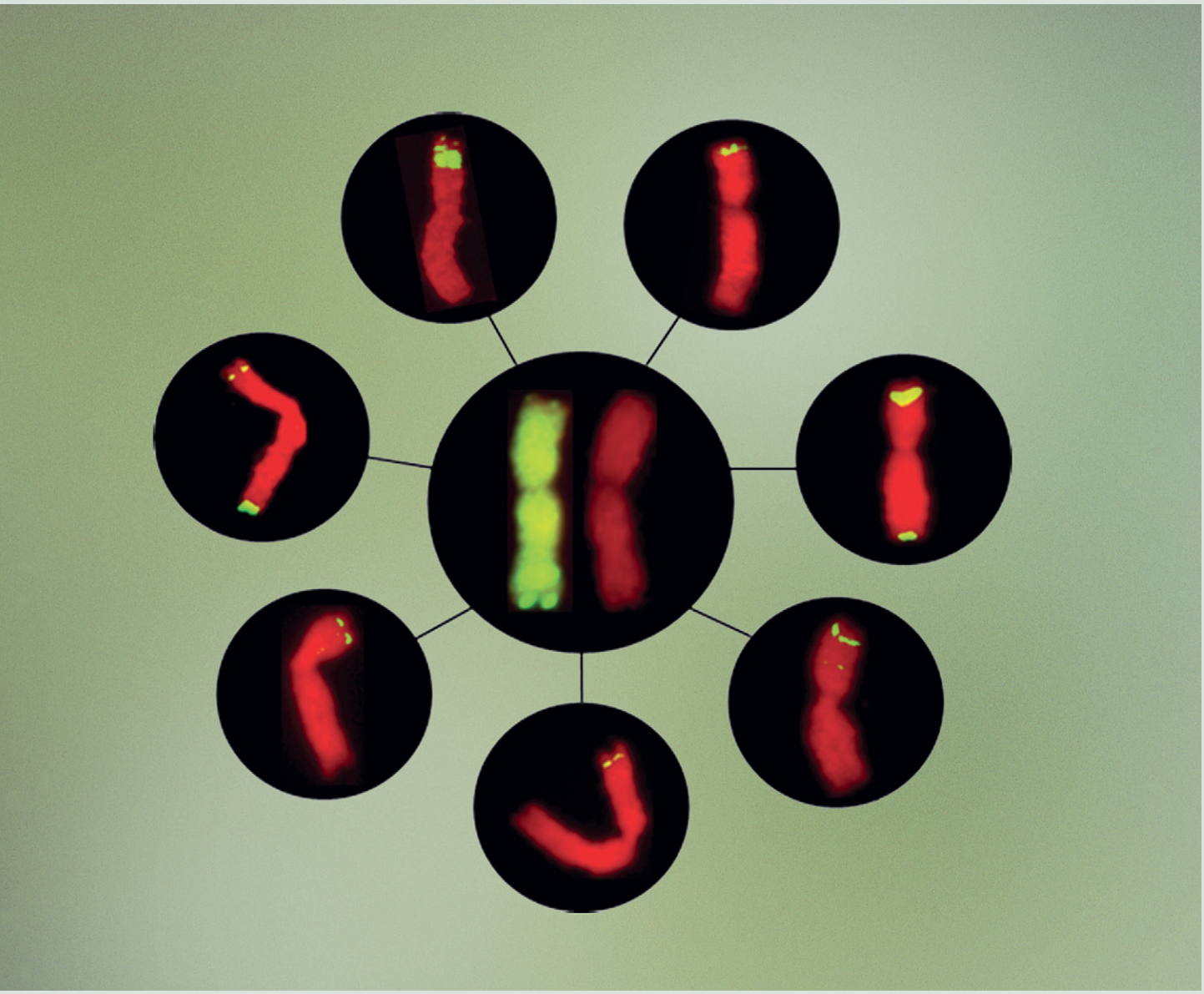

Introgression without the chaff

Efficient motif modeling

The mirage of neutrality

Muller's erratic ratchet

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Targeted Introgression of a Wheat Stem Rust Resistance Gene by DNA Marker-Assisted Chromosome Engineering

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ABSTRACT

Chromosome engineering is a useful strategy for transfer of alien genes from wild relatives into modern crops. However, this strategy has not been extensively used for alien gene introgression in most crops due to low efficiency of conventional cytogenetic techniques. Here, we report an improved scheme of chromosome engineering for efficient elimination of a large amount of goatgrass (*Aegilops speltoides*) chromatin surrounding *Sr39*, a gene that provides resistance to multiple stem rust races, including Ug99 (TTKSK) in wheat. The wheat *ph1b* mutation, which promotes meiotic pairing between homoeologous chromosomes, was employed to induce recombination between wheat chromosome 2B and goatgrass 2S chromatin using a backcross scheme favorable for inducing and detecting the homoeologous recombinants with small goatgrass chromosome segments. Forty recombinants with *Sr39* with reduced surrounding goatgrass chromatin were quickly identified from 1048 backcross progenies through disease screening and molecular marker analysis. Four of the recombinants carrying *Sr39* with a minimal amount of goatgrass chromatin (2.87–9.15% of the translocated chromosomes) were verified using genomic *in situ* hybridization. Approximately 97% of the goatgrass chromatin was eliminated in one of the recombinants, in which a tiny goatgrass chromosome segment containing *Sr39* was retained in the wheat genome. Localization of the goatgrass chromatin in the recombinants led to rapid development of three molecular markers tightly linked to *Sr39*. The new wheat lines and markers provide useful resources for the ongoing global effort to combat Ug99. This study has demonstrated great potential of chromosome engineering in genome manipulation for plant improvement.

MODERN genetic improvement has increased crop productivity worldwide, but it also erodes genetic variability of crops (Allard 1996; Hoisington *et al.* 1999). Narrowed genetic bases make modern crops fragile to global climate change and disease and insect epidemics (Tanksley and McCouch 1997; Chan 2010). Broadening the genetic variability will make crop production more sustainable under various biotic and abiotic stresses. One approach to increase genetic diversity is to incorporate genes from the crop's wild relatives using chromosome engineering (Brar and Khush 2005; Jellen and Leggett 2005; Singh 2005; Jauhar *et al.* 2009). However, progress of chromosome engineering has been limited due to difficulties in generating, recovering, and identifying meiotic recombinant chromosomes. Recent advances in genomics and high-throughput genotyping technologies have enhanced the competence of chromosome engineering for crop improvement (Ceoloni *et al.* 2005).

Supporting information is available online at http://www.genetics.org/cgi/content/full/genetics.110.123588/DC1.

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Wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD) is one of the major food crops and its production is currently threatened by a new stem rust (*Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn.) race, Ug99 (or TTKSK), identified in Uganda in 1999 (Pretorius *et al.* 2000; Wanyera *et al.* 2006; Singh *et al.* 2008; Jin *et al.* 2008; Jin *et al.* 2009). Although Ug99 has broad virulence to currently deployed *Sr* genes, it is avirulent to most of the *Sr* genes derived from relatives of wheat (Singh *et al.* 2006; Jin *et al.* 2007). However, large amounts of alien chromatin surround most of the alien *Sr* genes transferred to wheat (Xu *et al.* 2008). The genes other than the targeted gene on the alien chromatin usually cause linkage drag, a deleterious effect on yield and quality (The *et al.* 1988; Lukaszewski 2000; Labuschagne *et al.* 2002). Thus, additional chromosome engineering is needed to minimize alien chromatin before the alien *Sr* genes are deployed in wheat cultivars.

The alien *Sr* gene *Sr39* is effective against Ug99 and was transferred from goatgrass (*Aegilops speltoides* Tausch, $2n = 2x = 14$, SS) to wheat cultivar Marquis (Kerber and Dyck 1990). The original wheat line, RL5711, carried both *Sr39* and a gene for leaf rust resistance (*Lr35*) on a translocated chromosome between *Ae. speltoides* chromo-

some 2S and wheat chromosome 2B designated as T2B/2S#2 (Friebe *et al.* 1996). Later, *Sr39* and *Lr35* were transferred from RL5711 into wheat line RL6082 through six backcrosses using wheat cultivar Thatcher as the recurrent parent (Seyfarth *et al.* 1999). The translocated chromosome T2B/2S#2 consisted of the 2S long arm, a large portion (~85%) of the 2S short arm, and a small terminal 2B segment (Yu *et al.* 2010). To make *Sr39* usable to fight Ug99, the excess *Ae. speltoides* chromatin surrounding *Sr39* needs to be eliminated.

The common procedure for reducing alien chromatin in wheat is to induce meiotic recombination between the alien chromatin and its homoeologous (*i.e.*, partially homologous) region of wheat chromosome. Regular pairing between homologous chromosomes is ensured by the major gene *Ph1* on chromosome 5B in wheat (Riley and Chapman 1958; Gill *et al.* 1993; Martinez-Perez *et al.* 2001; Griffiths *et al.* 2006; Sidhu *et al.* 2008). Absence of *Ph1* due to nullisomy for chromosome 5B or mutation (*e.g.*, *ph1b* and *ph1c*) enhances meiotic pairing and recombination between homoeologous chromosomes (Sears 1954, 1966, 1977; Joppa and Williams 1988). The *Ph1*-deficient genetic stocks such as substitutions of chromosome 5B by 5D and *ph1b* mutant have been successfully employed for inducing meiotic recombination between wheat chromosomes and their alien homeologues (see review by Qi *et al.* 2007; Faris *et al.* 2008; Marais *et al.* 2010). By using the *ph1b* mutant, Mago *et al.* (2009) developed four wheat lines carrying *Sr39* on shortened *Ae. speltoides* chromosome segments. On the basis of their characterization of the two best lines, we estimate that the amount of *Ae. speltoides* chromatin has only been reduced by 20–25%.

In addition to the wheat lines reported by Mago *et al.* (2009), R. E. Knox developed a set of breeding lines carrying *Lr35/Sr39* from the original translocation line (Knox *et al.* 2000; Yu *et al.* 2010). One line had a slightly shortened *Ae. speltoides* chromosome segment, but the translocation chromosome still retained ~80–85% of the *Ae. speltoides* chromatin (Yu *et al.* 2010). All other lines contained similar amounts of *Ae. speltoides* chromatin as the original translocation lines. The *Ae. speltoides* chromatin present in the lines reported by Mago *et al.* (2009) and Yu *et al.* (2010) will still be unacceptable to many wheat breeders. Therefore, the objectives of this study were to develop an efficient chromosome engineering procedure to minimize *Ae. speltoides* chromatin surrounding *Sr39* in the wheat–*Ae. speltoides* translocation line.

MATERIALS AND METHODS

Plant materials: Wheat line RL6082 containing *Sr39* on the translocated chromosome T2B/2S#2, which was transferred from original wheat–*Ae. speltoides* translocation line RL5711 (Kerber and Dyck 1990) to Thatcher by P. L. Dyck in Winnipeg, Canada (Seyfarth *et al.* 1999), was used for chromosome engineering. Wheat 'Chinese Spring' (CS) and CS *ph1b* mutant were employed to induce meiotic recombination between the 2B and 2S homoeologous regions. Thatcher, *Ae. speltoides* accession RL5344 (donor of *Sr39*), CS N2A-T2B (nullisomic for 2A and tetrasomic for 2B), CS N2B-T2A (nullisomic for 2B and tetrasomic for 2A), and CS N2D-T2A (nullisomic for 2D and tetrasomic for 2A) were used as controls for stem rust evaluation, fluorescent genomic *in situ* hybridization (FGISH), and molecular marker analysis.

Chromosome manipulation: Wheat plants homozygous for *ph1b* and monosomic for both wheat chromosome 2B and translocated chromosome T2B/2S#2 were developed to induce meiotic recombination between 2B and T2B/2S#2 (Figure 1). These plants were created by crossing CS *ph1b* mutant (*ph1bph1b*) to RL6082 and backcrossing the F_1 plants to the *ph1b* mutant. The BC_1F_1 plants were tested for reactions to stem rust. Resistant BC_1F_1 plants were then analyzed with the molecular markers *PSR128*, *PSR574*, and *AWJL3* to select individuals homozygous for *ph1b* (Roberts *et al.* 1999). The resistant *ph1bph1b* plants, which were monosomic for both 2B and T2B/2S#2, were backcrossed to CS to efficiently recover the gametes containing a recombinant chromosome of 2B and T2B/2S#2. The BC_2F_1 plants were tested with stem rust and genotyped with a molecular marker. The BC_2F_2 progeny of the plants with a shortened *Ae. speltoides* chromosome segment were screened with stem rust and FGISH to confirm the lines carried *Sr39* on shortened *Ae. speltoides* chromosome segments. The sizes of the *Ae. speltoides* chromosome segments in the selected wheat lines were calculated as the average percentage of length of *Ae. speltoides* chromosome segment/total length of the translocation chromosome, which was measured in 18–20 cells with good-quality mitotic metaphases. To verify elimination of the *ph1b* allele in the selected wheat lines, 16 BC_2F_3 seeds from each of the selected BC_2F_2 plants were analyzed with markers linked to *ph1b*. The FGISH was performed as described by Yu *et al.* (2010). The methodologies for stem rust testing and molecular marker development, analysis, and validation are described below.

Stem rust resistance evaluation: The stem rust race TMLK, which can differentiate *Sr39* from the *Sr* genes in Thatcher and CS (Yu *et al.* 2010), was used for the inoculation of the BC_1F_1, BC_2F_1, and BC_2F_2 populations. The selected wheat lines with shortened *Ae. speltoides* chromosome segment were tested with six additional races, THTS, TPMK, RTQQ, RHTS, QFMQ, and QFCQ, along with TMLK and Ug99. The seedlings were grown in the greenhouse at 20–23° with a 16/8 hr (day/night) photoperiod. Seven-day-old seedlings were inoculated as described by Williams *et al.* (1992). Inoculated seedlings were transferred to either a greenhouse or growth chamber maintained at 20–23°. Infection types were scored on the 13th or 14th day after inoculation using the scale described by Stakman (1962), where 0 = immune, ; = necrotic flecks, 1 = small necrotic pustules, 2 = small to medium-sized chlorotic pustules with green island, 3 = medium-sized chlorotic pustules, and 4 = large pustules without chlorosis. Plants with infection type $\geq$3 were considered susceptible, and plants with an infection type $<$3 were considered resistant.

Molecular marker analysis: Yu *et al.* (2010) showed that microsatellite marker *Xgwm319* generated a 173-bp fragment from chromosome 2B and a 162-bp fragment from *Ae. speltoides* 2S chromatin. This marker was mapped to the centromeric region of chromosome 2B and 2S (Somers *et al.* 2004; Yu *et al.* 2010) and was used to select new 2B/2S recombinants. DNA samples of the BC_2F_1 population were extracted as described in the following steps. Young leaf tissue was collected in Qiagen 1.2 ml 96-well plates with addition of one 3-mm tungsten carbide bead (Qiagen, Valencia, CA) to each well. After loaded

plates were frozen in liquid nitrogen, the plates were shaken on a MM300 shaker (Retsch) at 30 Hz for 60–90 sec two to three times until the leaf tissue was ground into a fine powder. Four hundred microliters of preheated DNA extraction buffer (500 mM NaCl, 100 mM Tris-HCl, pH 8.0, 50 mM EDTA, 0.84% SDS, and 0.38 g/100 ml sodium bisulfate freshly added) was added to each well. Plates were briefly (5–10 sec) shaken, and the samples were incubated at 65° for 30–40 min. Chloroform (300–400 μl) was added to each well. After vigorously mixing, the samples were centrifuged at 4000–4500 rpm for 15 min and ~300 μl of supernatant from each well was transferred into new 96-well plates. About 0.8–0.9 volume of isopropanol was added to each well and plates were vigorously hand shaken. The plate was centrifuged at 4000–4500 rpm for 15 min and the supernatant was discarded. Six hundred microliters of 70% ethanol was added to each well and the plate was centrifuged at 4000–4500 rpm for 15 min. The supernatant was discarded and the pellet was air dried. The DNA was dissolved in 300 μl TE buffer (10 mM Tris, pH 8.0, 1 mM EDTA).

Marker genotyping was performed as described by Tsilo *et al.* (2009). Polymerase chain reaction (PCR) was performed at an annealing temperature of 50°. Amplified PCR products were labeled with four different fluorescent dyes (6-FAM, VIC, NED, and PET) and separated by a 16-capillary electrophoresis system ABI 3130xl genetic analyzer (Applied Biosystems, Foster City, CA), and genotype calls were analyzed using GeneMapper software v3.7 (Applied Biosystems). Following testing with marker *Xgwm319*, seven additional PCR-based markers (Sr39#22r, Sr39#50s, BE500705, *Xbarc18*, *Xbarc183*, *Xbarc200*, and *Xwmc025*) that detect the *Ae. speltoides* 2S chromatin carrying *Sr39* (Mago *et al.* 2009) were tested on the selected lines. In addition, STS (sequence-tagged site) markers developed as described below were used to test all BC_2F_1 plants in which *Sr39* disassociated from the *Ae. speltoides* allele of *Xgwm319*.

Development and validation of new STS markers linked to *Sr39*: On the basis of the physical location of *Ae. speltoides* chromatin detected with FGISH, the wheat EST (expressed sequence tag) sequences from the deletion bin 2BS4-0.75-0.84 (http://wheat.pw.usda.gov/data-bin/graingenes/report.data?class=breakpointinterval;name=2BS4-0.75-0.84;show=locus) were selected to design primers using Primer3Plus (Rozen and Skaletsky 2000; http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) under general settings. These primers were used to screen for polymorphisms among Thatcher, CS, RL6082, a bulk of four resistant BC_2F_2 plants, a bulk of four susceptible BC_2F_2 plants, CS N2A-T2D, CS N2B-T2A, and CS N2D-T2A. Genomic DNA was extracted using the 2× CTAB method (Li and Quiros 2001). PCR were carried out as follows: 95° for 5 min, 95° for 40 sec, 55° for 40 sec, 72° for 40 sec, repeated for 36 cycles, with a final extension at 72° for 10 min. The PCR products were separated on an 8% nondenaturing polyacrylamide gel and stained with 2× GelRed. The gel was scanned using a Typhoon 9410 scanner (GE Healthcare Biosciences, Pittsburgh, NJ). The EST sequences from primers detecting polymorphisms were then used to BLAST against the wheat EST database (http://wheat.pw.usda.gov/GG2/blast.shtml), and the top hit contig sequence (NSFT03P2_Contig11068) was selected to BLAST against the rice (*Oryza sativa* L.) genomic sequence (http://www.shigen.nig.ac.jp/wheat/komugi/blast/blast.jsp). The top hit clone sequence (tp1b0012I12) from the rice Indica group was selected to design additional primers. The polymorphic STS markers were tested on a BC_2F_2 population inoculated with TMLK. The new STS markers linked to *Sr39* on reduced *Ae. speltoides* chromatin were then validated in a set of 40 durum (*T. turgidum* L. subsp. *durum*) and common wheat cultivars/lines from China and the United States.

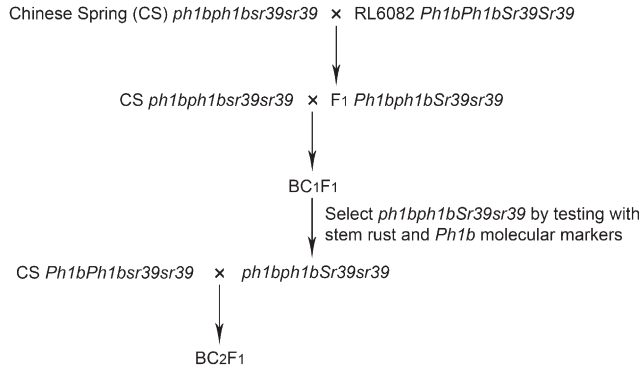

Figure 1.—Hybridization procedure to reduce the size of the *Aegilops speltoides* chromosome segment containing *Sr39* in the wheat–*Ae. speltoides* chromosome 2B/2S translocation line RL6082.

RESULTS

We used an improved procedure (Figure 1) to develop *ph1b*-induced homoeologous recombinants. From the cross and backcross of RL6082 with CS *ph1b* mutant, 93 BC_1F_1 plants (pedigree: CS *ph1bph1b**2/RL6082) were generated and tested with TMLK (supporting information, Table S1). The segregation of 53 resistant plants to 40 susceptible plants fit a 1:1 ratio ($\chi^2 = 1.82$), indicating that stem rust resistance was conditioned by a single gene. The 53 resistant plants were analyzed with markers *PSR128*, *PSR574*, and *AWJL3* to detect the presence of *ph1b*. Sixteen resistant plants were identified as homozygotes for *ph1b* (Figure S1 and Table S1) and were backcrossed to CS to develop 16 families composing a large BC_2F_1 population. Among 1048 BC_2F_1 plants from 12 families, 554 were resistant and 494 were susceptible to TMLK (Table 1 and Table S2). While this segregation was close to a 1:1 ratio, analysis of the populations on a family basis indicated that segregation in only three families fit a 1:1 ratio (Table 1). A heterogeneity χ^2 test of the families ($\chi^2 = 204.6$) indicated that the data could not be pooled. These results indicated that significant segregation distortion was present among families.

The 1048 BC_2F_1 plants tested with TMLK were screened for dissociation between marker *Xgwm319* and *Sr39*. *Xgwm319* amplified 193-bp and 191-bp fragments from chromosome 2B of CS and Thatcher, respectively, and a 182-bp fragment from RL5344 and RL6082 (fragment sizes include a 20-bp M13 primer tail) (Figure 2). The results showed that only 40 of 532 resistant BC_2F_1 plants carried the *Xgwm319* allele from CS, while the remaining 492 plants had the RL6082 allele (Table 2 and Table S3). Of 476 susceptible plants, 265 carried the RL6082 allele and the remaining 211 plants had the CS allele. The overall dissociation frequency of *Sr39* from *Xgwm319* was 30.3% (305/1008) (Table 2); however, the dissociation frequency was 7.5% (40/532) and 44.3%

TABLE 1

Segregation for resistance to stem rust race TMLK among BC_2F_1 plants in the 12 families derived from crossing Chinese Spring (CS) to 12 BC_1F_1 plants having the pedigree CS *ph1bph1b2/RL6082**

Family no.	Resistant	Susceptible	χ^2 (1:1)	Prob. (1:1)
81-3	49	35	2.33	0.127
81-5	27	63	14.40	<0.001
81-6	51	38	1.90	0.168
81-11	48	40	0.73	0.394
81-20	21	61	19.51	<0.001
81-35	60	29	10.80	0.001
81-38	72	16	35.64	<0.001
81-39	17	71	33.14	<0.001
81-40	66	24	19.6	<0.001
81-42	57	29	9.12	0.003
81-56	67	18	28.24	<0.001
81-63	19	70	29.22	<0.001
Total	554	494	3.44	0.064

Prob., probability.

(211/476) among resistant and susceptible plants, respectively. The difference in dissociation frequency among resistant and susceptible plants supports the conclusion that significant segregation distortion occurred in the populations.

The original translocation line RL6082 and the BC_2F_2 plants derived from 11 of the resistant BC_2F_1 plants exhibiting dissociation of *Sr39* from *Xgwm319* were screened with stem rust and FGISH to confirm the BC_2F_1 plants with *Sr39* on shortened *Ae. speltoides* chromosome segments. One BC_2F_1 plant from family 81-3 was identified to carry a 2B/2S translocation chromosome, but with only a slightly reduced *Ae. speltoides* chromatin in the short arm (Figure S2A). Six BC_2F_1 plants carried a telocentric chromosome, with the entire 2S long arm (2SL) being absent (Figure S2, B–G). Four BC_2F_1 plants were identified that carried a 2B/2S translocation chromosome with a very small amount of *Ae. speltoides* chromatin. From each of these four plants, new wheat lines (RWG1, RWG2, RWG3, and RWG4) with homozygous short translocations were selected from the BC_2F_2 progeny by rust testing (Table S4) followed by FGISH analysis (Figures 3 and 4A). The *Ae. speltoides* chromatin carrying *Sr39* in RWG1, RWG2, RWG3, RWG4, and RL6082, in average comprised 2.87%, 4.72%, 3.60%, 9.15%, and 95.03% of the interchanged chromosome, respectively (Table S5), indicating that ~90–97% of the *Ae. speltoides* chromatin surrounding *Sr39* had been eliminated. Except for the *Ae. speltoides* chromatin carrying *Sr39*, other small hybridization signals at the telomeres of some chromosomes were also frequently detected in RL6082 and the four new wheat lines (Figures 3 and 4A). We were unable to determine the identity of these signals, which could be caused by the *Ae.*

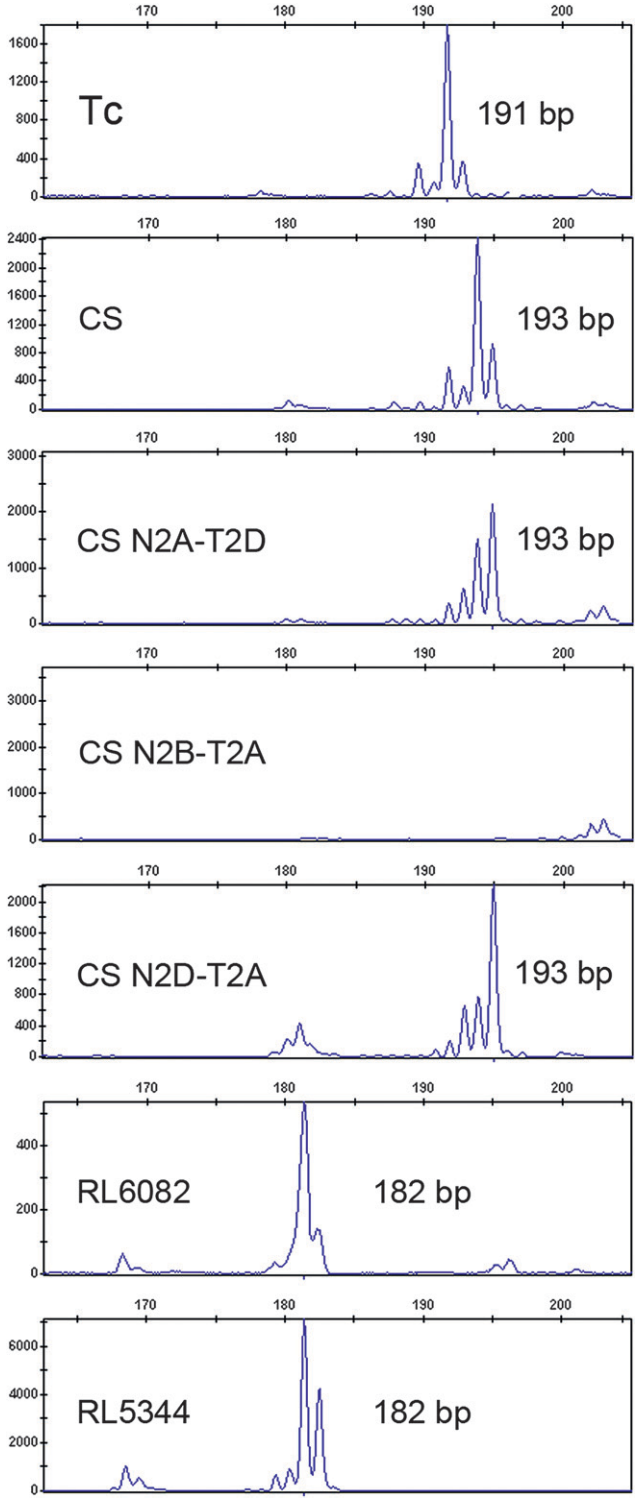

FIGURE 2.—Electropherograms showing the polymerase chain reaction (PCR) products of microsatellite marker *Xgwm319* in Thatcher (Tc), Chinese Spring (CS), three CS nullisomic-tetrasomic (N-T) lines, RL6082, and *Aegilops speltoides* RL5344 (*Sr39* source). The three CS N-T lines, including N2A-T2D (nullisomic 2A and tetrasomic for 2D), N2B-T2A, and N2D-T2A, were used as checks for the PCR amplification on chromosome 2B. Fragment sizes include a 20-bp M13 primer tail. The peak represents the PCR products, whereas the horizontal and vertical scales represent fragment sizes in base pairs and fluorescent signal intensity, respectively.

TABLE 2

Stem rust test and marker analysis for BC_2F_1 plants having the pedigree Chinese Spring (CS)//CS *ph1bph1b2/ RL6082**

Reaction to TMLK		No. of plants with marker (*Xgwm319*) allele from		
Type	No. of plants	RL6082	CS	Missing data
R	554	492	40	22
S	494	265	211	18
Total	1,048	755	253	40

R, resistant; S, susceptible.

speltoides chromatin or the highly repetitive sequences shared by the wheat and *Ae. speltoides* genomes.

The FGISH analysis of the new wheat lines indicated that the *Ae. speltoides* chromatin carrying *Sr39* was approximately located in chromosome bin 2BS4-0.75-0.84. To develop molecular markers closely associated with *Sr39*, we designed primers on the basis of the wheat ESTs mapped to 2BS4-0.75-0.84 and its collinear region of the rice genome. A total of 40 primer pairs (Table S6) were tested for polymorphisms on Thatcher, CS, RL6082, bulked resistant BC_2F_2 plants, bulked susceptible BC_2F_2 plants, CS N2A-T2D, CS N2B-T2A, and CS N2D-T2A. Three primer pairs detected polymorphisms and generated codominant STS markers, which were designated as *Xrwgs27*, *Xrwgs28*, and *Xrwgs29* (Table 3 and Figure 4C). The marker *Xrwgs27* amplified a 740-bp fragment in RL6082 and a 710-bp fragment in Thatcher and CS; *Xrwgs28* amplified 360-bp, 450-bp, and 520-bp fragments in RL6082 and a 350-bp fragment in Thatcher and CS; and *Xrwgs29* amplified a 540-bp fragment in RL6082 and a 550-bp fragment in Thatcher and CS. The four introgression lines therefore carried bands of 740, 360/450/520, and 540 bp for *Xrwgs27*, *Xrwgs28*, and *Xrwgs29*, respectively. These three markers were tested on a population of 65 BC_2F_2 plants, and all cosegregated with stem rust resistance (Table S7). Identification of markers based on ESTs mapped to bin 2BS4-0.75-0.84 confirmed that the short *Ae. speltoides* chromosome segments carrying *Sr39* was interstitially located in bin 2BS4-0.75-0.84.

Analysis of four introgression lines with the seven markers reported by Mago *et al.* (2009) revealed that marker Sr39#22r detected the *Ae. speltoides* chromatin carrying *Sr39* for all four lines, but Sr39#50s and *Xbarc183* detected the *Ae. speltoides* chromatin only for RWG4 (Figure 4, D and E). The four remaining markers (BE500705, *Xbarc18*, *Xbarc200*, and *Xwmc025*) produced no polymorphism between RL6082 and CS or Thatcher. Because we only analyzed the BC_2F_2 plants derived from 11 of the 40 BC_2F_1 plants exhibiting dissociation of *Sr39* from *Xgwm319* with FGISH, the remaining 29 BC_2F_1 plants were tested with markers Sr39#22r, *Xrwgs27*, *Xrwgs28*, and *Xrwgs29*. When compared to RL6082 and the four introgression lines, no polymorphisms were observed in the 29 plants (Table S8), and therefore none of the 29 plants had *Ae. speltoides* chromosome segments even shorter than the four selected introgression lines.

The four introgression lines (RWG1, RWG2, RWG3, and RWG4) all exhibited a similar level of resistance to seven local stem rust races (THTS, TPMK, RTQQ, RHTS, QFMQ, QFCQ, and TMLK) and Ug99 as the original stock RL6082 (Table 4 and Figure 4A, right). They were morphologically similar to CS with normal seed fertility and had bigger spikes than RL6082 (Figure 4B). Analysis of 16 single BC_2F_3 seeds from the original BC_2F_2 plants for each of four lines with *ph1b* markers showed that plants homozygous for *ph1b* were not detected in any line (Figure S3), suggesting that the four introgression lines did not carry the *ph1b* allele.

The three STS markers (*Xrwgs27*, *Xrwgs28*, and *Xrwgs29*) were validated with 23 spring wheat, 9 winter wheat, and 8 durum cultivars/lines (Table 5, Figure 4F). *Xrwgs27* amplified a 710-bp or a 725-bp fragment in 28 or 12 cultivars/lines, respectively (Table 5). For *Xrwgs28* and *Xrwgs29*, amplicons of different sizes were observed between wheat classes (durum wheat *vs.* common wheat), but were uniform within class. Amplicons derived from *Ae. speltoides* chromatin could be differentiated from all the durum and common wheat cultivars/lines tested, and these three markers are thus suitable for breeding resistant cultivars using marker-assisted selection of *Sr39*.

DISCUSSION

Success of chromosome engineering for targeted introgression of alien genes is dependent upon elimination of the deleterious effects of the introgressed alien chromatin in the crop plant. Thus far, it has been a challenge to transfer a small amount of alien chromatin containing the gene of interest from one genome to another nonhomologous genome. Only a few genes have been isolated from large alien chromosomal segments, such as *Sr26* (Dundas *et al.* 2007) and *SrR* (Anugrahwati *et al.* 2008) for stem rust resistance and *Pm21* (Chen *et al.* 1995) for powdery mildew (*Erysiphe graminis* DC. f. sp. *tritici* Em. Marchal) resistance transferred into wheat, and *Rfo* for fertility restoration transferred from radish (*Raphanus sativus* L.) into canola (*Brassica napus* L.) (Feng *et al.* 2009). The end products of most chromosome engineering studies have been chromosome additions or translocations involving large alien chromosomal segments (see reviews by Jiang *et al.* 1994; Friebe *et al.* 1996; Fedak 1999; Xu *et al.* 2009). The present study demonstrated that targeted introgression of alien genes could be more efficiently and precisely accomplished than in the past by improving the protocol in inducing and identifying homoeologous recombinants.

Construction of an optimal population of homoeologous recombinants: Development of a population of

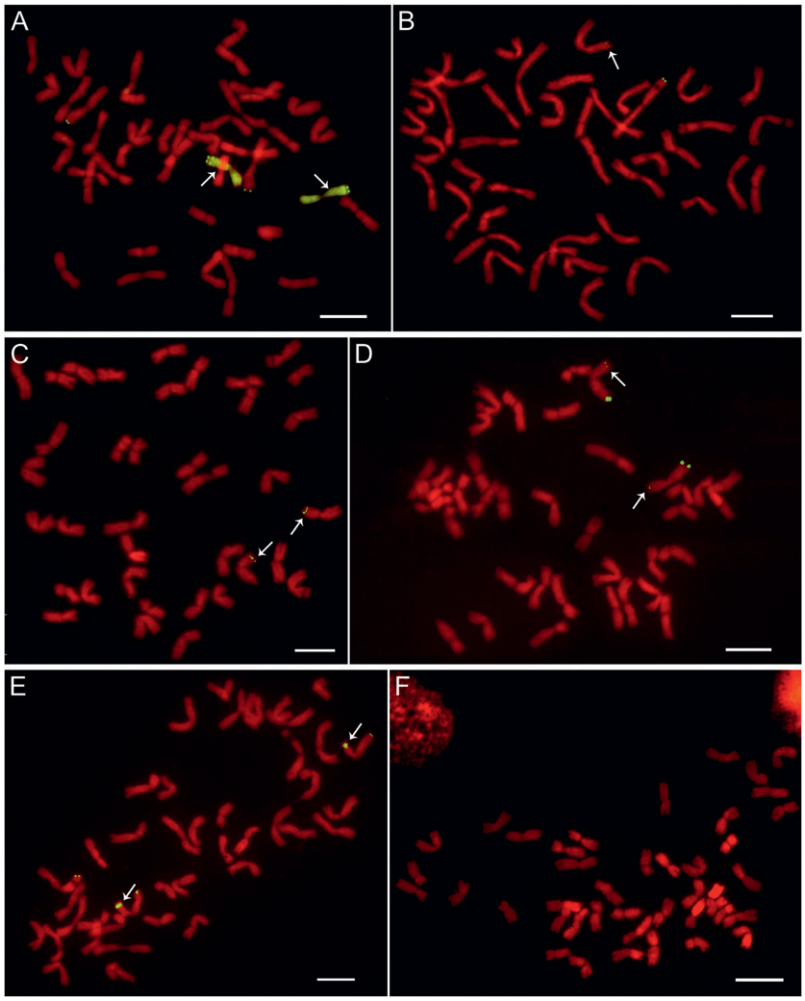

FIGURE 3.—Fluorescent genomic *in situ* hybridization (FGISH) results for RL6082 (A), four new wheat lines RWG1 (B), RWG2 (C), RWG3 (D), and RWG4 (E) with *Sr39*, and Chinese Spring (F). The FGISH result for RWG1 (B) was obtained from a BC_2F_2 plant that was heterozygous for the translocation chromosome. The *Ae. speltoides* chromatin (green) is indicated by arrows. The green signals on the telomeres of the chromosomes could be caused by the *Ae. speltoides* chromatin or the highly repetitive sequences shared by wheat and *Ae. speltoides*. Bar, 10 μm.

spontaneous or artificially induced homoeologous recombinants containing an alien gene through a proper hybridization scheme is the first step in eliminating unwanted alien chromatin. Such populations should be favorable for inducing homoeologous recombination and should be easily phenotyped and genotyped. The population size should be large enough (*e.g.*, 1000–2000 individuals) to include desired recombinants carrying the gene of interest. On the basis of these considerations, we used a stem rust-susceptible wheat cultivar CS and its *ph1b* mutant to develop a large backcross population for *Sr39* so that the homoeologous recombinants carrying the gene could be quickly identified using a stem rust test at the seedling stage.

The F_2 populations from crosses involving the CS *ph1b* mutant have been commonly used to reduce alien chromatin in the wheat genome (FRIEBE *et al.* 1996; QI *et al.* 2007; MAGO *et al.* 2009). In our study, we used a BC_2F_1 population instead of an F_2 population for induction of new homoeologous recombinants. The BC_2F_1 population was developed by backcrossing the resistant BC_1F_1 plants that were homozygous for *ph1b* and hemizygous for the translocated alien chromosome segment. By making this backcross, any plant detected to have a reduced alien chromatin was selfed, and homozygous progeny for the reduced alien chromatin were selected. Most importantly, the hemizygous alien chromatin was easily detected by marker screening. Selection in a BC_2F_1 instead of a F_2 or F_3 population may have an advantage beyond ease of selection of homozygotes. In our study, we used a BC_1F_1 plant as male to produce the BC_2F_1 population. As a consequence, pollen competition may be a factor in producing lines with shortened alien chromosome segments. CHEN *et al.* (2005) produced wheat–*Leymus racemosus* translocation lines using irradiated monosomic-addition plants as male in crosses to cultivars susceptible to Fusarium head blight (caused by *Fusarium graminearum* Schw.). They concluded that preferential transmission favored selection of gametes carrying a translocated chromosome over gametes carrying a complete alien chromosome. In our study, RL6082 carried a pair of translocated chromosomes with a very large *Ae. speltoides* chromosome segment, and pollen competition may therefore favor gametes carrying a small *Ae. speltoides* chromosome segment.

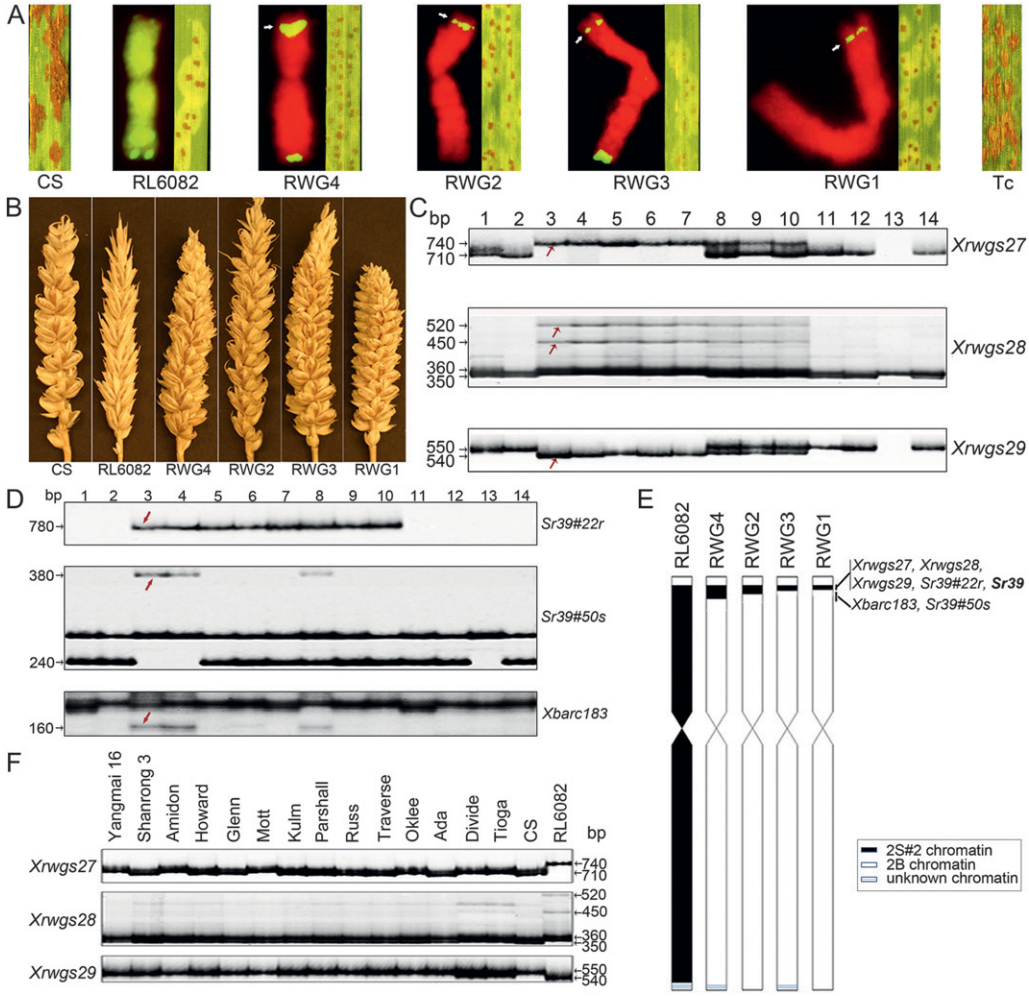

FIGURE 4.—Molecular, phenotypic, and cytogenetic characteristics of four new wheat lines RWG1, RWG2, RWG3, and RWG4 and their parents RL6082, Chinese Spring (CS), and Thatcher (Tc). (A) Fluorescent genomic *in situ* hybridization results and stem rust reactions to TMLK. The *Ae. speltoides* chromatin (green signal) is indicated by arrows. (B) Spike morphology. (C) Images of the polymerase chain reaction (PCR) amplicons of three codominant sequence-tagged site (STS) markers (*Xrwgs27*, *Xrwgs28*, and *Xrwgs29*) associated with *Sr39*. The numbers at the top of the gels are lane numbers: 1, Tc; 2, CS; 3, RL6082; 4, RWG4; 5, RWG3; 6, RWG2; 7, RWG1; 8-10, heterozygous BC_2F_2 plants for RWG4, RWG3, and RWG2, respectively; 11, one BC_2F_2 susceptible plant; 12, CS N2A-T2D (nullisomic for chromosome 2A and tetrasomic for 2D); 13, CS N2B-T2A; and 14, CS N2D-T2A. Diagnostic bands for the *Ae. speltoides* chromatin carrying *Sr39* are indicated by arrows in RL6082. The numbers on the left represent the fragment size in base pairs. (D) Images of PCR amplicons for markers Sr39#22r, Sr39#50s, and *Xbarc183*. The lane numbers and genotype designations were the same as those for the markers *Xrwgs27*, *Xrwgs28*, and *Xrwgs29* in image C. (E) Schematic representation of the interchanged chromosomes in RL6082, RWG1, RWG2, RWG3, and RWG4, showing sizes of shortened *Ae. speltoides* chromosome segments and locations of *Sr39* and six PCR-based markers. (F) A portion of the gel image from validation of three STS markers in a set of durum and common wheat cultivars and lines. The numbers on the right represent the fragment size in base pairs.

Rapid identification of homoeologous recombinants with reduced alien chromatin using robust DNA marker: The low output of short translocations from most previous chromosome engineering efforts are largely due to the conventional cytogenetic approaches used for chromosome identification (*e.g.*, chromosome karyotype and pairing analysis, banding, and *in situ* hybridization) that are tedious, laborious, and not suitable for handling large populations. In this study, we quickly selected individuals with reduced alien chromatin by screening a large segregating population with a single molecular marker, *Xgwm319*, instead of cytogenetic approaches. Our success in using this marker may be partially attributed to the relative position between *Xgwm319* and *Sr39*. MAGO *et al.* (2009) found that *Sr39* was located near the original translocation breakpoint in RL5711; and, as a consequence, the *Ae. speltoides* chromatin needed little or no reduction distal to *Sr39*. *Xgwm319* is close to the centromere of wheat chromosome 2B (SOMERS *et al.* 2004; SOURDILLE *et al.* 2004), where recombination rates are reduced and linkage blocks are large (WU *et al.* 2003). Thus, any resistant plant that lacks the *Ae. speltoides* allele at the *Xgwm319* locus must have lost a relatively large *Ae. speltoides* chromosome segment. At the same time, *Xgwm319* is sufficiently remote from *Sr39* to allow for multiple recombination events between *Xgwm319* and *Sr39*. Using this rationale, only 40 resistant recombinants were identified from the original population of 1048 plants.

Segregation distortion of stem rust resistance in a population of *ph1b*-induced homoeologous recombinants: We observed significant segregation distortion of stem rust resistance in the BC_2F_1 population in the study. Segregation distortion is a common feature associated with alien chromosomes in the wheat background (CEOLONI *et al.* 1996; MARAIS *et al.* 2010). It is

TABLE 3

Three sequence-tagged site (STS) markers linked to *Sr39* located on short *Aegilops speltoides* chromosome segment in the four new wheat lines

Marker	Primer sequence	Tm (50 mM Na^+) (°C)[a]	Band size (bp)		EST accession or genomic group[c]
			RL6082	CS[b]	
Xrwgs27	5′ GCCTTGGTGGATTTTGTGAT 3′	60	740	710	BG275030
	5′ GCGCTTTCAGTACAGGGTTC 3′	60			
Xrwgs28	5′ AGAGCCTGGGACTGTTGCTA 3′	60	360/450/520	350	tplb0012l12
	5′ CAATGGCACTCTTCAAAGCA 3′	60			
Xrwgs29	5′ CGGCTATTGCTCAAAGAAGG 3′	60	540	550	tplb0012l12
	5′ TGTTTCTGTCAGAGGCAACG 3′	60			

[a] Melting temperature.
[b] CS, Chinese Spring.
[c] Wheat EST (expressed sequence tag) accessions were obtained from Website: http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi (verified on November 27, 2010). The genomic clone tplb0012l12 sequence (4162 bp) was obtained from Website: http://www.shigen.nig.ac.jp/wheat/komugi/ests/cdnaQueryAction.do?cloneName=tplb0012l12&resourceTypeId=2 (verified on November 27, 2010), which contains a hypothetical protein OsI_27446 of rice (*Oryza sativa* L.) Indica Group.

caused by the presence of segregation distortion (*Sd*) and gametocidal (*Gc*) factors. Kerber and Dyck (1990) reported distorted segregation ratios in the breeding of RL5711, which resulted in preferential transmission of the rust resistance genes through male gametes. Our observation that the dissociation frequency was only 7.5% in resistant plants indicated a preferential transmission of the alien chromatin carrying both the *Ae. speltoides* allele at the *Xgwm319* locus and *Sr39*. The higher dissociation frequency of 44.3% among susceptible plants indicated that there was reduced preferential transmission when only the *Ae. speltoides* segment carrying *Sr39* is present. In the present experiment, segregation ratios may also have been altered due to chromosome breaks, which produced telocentric chromosomes having reduced transmission. Finally, the CS *ph1bph1b* line used in this experiment had been maintained in a homozygous recessive condition, and the line may have accumulated translocations that, despite not seriously affecting fertility of the CS *ph1bph1b* line, may have affected meiosis to alter transmission of some chromosome segments. Thus, backcross of the selected lines to agronomically acceptable cultivars is important to eliminate *ph1b* and remove other unwanted translocations from the wheat lines.

Development of new molecular markers linked to *Sr39* in new introgression lines: Pyramiding of multiple stem rust resistant genes into one cultivar will be necessary for long-term control of stem rust. One of the major factors for successful control of stem rust in North America has been the development of cultivars carrying multiple *Sr* genes (Zhong *et al.* 2009). To pyramid multiple resistance genes into one cultivar using stem rust testing, multiple races are needed to differentiate resistance genes. However, because *Sr* genes are usually effective against multiple races, it is difficult, or even impossible, to select specific races for screening the desired genes. Therefore, molecular markers could serve as an important alternate tool for gene pyramiding. Mago *et al.* (2009) reported several markers that could

TABLE 4

Infection types produced by four wheat lines and their parental lines to Ug99 (TTKSK) and seven locally maintained races of stem rust

Genotype	Infection types to races[a]							
	TTKSK	TPMK	TMLK	THTS	RTQQ	RHTS	QFMQ	QFCQ
Thatcher		432	4	43	34	43	32	34
Chinese Spring	4	4	43	4	4	43	43	4
RL6082	2–	12	12	12	12	1	12	12
RWG1	2–	123	12	12	12	12	12	12
RWG2	2–2	21	12	12	12	12	12	12
RWG3	2–	21	12	12	12	12	12	21
RWG4	2+	12	12	12	12	12	12	12

[a] Infection types follow Stakman (1962) where 0, ;, 1, and 2 were considered low infection types, and 3 to 4 were considered high infection types. For combinations, order indicates predominant types, hence 432 is predominantly infection type (IT) 4, with decreasing amounts of IT 3 and IT 2. 2–, small IT 2; 2+, large IT 2.

TABLE 5

Band size of three marker loci in 40 durum and common wheat cultivars or lines

Cultivar or line	Origin[a]	Growth habit	Type	Marker band size (bp)[b]		
				Xrwgs27	*Xrwgs28*	*Xrwgs29*
Jimai 22	China	Winter	Common wheat	725	350	550
Yangmai 16	China	Winter	Common wheat	725	350	550
Shanrong 1	China	Winter	Common wheat	710	350	550
Shanrong 3	China	Winter	Common wheat	710	350	550
Jinan 17	China	Winter	Common wheat	710	350	550
Jinan 177	China	Winter	Common wheat	725	350	550
Zhengmai 9023	China	Winter	Common wheat	725	350	550
Amidon	ND	Spring	Common wheat	725	350	550
Howard	ND	Spring	Common wheat	710	350	550
Alsen	ND	Spring	Common wheat	710	350	550
Grandin	ND	Spring	Common wheat	725	350	550
Glenn	ND	Spring	Common wheat	710	350	550
Faller	ND	Spring	Common wheat	710	350	550
Glupro	ND	Spring	Common wheat	725	350	550
Ernest	ND	Spring	Common wheat	725	350	550
Steele-ND	ND	Spring	Common wheat	710	350	550
Reeder	ND	Spring	Common wheat	710	350	550
Mott	ND	Spring	Common wheat	725	350	550
Kulm	ND	Spring	Common wheat	710	350	550
Parshall	SD	Spring	Common wheat	710	350	550
Granger	SD	Spring	Common wheat	710	350	550
Brick	SD	Spring	Common wheat	710	350	550
Russ	SD	Spring	Common wheat	710	350	550
Briggs	SD	Spring	Common wheat	710	350	550
Traverse	SD	Spring	Common wheat	710	350	550
Sabin	MN	Spring	Common wheat	710	350	550
Oklee	MN	Spring	Common wheat	725	350	550
Ulen	MN	Spring	Common wheat	710	350	550
Ada	MN	Spring	Common wheat	710	350	550
Tom	MN	Spring	Common wheat	725	350	550
Newton	KS	Winter	Common wheat	725	350	550
IL06-14262	IL	Winter	Common wheat	710	350	550
Divide	ND	Spring	Durum wheat	710	355	545 + 550
Ben	ND	Spring	Durum wheat	710	355	545 + 550
Tioga	ND	Spring	Durum wheat	710	355	545 + 550
Grenora	ND	Spring	Durum wheat	710	355	545 + 550
Lebsock	ND	Spring	Durum wheat	710	355	545 + 550
Monroe	ND	Spring	Durum wheat	710	355	545 + 550
Alkabo	ND	Spring	Durum wheat	710	355	545 + 550
Mountrail	ND	Spring	Durum wheat	710	355	545 + 550

[a] ND, North Dakota; SD, South Dakota; MN, Minnesota; IL, Illinois; KS, Kansas.

[b] The fragment sizes from *Aegilops speltoides* for *Xrwgs27*, *Xrwgs28*, and *Xrwgs29* are 740 bp, 360/450/520 bp, and 540 bp, respectively.

detect *Ae. speltoides* chromatin carrying *Sr39*. Of those markers, we found that only Sr39#22r could detect the *Ae. speltoides* chromatin in our three shortest translocation lines, RWG1, RWG2, and RWG3. Sr39#22r is a dominant marker, which is used with Sr39#50s or *Xbarc183* to detect heterozygous plants. Because Sr39#50s and *Xbarc183* cannot be detected in RWG1, RWG2, and RWG3, additional codominant markers are needed to detect heterozygous plants in these lines. In our study, we utilized information from FGISH, chromosome bin-mapped wheat ESTs, the rice genomic sequence, and the BLAST tool to quickly develop three codominant STS markers tightly linked to *Sr39*. These markers should be useful for pyramiding of *Sr39* with other *Sr* genes into commercial cultivars for controlling Ug99.

This study demonstrated that alien gene introgression could be efficiently accomplished using an improved scheme of chromosome engineering. In <2 years, we successfully developed four wheat lines with *Sr39* residing in a small *Ae. speltoides* chromosome segment. Over 90% of the *Ae. speltoides* chromatin was eliminated in these wheat lines. We believe that introgression of stem rust resistance genes from wild relatives into wheat

will no longer be a challenging task using the optimal procedure reported in this study. The effective *Sr* genes present in the secondary and tertiary gene pool will become available for wheat breeding when they are needed for fighting newly emerging races. The successful integration of modern DNA marker technology into chromosome engineering will set a benchmark for future alien gene introgression in wheat and other plant species.

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LITERATURE CITED

Allard, R. W., 1996 Genetic basis of the evolution of adaptedness in plants. Euphytica **92:** 1–11.

Anugrahwati, D. R., K. W. Shepherd, D. C. Verlin, P. Zhang, G. Mirzaghaderi *et al.*, 2008 Isolation of wheat-rye 1RS recombinants that break the linkage between the stem rust resistance gene *SrR* and secalin. Genome **51:** 341–349.

Brar, D. S., and G. S. Khush, 2005 Cytogenetic manipulation and germplasm enhancement of rice (*Oryza sativa* L.), pp. 115–158 in *Genetic Resources, Chromosome Engineering, and Crop Improvement Series: Cereals*, Vol. 2, edited by R. J. Singh and P. P. Jauhar. CRC Press, Boca Raton, FL.

Ceoloni, C., M. Biagetti, M. Ciaffi, P. Forte and M. Pasquini, 1996 Wheat chromosome engineering at the 4x level: the potential of different alien gene transfers into durum wheat. Euphytica **89:** 87–97.

Ceoloni, C., P. Forte, A. Gennaro, S. Micali, R. Carozza *et al.*, 2005 Recent developments in durum wheat chromosome engineering. Cytogenet. Genome Res. **109:** 328–334.

Chan, S. W. L., 2010 Chromosome engineering: power tools for plant genetics. Trends Biotechnol. **28:** 605–610.

Chen, P. D., L. L. Qi, B. Zhou, S. Z. Zhang and D. J. Liu, 1995 Development and molecular cytogenetic analysis of wheat-*Haynaldia villosa* 6VS/6AL translocation lines specifying resistance to powdery mildew. Theor. Appl. Genet. **91:** 1125–1128.

Chen, P. D., W. X. Liu, J. H. Yuan, X. E. Wang, B. Zhou *et al.*, 2005 Development and characterization of wheat-*Leymus racemosus* translocation lines with resistance to *Fusarium* head blight. Theor. Appl. Genet. **111:** 941–948.

Dundas, I. S., D. R. Anugrahwati, D. C. Verlin, R. F. Park, H. S. Bariana *et al.*, 2007 New sources of rust resistance from alien species: meliorating linked defects and discovery. Aust. J. Agric. Res. **58:** 545–549.

Faris, J. D., S. S. Xu, X. Cai, T. L. Friesen and Y. Jin, 2008 Molecular and cytogenetic characterization of a durum wheat-*Aegilops speltoides* chromosome translocation conferring resistance to stem rust. Chromosome Res. **16:** 1097–1105.

Fedak, G., 1999 Molecular aids for integration of alien chromatin through wide crosses. Genome **42:** 584–591.

Feng, J., V. Primomo, Z. Li, Y. Zhang, C.-C. Jan *et al.*, 2009 Physical localization and genetic mapping of the fertility restoration gene *Rfo* in canola (*Brassica napus* L.). Genome **52:** 401–407.

Friebe, B., J. Jiang, W. J. Raupp, R. A. McIntosh and B. S. Gill, 1996 Characterization of wheat-alien translocations conferring resistance to diseases and pests: current status. Euphytica **91:** 59–87.

Gill, K. S., B. S. Gill, T. R. Endo and Y. Mukai, 1993 Fine physical mapping of *Ph1*, a chromosome pairing regulator gene in polyploid wheat. Genetics **134:** 1231–1236.

Griffiths, S., R. Sharp, T.N. Foote, I. Bertin, M. Wanous *et al.*, 2006 Molecular characterization of *Ph1* as a major chromosome pairing locus in polyploid wheat. Nature **439:** 749–752.

Hoisington, D., M. Khairallah, T. Reeves, J. M. Ribaut, B. Skovmand *et al.*, 1999 Plant genetic resources: What can they contribute toward increased crop productivity? Proc. Natl. Acad. Sci. USA **96:** 5937–5943.

Jauhar, P. P., T. S. Peterson and S. S. Xu, 2009 Cytogenetic and molecular characterization of a durum alien disomic addition line with enhanced tolerance to Fusarium head blight. Genome **52:** 467–483.

Jellen, E. N., and J. M. Leggett, 2005 Cytogenetic manipulation in oat improvement, pp. 199–231 in *Genetic Resources, Chromosome Engineering, and Crop Improvement Series: Cereals*, Vol. 2, edited by R. J. Singh and P. P. Jauhar. CRC Press, Boca Raton, FL.

Jiang, J., B. Friebe and B. S. Gill, 1994 Recent advances in alien gene transfer in wheat. Euphytica **73:** 199–212.

Jin, Y., R. P. Singh, R. W. Ward, R. Wanyera, M. Kinyua *et al.*, 2007 Characterization of seedling infection types and adult plant infection responses of monogenic *Sr* gene lines to race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Dis. **91:** 1096–1099.

Jin, Y., L. J. Szabo, Z. A. Pretorius, R. P. Singh, R. Ward *et al.*, 2008 Detection of virulence to resistance gene *Sr24* within race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Dis. **92:** 923–926.

Jin, Y., L. J. Szabo, M. N. Rouse, T. Fetch, Z. A. Pretorius *et al.*, 2009 Detection of virulence to resistance gene *Sr36* within the TTKS race lineage of *Puccinia graminis* f. sp *tritici*. Plant Dis. **93:** 367–370.

Joppa, L. R., and N. D. Williams, 1988 Langdon durum disomic substitution lines and aneuploid analysis in tetraploid wheat. Genome **30:** 222–228.

Kerber, E. R., and P. L. Dyck, 1990 Transfer to hexaploid wheat of linked genes for adult-plant leaf rust and seedling stem rust resistance from an amphiploid of *Aegilops-speltoides* × *Triticum monococcum*. Genome **33:** 530–537.

Knox, R. E., H. L. Campbell, R. M. DePauw, J. M. Clarke and J. J. Gold, 2000 Registration of P8810–B5B3A2A2 white-seeded spring wheat germplasm with *Lr35* leaf and *Sr39* stem rust resistance. Crop Sci. **40:** 1512–1513.

Labuschagne, M. T., Z. A. Pretorius and B. Grobbelaar, 2002 The influence of leaf rust resistance genes *Lr29, Lr34, Lr35* and *Lr37* on breadmaking quality in wheat. Euphytica **124:** 65–70.

Li, G., and C. F. Quiros, 2001 Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. Theor. Appl. Genet. **103:** 455–461.

Lukaszewski, A. J., 2000 Manipulation of the 1RS.1BL translocation in wheat by induced homoeologous recombination. Crop Sci. **40:** 216–225.

Mago, R., P. Zhang, H. S. Bariana, D. C. Verlin, U. K. Bansal *et al.*, 2009 Development of wheat lines carrying stem rust resistance gene *Sr39* with reduced *Aegilops speltoides* chromatin and simple PCR markers for marker-assisted selection. Theor. Appl. Genet. **119:** 1441–1450.

Marais, G. F., L. Kotze and A. Eksteen, 2010 Allosyndetic recombinants of the *Aegilops peregrina*-derived *Lr59* translocation in common wheat. Plant Breed. **129:** 356–361.

Martinez-Perez, E., P. Shaw and G. Moore, 2001 The *Ph1* locus is needed to ensure specific somatic and meiotic centromere association. Nature **411:** 204–207.

Pretorius, Z. A., R. P. Singh, W. W. Wagoire and T. S. Payne, 2000 Detection of virulence to wheat stem rust resistance gene *Sr31* in *Puccinia graminis* f. sp. *tritici* in Uganda. Plant Dis. **84:** 203.

Qi, L. L., B. Friebe, P. Zhang and B. S. Gill, 2007 Homoeologous recombination, chromosome engineering and crop improvement. Chromosome Res. **15:** 3–19.

Riley, R., and V. Chapman, 1958 Genetic control of cytologically diploid behaviour of hexaploid wheat. Nature **182:** 713–715.

Roberts, M. A., S. M. Reader, C. Dalgliesh, T. E. Miller, T. N. Foote *et al.*, 1999 Induction and characterization of *Ph1* wheat mutants. Genetics **153:** 1909–1918.

Rozen, S., and H. J. Skaletsky, 2000 Primer3 on the WWW for general users and for biologist programmers, pp. 365–386 in *Methods in Molecular Biology*, Vol. 132: *Bioinformatics Methods and Protocols*, edited by S. Misener and S. A. Krawetz. Humana Press, Totowa, NJ.

Sears, E. R., 1954 The aneuploids of common wheat. University of Missouri Research Bulletin No. 572, 1–58.

Sears, E. R., 1966 Nullisomic-tetrasomic combinations in hexaploid wheat, pp. 29–45 in *Chromosome Manipulation and Plant Genetics*, edited by R. Riley and K. R. Lewis. Oliver & Boyd, Edinburgh.

Sears, E. R., 1977 An induced mutant with homoeologous pairing in common wheat. Can. J. Genet. Cytol. **19:** 585–593.

Seyfarth, R., C. Feuillet, G. Schachermayr, M. Winzeler and B. Keller, 1999 Development of a molecular marker for the adult plant leaf rust resistance gene *Lr35* in wheat. Theor. Appl. Genet. **99:** 554–560.

Sidhu, G. K., S. Rustgi, M. N. Shafqat, D. von Wettstein and K. S. Gill, 2008 Fine structure mapping of a gene-rich region of wheat carrying *Ph1*, a suppressor of crossing over between homoeologous chromosomes. Proc. Natl. Acad. Sci. USA **105:** 5815–5820.

Singh, R. J., 2005 Utilization of genetic resources for barley improvement, pp. 233–255 in *Genetic Resources, Chromosome Engineering, and Crop Improvement Series: Cereals*, Vol. 2, edited by R. J. Singh and P. P. Jauhar. CRC Press, Boca Raton, FL.

Singh, R. P., D. P. Hodson, Y. Jin, J. Huerta-Espino, M. G. Kinyua *et al.*, 2006 Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. CAB Reviews: Perspectives in Agriculture, Veterinary Science. Nutr. Nat. Res. **1**(54): 1–13.

Singh, R. P., D. P. Hodson, J. Huerta-Espino, Y. Jin, P. Njau *et al.*, 2008 Will stem rust destroy the world's wheat crop? Adv. Agron. **98:** 271–309.

Somers, D. J., P. Isaac and K. Edwards, 2004 A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor. Appl. Genet. **109:** 1105–1114.

Sourdille, P., S. Singh, T. Cadalen, G. L. Brown-Guedira, G. Gay *et al.*, 2004 Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). Funct. Integr. Genomics **4:** 12–25.

Stakman, E. C., 1962 Identification of physiologic races of *Puccinia graminis* var. *tritic*, p. 53, edited by E. C. Stakman, D. M. Stewart and W. Q. Loegering. U.S. Agricultural Research Service, Entomology Research Branch, E617, Washington, DC.

Tanksley, S. D., and S. R. McCouch, 1997 Seed banks and molecular maps: unlocking genetic potential from the wild. Science **277:** 1063–1066.

The, T. T., B. D. H. Latter, R. A. McIntosh, F. W. Ellison, P. S. Brennan *et al.*, 1988 Grain yields of near-isogenic lines with added genes for stem rust resistance, pp. 901–906 in *Proceedings of the 7th International Wheat Genetics Symposium*, Vol. 2, Cambridge, England, edited by T. E. Miller and R. M. D. Koebner. Cambridge, England.

Tsilo, T. J., S. Chao, Y. Jin and J. A. Anderson, 2009 Identification and validation of SSR markers linked to the stem rust resistance gene *Sr6* on the short arm of chromosome 2D in wheat. Theor. Appl. Genet. **118:** 515–524.

Wanyera, R., M. G. Kinyua, Y. Jin and R. P. Singh, 2006 The spread of stem rust caused by *Puccinia graminis* f. sp. *tritici*, with virulence on *Sr31* in wheat in Eastern Africa. Plant Dis. **90:** 113.

Williams, N. D., J. D. Miller and D. L. Klindworth, 1992 Induced mutations of a genetic suppressor of resistance to wheat stem rust. Crop Sci. **32:** 612–616.

Wu, J. Z., H. Mizuno, M. Hayashi-Tsugane, Y. Ito, Y. Chiden *et al.*, 2003 Physical maps and recombination frequency of six rice chromosomes. Plant J. **36:** 720–730.

Xu, S. S., I. S. Dundas, M.O. Pumphrey, Y. Jin, J. D. Faris *et al.*, 2008 Chromosome engineering to enhance utility of alien-derived stem rust resistance, pp. 12–14 in *Proceedings of the 11th International Wheat Genetics Symposium*, Vol. 1, Brisbane, Queensland, Australia, edited by R. Appels, R. Eastwood, E. Lagudah, P. Langridge, M. Mackay *et al.* Sydney University Press, Sydney, Australia.

Xu, S. S., Y. Jin, D. L. Klindworth, R. R.-C. Wang and X. Cai, 2009 Evaluation and characterization of seedling resistances to stem rust Ug99 races in wheat-alien species derivatives. Crop Sci. **49:** 2167–2175.

Yu, G., Q. Zhang, D. L. Klindworth, T. L. Friesen, R. Knox *et al.*, 2010 Molecular and cytogenetic characterization of wheat introgression lines carrying the stem rust resistance gene *Sr39*. Crop Sci. **50:** 1393–1400.

Zhong, S., Y. Leng, T. L. Friesen, J. D. Faris and L. J. Szabo, 2009 Development and characterization of expressed sequence tag-derived microsatellite markers for the wheat stem rust fungus *Puccinia graminis* f. sp. *tritici*. Phytopathology **99:** 282–289.

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GENETICS

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Targeted Introgression of a Wheat Stem Rust Resistance Gene by DNA Marker-Assisted Chromosome Engineering

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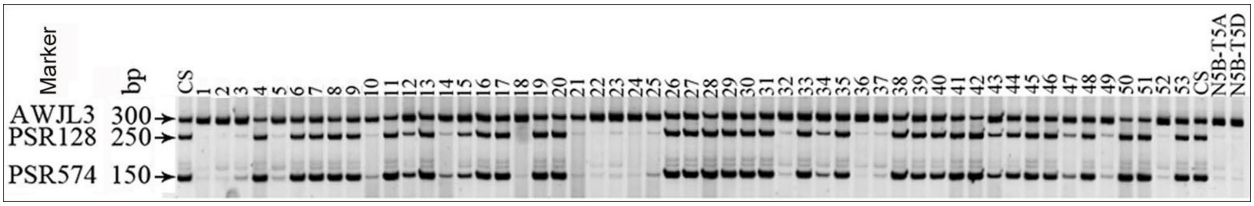

FIGURE S1.—Detection of *ph1bph1b* homozygotes among stem rust-resistant BC_1F_1 plants having the pedigree Chinese Spring (CS) *ph1bph1b**2/RL6082. Plants 1-53 were resistant to stem rust. CS, CS N5B-T5A (nullisomic for chromosome 5B and tetrasomic for 5A) and CS N5B-T5D (nullisomic for chromosome 5B and tetrasomic for 5D) were used as checks. PSR128 and PSR574 were the markers for detecting *ph1bph1b* plants and AWJL3 was the positive check marker. PSR128 and PSR574 are located in the deleted region of chromosome 5B that carries the *Ph1* gene (ROBERTS *et al.* 1999). The *ph1bph1b* plants (1, 2, 3, 5, 10, 18, 21, 22, 23, 24, 25, 32, 36, 37, 49, and 52) were detected by the absence of the bands produced by PSR128 and PSR574.

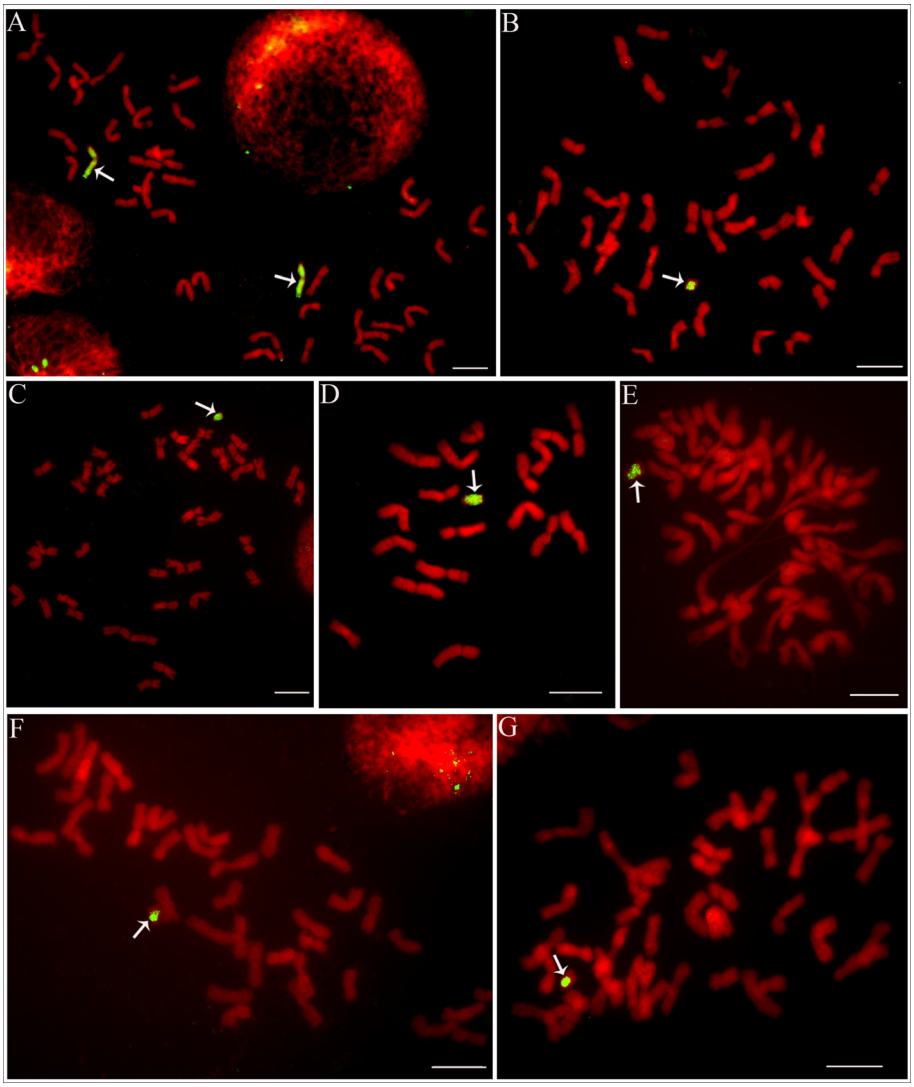

FIGURE S2.—Fluorescent genomic *in situ* hybridization images of BC_2F_2 plants from seven BC_2F_1 plants [Pedigree: Chinese Spring (CS)//CS *ph1bph1b**2/RL6082] exhibiting dissociation of *Sr39* from *Xgwm319*. A) One BC_2F_2 plant from 09N87 carried a translocated chromosome, but with only a slightly reduced *Aegilops speltoides* chromatin in the short arm. B-G) The BC_2F_2 plants from six BC_2F_1 plants (B, 09N236; C, 09N374; D, 09N272; E, 09N232; F, 09N280; G, 09N300) carried a telocentric chromosome, with the entire long arm being absent. Five of the BC_2F_1 plants (09N236, 09N272, 09N232, 09N280, and 09N300) originated from Family 81-5 and one plant (09N374) from Family 81-6. The *Ae. speltoides* chromatin (green) is indicated by arrows. Bar = 10 μm.

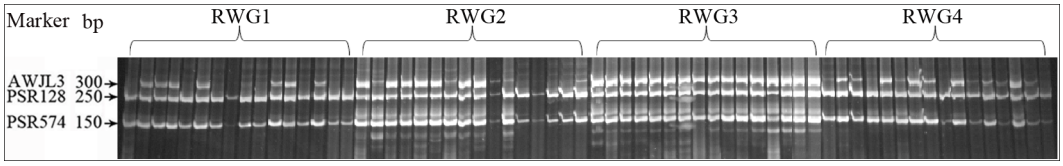

FIGURE S3.—Gel image of the four wheat lines (RWG1, RWG2, RWG3, and RWG4) analyzed with markers *PSR128*, *PSR574*, and *AWJL3*. The DNA samples were extracted from 16 single BC_2F_3 seeds derived from the original BC_2F_2 plants for each of the four lines. PSR128 and PSR574 were the markers for detecting *ph1bph1b* plants and AWJL3 was the positive check marker. The *ph1bph1b* plants were not detected in the four lines.

TABLE S1

Infection types (IT)) to stem rust race TMLK and *Ph1* gene genotypes of 93 BC_1F_1 plants from backcross of RL6082 (*Sr39*) with Chinese Spring (CS) *ph1b* mutant.

Plant No	Source [a]	Pedigree	IT	*Ph1* genotype
81-01	08A50-6/08A52-1 #1	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-02	08A50-6/08A52-1 #2	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-03	08A50-6/08A52-1 #3	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*ph1bph1b*
81-04	08A50-6/08A52-1 #4	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-05	08A50-6/08A52-1 #5	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*ph1bph1b*
81-06	08A50-6/08A52-1 #6	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*ph1bph1b*
81-07	08A50-6/08A52-1 #7	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-08	08A50-6/08A52-1 #8	CS *ph1bph1b**2/RL6082 (*Sr39*)	12	*Ph1bph1b*
81-09	08A50-6/08A52-1 #9	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-10	08A50-6/08A52-1 #10	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-11	08A50-6/08A52-1 #11	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*ph1bph1b*
81-12	08A50-6/08A52-1 #12	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-13	08A50-6/08A52-1 #13	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-14	08A50-6/08A52-1 #14	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-15	08A50-6/08A52-1 #15	CS *ph1bph1b**2/RL6082 (*Sr39*)	12	*Ph1bph1b*
81-16	08A50-6/08A52-1 #16	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-17	08A50-6/08A52-1 #17	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-18	08A50-6/08A52-1 #18	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-19	08A50-6/08A52-1 #19	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-20	08A50-6/08A52-1 #20	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*ph1bph1b*
81-21	08A50-6/08A52-1 #21	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-22	08A50-6/08A52-1 #22	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-23	08A50-6/08A52-1 #23	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-24	08A50-6/08A52-1 #24	CS *ph1bph1b**2/RL6082 (*Sr39*)	34	
81-25	08A50-6/08A52-1 #25	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-26	08A50-6/08A52-1 #26	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-27	08A50-6/08A52-1 #27	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-28	08A50-6/08A52-1 #28	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*?*
81-29	08A50-6/08A52-1 #29	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-30	08A50-6/08A52-1 #30	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-31	08A50-6/08A52-1 #31	CS *ph1bph1b**2/RL6082 (*Sr39*)	23*	*Ph1bph1b*
81-32	08A50-6/08A52-1 #32	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-33	08A50-6/08A52-1 #33	CS *ph1bph1b**2/RL6082 (*Sr39*)	23*	*Ph1bph1b*
81-34	08A50-6/08A52-1 #34	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-35	08A50-6/08A52-1 #35	CS *ph1bph1b**2/RL6082 (*Sr39*)	23*	*ph1bph1b*
81-36	08A50-13/08A52-3 #1	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-37	08A50-13/08A52-3 #2	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-38	08A50-13/08A52-3 #3	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*ph1bph1b*
81-39	08A50-13/08A52-3 #4	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*ph1bph1b*

81-40	08A50-13/08A52-3 #5	CS *ph1bph1b**2/RL6082 (*Sr39*)	23	*ph1bph1b*
81-41	08A50-13/08A52-3 #6	CS *ph1bph1b**2/RL6082 (*Sr39*)	23	*ph1bph1b*
81-42	08A50-13/08A52-3 #7	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*ph1bph1b*
81-43	08A50-13/08A52-3 #8	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-44	08A50-13/08A52-3 #9	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-45	08A50-13/08A52-3 #10	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-46	08A50-13/08A52-3 #11	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-47	08A50-13/08A52-3 #12	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-48	08A50-13/08A52-3 #13	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-49	08A50-13/08A52-3 #14	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-50	08A50-13/08A52-3 #15	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-51	08A50-13/08A52-3 #16	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-52	08A50-13/08A52-3 #17	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-53	08A50-13/08A52-3 #18	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-54	08A50-13/08A52-3 #19	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-55	08A50-13/08A52-3 #20	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-56	08A50-13/08A52-3 #21	CS *ph1bph1b**2/RL6082 (*Sr39*)	1	*ph1bph1b*
81-57	08A50-13/08A52-3 #22	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-58	08A50-13/08A52-3 #23	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-59	08A50-10/08A52-4 #1	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-60	08A50-10/08A52-4 #2	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-61	08A50-10/08A52-4 #3	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-62	08A50-10/08A52-4 #4	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-63	08A50-10/08A52-4 #5	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*ph1bph1b*
81-64	08A50-10/08A52-4 #6	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-65	08A50-10/08A52-4 #7	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-66	08A50-10/08A52-4 #8	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*ph1bph1b*
81-67	08A50-10/08A52-4 #9	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-68	08A50-10/08A52-4 #10	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-69	08A50-10/08A52-4 #11	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-70	08A50-10/08A52-4 #12	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-71	08A50-10/08A52-4 #13	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-72	08A50-10/08A52-4 #14	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-73	08A50-10/08A52-4 #15	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-74	08A50-10/08A52-4 #16	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-75	08A50-10/08A52-4 #17	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-76	08A50-10/08A52-4 #18	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-77	08A50-10/08A52-4 #19	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-78	08A50-10/08A52-4 #20	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-79	08A50-10/08A52-4 #21	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-80	08A50-10/08A52-4 #22	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-81	08A50-7/08A52-4 #1	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-82	08A50-7/08A52-4 #2	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-83	08A50-7/08A52-4 #3	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*

81-84	08A50-7/08A52-4 #4	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-85	08A50-7/08A52-4 #5	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-86	08A50-7/08A52-4 #6	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-87	08A50-7/08A52-4 #7	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
81-88	08A50-7/08A52-4 #8	CS *ph1bph1b**2/RL6082 (*Sr39*)	23	*ph1bph1b*
81-89	08A50-7/08A52-4 #9	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-90	08A50-7/08A52-4 #10	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-91	08A50-7/08A52-4 #11	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*ph1bph1b*
81-92	08A50-7/08A52-4 #12	CS *ph1bph1b**2/RL6082 (*Sr39*)	2	*Ph1bph1b*
81-93	08A50-7/08A52-4 #13	CS *ph1bph1b**2/RL6082 (*Sr39*)	4	
Checks	07C 65-1	RL6082	1	*Ph1bPh1b*
Checks	07C 65-1	RL6082	12	*Ph1bPh1b*
Checks		CS	4	*Ph1bPh1b*
Checks		CS	34	*Ph1bPh1b*
Checks		CS	4	*Ph1bPh1b*

[a] Source: 08A50 and 08A52 are CS *ph1bph1b* and F_1 hybrid (CS *ph1bph1b*/RL6082) plants, respectively.

TABLE S2

Infection types (IT) of 1,048 BC_2F_1 plants having the pedigree Chinese Spring (CS)//CS *ph1bph1b2/RL6082 and three parental lines to stem rust race TMLK.**

Plant No.	Source [a]	IT	Plant No.	Source	IT	Plant No.	Source	IT
09N1	08C3-84/08C81-3	1	09N744	08C3-34/08C81-20	43	09N1174	08C3-20/08C81-40	21
09N2	08C3-84/08C81-3	1	09N745	08C3-34/08C81-20	12	09N1175	08C3-20/08C81-40	1
09N3	08C3-84/08C81-3	1	09N746	08C3-34/08C81-20	12	09N1176	08C3-20/08C81-40	34
09N4	08C3-84/08C81-3	1	09N747	08C3-34/08C81-20	43	09N1177	08C3-20/08C81-40	12
09N5	08C3-84/08C81-3	1	09N748	08C3-34/08C81-20	34	09N1178	08C3-20/08C81-40	43
09N6	08C3-84/08C81-3	32	09N749	08C3-34/08C81-20	1	09N1179	08C3-20/08C81-40	21
09N7	08C3-84/08C81-3	4	09N750	08C3-34/08C81-20	43	09N1180	08C3-20/08C81-40	34
09N8	08C3-84/08C81-3	34	09N751	08C3-34/08C81-20	43	09N1181	08C3-20/08C81-40	21
09N9	08C3-84/08C81-3	4	09N752	08C3-34/08C81-20	43	09N1182	08C3-20/08C81-40	1
09N10	08C3-84/08C81-3	34	09N753	08C3-34/08C81-20	12	09N1183	08C3-20/08C81-40	21
09N11	08C3-84/08C81-3	1	09N754	08C3-34/08C81-20	43	09N1184	08C3-20/08C81-40	21
09N12	08C3-84/08C81-3	1	09N755	08C3-44/08C81-20	43	09N1185	08C3-20/08C81-40	21
09N13	08C3-84/08C81-3	12	09N756	08C3-44/08C81-20	34	09N1186	08C3-20/08C81-40	21
09N14	08C3-84/08C81-3	1	09N757	08C3-44/08C81-20	1	09N1187	08C3-20/08C81-40	21
09N15	08C3-84/08C81-3	1	09N758	08C3-44/08C81-20	43	09N1188	08C3-20/08C81-40	21
09N16	08C3-84/08C81-3	1	09N759	08C3-44/08C81-20	43	09N1189	08C3-20/08C81-40	2
09N17	08C3-84/08C81-3	4	09N760	08C3-44/08C81-20	34	09N1190	08C3-24/08C81-40	21
09N18	08C3-84/08C81-3	1	09N761	08C3-44/08C81-20	43	09N1191	08C3-24/08C81-40	43
09N19	08C3-84/08C81-3	4	09N763	08C3-44/08C81-20	43	09N1192	08C3-24/08C81-40	12
09N20	08C3-84/08C81-3	1	09N764	08C3-44/08C81-20	43	09N1193	08C3-24/08C81-40	21
09N21	08C3-84/08C81-3	1	09N765	08C3-44/08C81-20	43	09N1194	08C3-24/08C81-40	21
09N22	08C3-84/08C81-3	43	09N766	08C3-44/08C81-20	43	09N1195	08C3-24/08C81-40	21
09N23	08C3-84/08C81-3	1	09N767	08C3-44/08C81-20	1	09N1196	08C3-24/08C81-40	1
09N24	08C3-84/08C81-3	1	09N768	08C3-44/08C81-20	43	09N1197	08C3-24/08C81-40	34
09N25	08C3-84/08C81-3	34	09N769	08C3-44/08C81-20	34	09N1198	08C3-24/08C81-40	34
09N26	08C3-84/08C81-3	1	09N770	08C3-44/08C81-20	34	09N1199	08C3-24/08C81-40	21
09N27	08C3-84/08C81-3	1	09N771	08C3-48/08C81-20	4	09N1200	08C3-24/08C81-40	21
09N28	08C3-84/08C81-3	1	09N772	08C3-48/08C81-20	43	09N1201	08C3-24/08C81-40	43
09N29	08C3-84/08C81-3	12	09N773	08C3-48/08C81-20	43	09N1202	08C3-24/08C81-40	21
09N30	08C3-84/08C81-3	34	09N774	08C3-48/08C81-20	43	09N1203	08C3-24/08C81-40	34
09N31	08C3-84/08C81-3	1	09N775	08C3-48/08C81-20	43	09N1204	08C3-24/08C81-40	12
09N32	08C3-84/08C81-3	4	09N776	08C3-48/08C81-20	43	09N1205	08C3-24/08C81-40	12
09N33	08C3-84/08C81-3	34	09N777	08C3-48/08C81-20	34	09N1206	08C3-24/08C81-40	21
09N34	08C3-84/08C81-3	1	09N778	08C3-48/08C81-20	4	09N1207	08C3-24/08C81-40	43
09N35	08C3-84/08C81-3	1	09N779	08C3-48/08C81-20	34	09N1208	08C3-24/08C81-40	34
09N36	08C3-84/08C81-3	4	09N780	08C3-48/08C81-20	43	09N1209	08C3-24/08C81-40	21
09N37	08C3-84/08C81-3	4	09N781	08C3-48/08C81-20	1	09N1210	08C3-24/08C81-40	34
09N38	08C3-84/08C81-3	1	09N782	08C3-48/08C81-20	43	09N1211	08C3-31/08C81-40	1

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09N42	08C3-84/08C81-3	4	09N785	08C3-48/08C81-20	1	09N1214	08C3-31/08C81-40	34
09N43	08C3-95/08C81-3	34	09N786	08C3-48/08C81-20	43	09N1215	08C3-31/08C81-40	1
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09N47	08C3-95/08C81-3	1	09N790	08C3-48/08C81-20	43	09N1219	08C3-31/08C81-40	21
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09N53	08C3-95/08C81-3	12	09N796	08C3-48/08C81-20	43	09N1225	08C3-31/08C81-40	21
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09N67	08C3-95/08C81-3	34	09N810	08C3-120/08C81-20	34	09N1239	08C3-33/08C81-40	21
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09N72	08C3-95/08C81-3	43	09N814	08C3-120/08C81-20	43	09N1243	08C3-33/08C81-40	12
09N73	08C3-95/08C81-3	34	09N815	08C3-120/08C81-20	1	09N1244	08C3-33/08C81-40	21
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09N84	08C3-95/08C81-3	43	09N825	08C3-120/08C81-20	34	09N1254	08C3-33/08C81-40	21
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09N225	08C3-44/08C81-5	34	09N830	08C3-16/08C81-35	1	09N1259	08C3-33/08C81-40	43
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09N252	08C3-71/08C81-5	4	09N854	08C3-91/08C81-35	43	09N1434	08C3-16/08C81-42	32
09N253	08C3-71/08C81-5	43	09N855	08C3-91/08C81-35	43	09N1435	08C3-16/08C81-42	12
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09N255	08C3-71/08C81-5	1	09N857	08C3-91/08C81-35	1	09N1437	08C3-16/08C81-42	12
09N256	08C3-71/08C81-5	23	09N858	08C3-91/08C81-35	12	09N1438	08C3-16/08C81-42	21
09N258	08C3-71/08C81-5	4	09N859	08C3-91/08C81-35	12	09N1439	08C3-16/08C81-42	34
09N260	08C3-71/08C81-5	34	09N860	08C3-91/08C81-35	12	09N1440	08C3-16/08C81-42	21
09N261	08C3-71/08C81-5	34	09N861	08C3-91/08C81-35	12	09N1441	08C3-16/08C81-42	12
09N262	08C3-71/08C81-5	4	09N862	08C3-91/08C81-35	21	09N1442	08C3-16/08C81-42	34
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09N265	08C3-71/08C81-5	1	09N865	08C3-91/08C81-35	12	09N1444	08C3-16/08C81-42	21
09N266	08C3-71/08C81-5	34	09N866	08C3-91/08C81-35	43	09N1445	08C3-16/08C81-42	32
09N267	08C3-71/08C81-5	12	09N867	08C3-91/08C81-35	12	09N1446	08C3-16/08C81-42	1
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09N270	08C3-71/08C81-5	1	09N870	08C3-91/08C81-35	1	09N1449	08C3-16/08C81-42	21
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09N272	08C3-71/08C81-5	1	09N872	08C3-91/08C81-35	1	09N1451	08C3-20/08C81-42	34
09N273	08C3-71/08C81-5	4	09N873	08C3-91/08C81-35	1	09N1452	08C3-20/08C81-42	43
09N274	08C3-71/08C81-5	43	09N874	08C3-91/08C81-35	12	09N1453	08C3-20/08C81-42	34
09N275	08C3-71/08C81-5	4	09N875	08C3-91/08C81-35	1	09N1454	08C3-20/08C81-42	43
09N277	08C3-140/08C81-5	4	09N876	08C3-96/08C81-35	2	09N1455	08C3-20/08C81-42	12
09N278	08C3-140/08C81-5	4	09N877	08C3-96/08C81-35	1	09N1456	08C3-20/08C81-42	21
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09N285	08C3-140/08C81-5	4	09N883	08C3-96/08C81-35	12	09N1462	08C3-20/08C81-42	32
09N286	08C3-140/08C81-5	34	09N884	08C3-96/08C81-35	43	09N1463	08C3-20/08C81-42	21
09N287	08C3-140/08C81-5	4	09N885	08C3-96/08C81-35	43	09N1464	08C3-20/08C81-42	2
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09N289	08C3-140/08C81-5	1	09N887	08C3-96/08C81-35	43	09N1466	08C3-132/08C81-42	21
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09N292	08C3-140/08C81-5	1	09N890	08C3-96/08C81-35	12	09N1469	08C3-132/08C81-42	21
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09N358	08C3-16/08C81-6	2	09N976	08C3-48/08C81-38	1	09N1510	08C3-78/08C81-56	12
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09N361	08C3-16/08C81-6	12	09N978	08C3-48/08C81-38	12-	09N1512	08C3-78/08C81-56	43
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09N369	08C3-33/08C81-6	43	09N986	08C3-48/08C81-38	1	09N1520	08C3-88/08C81-56	21
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09N561	08C3-19/08C81-11	34	09N1130	08C3-91/08C81-39	34	09N1752	08C3-99/08C81-63	32
09N562	08C3-19/08C81-11	34	09N1131	08C3-91/08C81-39	43	09N1753	08C3-99/08C81-63	34
09N563	08C3-19/08C81-11	43	09N1132	08C3-91/08C81-39	43	09N1754	08C3-99/08C81-63	21
09N564	08C3-19/08C81-11	1	09N1133	08C3-91/08C81-39	4	09N1755	08C3-99/08C81-63	32
09N565	08C3-19/08C81-11	21	09N1134	08C3-91/08C81-39	21	09N1756	08C3-99/08C81-63	34
09N566	08C3-19/08C81-11	43	09N1135	08C3-91/08C81-39	34	09N1758	08C3-99/08C81-63	32
09N567	08C3-19/08C81-11	12	09N1136	08C3-91/08C81-39	43	09N1759	08C3-99/08C81-63	34
09N568	08C3-19/08C81-11	1	09N1137	08C3-91/08C81-39	34	09N1760	08C3-99/08C81-63	21
09N569	08C3-19/08C81-11	12	09N1138	08C3-91/08C81-39	43	09N1761	08C3-99/08C81-63	34
09N570	08C3-19/08C81-11	12	09N1139	08C3-91/08C81-39	4	09N1762	08C3-100/08C81-63	34
09N571	08C3-19/08C81-11	2	09N1140	08C3-91/08C81-39	43	09N1763	08C3-100/08C81-63	32
09N572	08C3-19/08C81-11	12	09N1141	08C3-91/08C81-39	21	09N1764	08C3-100/08C81-63	34
09N573	08C3-19/08C81-11	34	09N1142	08C3-91/08C81-39	43	09N1765	08C3-100/08C81-63	12
09N574	08C3-19/08C81-11	34	09N1143	08C3-91/08C81-39	43	09N1766	08C3-100/08C81-63	21
09N575	08C3-19/08C81-11	34	09N1144	08C3-91/08C81-39	43	09N1767	08C3-100/08C81-63	34
09N576	08C3-19/08C81-11	43	09N1145	08C3-132/08C81-39	4	09N1768	08C3-100/08C81-63	34
09N577	08C3-19/08C81-11	12	09N1146	08C3-132/08C81-39	43	09N1769	08C3-100/08C81-63	12
09N578	08C3-43/08C81-11	1	09N1147	08C3-132/08C81-39	21	09N1770	08C3-100/08C81-63	12
09N579	08C3-43/08C81-11	1	09N1148	08C3-132/08C81-39	43	09N1771	08C3-100/08C81-63	34
09N580	08C3-43/08C81-11	12	09N1149	08C3-132/08C81-39	1	09N1772	08C3-100/08C81-63	43
09N581	08C3-43/08C81-11	12	09N1150	08C3-132/08C81-39	1	09N1773	08C3-100/08C81-63	43
09N582	08C3-43/08C81-11	21	09N1151	08C3-132/08C81-39	32	09N1774	08C3-100/08C81-63	34
09N583	08C3-43/08C81-11	34	09N1152	08C3-132/08C81-39	4	09N1775	08C3-100/08C81-63	34
09N584	08C3-43/08C81-11	12	09N1153	08C3-132/08C81-39	43	09N1776	08C3-100/08C81-63	43
09N585	08C3-43/08C81-11	12	09N1154	08C3-132/08C81-39	43	09N1777	08C3-100/08C81-63	43
09N586	08C3-43/08C81-11	1	09N1155	08C3-132/08C81-39	12	09N1778	08C3-100/08C81-63	1-
09N587	08C3-43/08C81-11	12	09N1156	08C3-132/08C81-39	34	09N1779	08C3-100/08C81-63	34

09N588	08C3-43/08C81-11	34	09N1157	08C3-132/08C81-39	43	RL6082-1	1
09N589	08C3-43/08C81-11	1	09N1170	08C3-20/08C81-40	32	RL6082-2	1
09N590	08C3-43/08C81-11	0;1-	09N1171	08C3-20/08C81-40	43	CS-1	43
09N591	08C3-43/08C81-11	1	09N1172	08C3-20/08C81-40	12	CS-2	43
09N743	08C3-34/08C81-20	34	09N1173	08C3-20/08C81-40	12		

[a] Source: 08C3 and 08C81 are CS and BC_1F_1 (CS *ph1bph1b**2/RL6082) plants, respectively.

TABLE S3

Marker data of BC_2F_1 plants (pedigree: Chinese Spring//Chinese Spring *ph1bph1b2/RL6082) analyzed with microsatellite marker *Xgwm319*.**

Plant No. [a]	IT [b]	Marker allele (bp)		Plant No.	IT	Marker allele (bp)		Plant No.	IT	Marker allele (bp)	
		RL6082	CS [c]			RL6082	CS			RL6082	CS
09N1	1	182	193	09N747	43	182	193	09N1185	21	182	193
09N2	1	182	193	09N748	34	182	193	09N1186	21	182	193
09N3	1	182	193	09N749	1	182	193	09N1187	21	182	193
09N4	1	182	193	09N750	43	182	193	09N1188	21	182	193
09N5	1	182	193	09N751	43	182	193	09N1189	2	182	193
09N6	32		193	09N752	43		193	09N1190	21	182	193
09N7	4		193	09N753	12	182	193	09N1191	43	182	193
09N8	34		193	09N754	43	182	193	09N1192	12	182	193
09N9	4		193	09N755	43	182	193	09N1193	21	182	193
09N10	34		193	09N756	34		193	09N1194	21	182	193
09N11	1	182	193	09N757	1	182	193	09N1195	21	182	193
09N12	**1**		**193**	09N758	43		193	09N1196	1	182	193
09N13	12	182	193	09N759	43	182	193	09N1197	34	182	193
09N14	1	182	193	09N760	34	182	193	09N1198	34	182	193
09N15	1	182	193	09N761	43		193	09N1199	21	182	193
09N16	1	182	193	09N763	43		193	09N1200	21	182	193
09N17	4		193	09N764	43	182	193	09N1201	43	182	193
09N18	1	182	193	09N765	43		193	09N1202	21	182	193
09N19	4		193	09N766	43		193	09N1203	34	182	193
09N20	1	182	193	09N767	1		193	09N1204	12	182	193
09N21	1	182	193	09N768	43	182	193	09N1205	12	182	193
09N22	43		193	09N770	34		193	09N1206	21	182	193
09N23	1	182	193	09N771	4		193	09N1207	43	182	193
09N24	1	182	193	09N772	43		193	09N1208	34	182	193
09N25	34		193	09N773	43	182	193	09N1209	21	182	193
09N26	1	182	193	09N774	43		193	09N1210	34	182	193
09N28	1	182	193	09N775	43	182	193	09N1211	1	182	193
09N29	12	182	193	09N776	43	182	193	09N1212	21	182	193
09N30	34		193	09N778	4	182	193	09N1213	34	182	193
09N31	1	182	193	09N779	34	182	193	09N1214	34	182	193
09N32	4		193	09N780	43	182	193	09N1215	1	182	193
09N33	34		193	09N781	1	182	193	09N1216	21	182	193
09N34	1	182	193	09N782	43	182	193	09N1217	21	182	193
09N35	1	182	193	09N783	43	182	193	09N1218	12	182	193
09N36	4		193	09N784	1	182	193	09N1219	21	182	193
09N37	4		193	09N786	43	182	193	09N1220	2	182	193
09N38	1	182	193	09N787	43		193	09N1221	12	182	193

09N39	34		193	09N788	12	182	193	09N1222	12	182	193
09N40	4		193	09N789	34	182	193	09N1223	34	182	193
09N42	4		193	09N790	43	182	193	09N1224	12	182	193
09N43	34		193	09N791	43	182	193	09N1225	21	182	193
09N44	34		193	09N794	43		193	09N1226	12	182	193
09N45	1	182	193	09N795	4	182	193	09N1227	12	182	193
09N46	34		193	09N796	43	182	193	09N1228	21	182	193
09N47	1	182	193	09N797	12		193	09N1229	12	182	193
09N48	1	182	193	09N798	1		193	09N1230	12	182	193
09N49	1	182	193	09N799	1		193	09N1231	21	182	193
09N50	34		193	09N800	12	182	193	09N1232	43	182	193
09N51	12	182	193	09N801	43		193	09N1233	23	182	193
09N52	12	182	193	09N802	4	182	193	09N1234	21	182	193
09N53	12	182	193	09N804	43	182	193	09N1235	21	182	193
09N54	43		193	09N805	43	182	193	09N1236	21	182	193
09N55	4		193	09N806	0;1-	182	193	09N1237	1	182	193
09N56	43		193	09N807	43	182	193	09N1238	43	182	193
09N57	1	182	193	09N808	43	182	193	09N1239	21	182	193
09N58	1	182	193	09N809	34	182	193	09N1240	34	182	193
09N59	1	182	193	09N811	43	182	193	09N1241	21	182	193
09N60	34		193	09N812	1	182	193	09N1242	43	182	193
09N61	1	182	193	09N813	43		193	09N1243	12	182	193
09N62	4		193	09N814	43		193	09N1244	21	182	193
09N63	1	182	193	09N815	1	182	193	09N1245	12	182	193
09N64	12	182	193	09N816	43	182	193	09N1246	34	182	193
09N65	1	182	193	09N817	12	182	193	09N1247	43	182	193
09N66	1	182	193	09N819	4	182	193	09N1248	21	182	193
09N67	34		193	09N820	43	182	193	09N1249	21	182	193
09N68	1	182	193	09N821	43	182	193	09N1250	1	182	193
09N70	34		193	09N822	1	182	193	09N1251	21	182	193
09N71	12	182	193	09N823	43	182	193	09N1252	21	182	193
09N72	43		193	09N824	43	182	193	09N1253	21	182	193
09N73	34		193	09N825	34	182	193	09N1254	21	182	193
09N74	1	182	193	09N317	43	182	193	09N1255	21	182	193
09N75	4		193	09N318	4		193	09N1256	21	182	193
09N77	12	182	193	09N319	43	182	193	09N1257	43	182	193
09N78	12	182	193	09N320	34	182	193	09N1258	;1	182	193
09N79	4		193	09N321	12	182	193	09N1259	43	182	193
09N80	1	182	193	09N826	43	182	193	09N1411	1	182	193
09N81	43		193	09N827	12	182	193	09N1412	34	182	193
09N82	12	182	193	09N828	43	182	193	09N1413	43	182	193
09N84	43		193	09N829	12	182	193	09N1414	34	182	193
09N85	34		193	09N830	1	182	193	09N1415	1	182	193
09N86	12	182	193	09N831	43	182	193	09N1416	34	182	193

09N87	21		193	09N832	1	182	193	09N1417	34	182	193
09N224	34	182	193	09N833	43	182	193	09N1418	32	182	193
09N225	34		193	09N834	1	182	193	09N1419	12	182	193
09N226	34		193	09N835	43		193	09N1420	12	182	193
09N227	34		193	09N837	12	182	193	09N1421	12	182	193
09N228	34		193	09N838	43	182	193	09N1422	12	182	193
09N229	12		193	09N839	1	182	193	09N1423	12	182	193
09N230	12		193	09N840	12	182	193	09N1424	34	182	193
09N231	1		193	09N841	1	182	193	09N1425	21	182	193
09N232	1		193	09N842	43	182	193	09N1426	43	182	193
09N233	43		193	09N843	43	182	193	09N1427	21	182	193
09N235	12		193	09N844	1	182	193	09N1428	12	182	193
09N236	1		193	09N845	43	182	193	09N1429	34	182	193
09N237	12		193	09N846	43		193	09N1430	12	182	193
09N238	34		193	09N847	1	182	193	09N1431	12	182	193
09N239	4		193	09N848	1	182	193	09N1432	12	182	193
09N240	34		193	09N849	43	182	193	09N1433	32	182	193
09N241	1		193	09N850	2	182	193	09N1434	32	182	193
09N242	4		193	09N851	21	182	193	09N1435	12	182	193
09N243	43		193	09N853	12	182	193	09N1436	21	182	193
09N244	43		193	09N854	43		193	09N1437	12	182	193
09N245	43		193	09N855	43		193	09N1438	21	182	193
09N246	34		193	09N856	43	182	193	09N1439	34	182	193
09N250	4		193	09N857	1	182	193	09N1440	21	182	193
09N252	4		193	09N858	12	182	193	09N1441	12	182	193
09N253	43		193	09N859	12	182	193	09N1442	34	182	193
09N254	4		193	09N861	12	182	193	09N1443	34	182	193
09N255	1		193	09N862	21	182	193	09N1444	21	182	193
09N256	23		193	09N864	12	182	193	09N1445	32	182	193
09N258	4		193	09N865	12	182	193	09N1446	1	182	193
09N260	34		193	09N866	43	182	193	09N1447	32	182	193
09N261	34		193	09N867	12	182	193	09N1448	12	182	193
09N262	4		193	09N869	12	182	193	09N1449	21	182	193
09N263	4		193	09N870	1	182	193	09N1450	43	182	193
09N265	1		193	09N871	43	182	193	09N1451	34	182	193
09N266	34		193	09N872	1	182	193	09N1452	43	182	193
09N267	12		193	09N873	1	182	193	09N1453	34	182	193
09N268	4		193	09N874	12	182	193	09N1454	43	182	193
09N270	1		193	09N875	1	182	193	09N1455	12	182	193
09N271	4		193	09N877	1	182	193	09N1456	21	182	193
09N272	1		193	09N878	21	182	193	09N1457	43	182	193
09N273	4		193	09N879	21	182	193	09N1458	21	182	193
09N274	43		193	09N880	12	182	193	09N1459	21	182	193
09N275	4		193	09N881	21	182	193	09N1460	12	182	193

09N277	4		193	09N882	1	182	193	09N1461	43	182	193
09N278	4		193	09N883	12	182	193	09N1462	32	182	193
09N279	4		193	09N884	43		193	09N1463	21	182	193
09N280	1		193	09N885	43	182	193	09N1464	2	182	193
09N281	4		193	09N886	43	182	193	09N1465	12	182	193
09N282	4		193	09N887	43	182	193	09N1466	21	182	193
09N284	34		193	09N888	21	182	193	09N1467	21	182	193
09N285	4		193	09N889	12	182	193	09N1468	43	182	193
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09N288	34		193	09N891	21	182	193	09N1470	12	182	193
09N289	1		193	09N893	1	182	193	09N1471	23-	182	193
09N290	4		193	09N894	34	182	193	09N1472	21	182	193
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09N292	1		193	09N896	3		193	09N1474	34	182	193
09N293	43		193	09N897	43	182	193	09N1475	2	182	193
09N294	43		193	09N898	12	182	193	09N1476	21	182	193
09N295	1		193	09N899	1	182	193	09N1477	2	182	193
09N296	4		193	09N900	43	182	193	09N1478	21	182	193
09N297	43		193	09N901	21	182	193	09N1479	32	182	193
09N298	21		193	09N902	21	182	193	09N1480	21	182	193
09N299	34		193	09N903	21	182	193	09N1481	21	182	193
09N300	1		193	09N904	43	182	193	09N1482	21	182	193
09N301	1		193	09N905	12	182	193	09N1483	23-	182	193
09N303	4		193	09N906	43	182	193	09N1484	21	182	193
09N304	43		193	09N907	1	182	193	09N1485	21	182	193
09N305	4		193	09N909	1	182	193	09N1486	32	182	193
09N306	12		193	09N910	12	182	193	09N1487	23-	182	193
09N307	43		193	09N911	1	182	193	09N1488	2	182	193
09N308	43		193	09N912	43	182	193	09N1489	23-	182	193
09N309	43		193	09N913	21	182	193	09N1490	43	182	193
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09N311	43		193	09N915	21	182	193	09N1492	2	182	193
09N312	43		193	09N961	12-	182	193	09N1493	2	182	193
09N313	21		193	09N962	4	182	193	09N1494	21	182	193
09N314	43		193	09N963	1	182	193	09N1495	2	182	193
09N315	43		193	09N964	43	182	193	09N1496	23-	182	193
09N346	43		193	09N965	1	182	193	09N1497	1	182	193
09N347	1	182	193	09N966	12-	182	193	09N1498	1	182	193
09N348	21	182	193	09N967	12-	182	193	09N1499	1	182	193
09N349	21	182	193	09N968	1	182	193	09N1500	23	182	193
09N350	21	182	193	09N969	21	182	193	09N1501	21	182	193
09N351	2	182	193	09N970	12	182	193	09N1502	1	182	193
09N352	1	182	193	09N971	1		193	09N1503	4		193
09N353	21	182	193	09N972	4	182	193	09N1504	1	182	193

09N354	34		193	09N973	1	182	193	09N1505	21	182	193
09N355	43		193	09N974	1	182	193	09N1506	43		193
09N356	12	182	193	09N975	4	182	193	09N1507	12	182	193
09N357	43		193	09N976	1	182	193	09N1508	4	182	193
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09N361	12	182	193	09N979	12	182	193	09N1511	21	182	193
09N362	4		193	09N980	12	182	193	09N1512	43	182	193
09N363	1	182	193	09N981	4		193	09N1513	21	182	193
09N364	1	182	193	09N982	21	182	193	09N1514	34	182	193
09N365	1	182	193	09N983	12	182	193	09N1515	21	182	193
09N366	1	182	193	09N984	43	182	193	09N1516	21	182	193
09N367	4		193	09N985	4		193	09N1517	2	182	193
09N368	2	182	193	09N986	1	182	193	09N1518	34	182	193
09N369	43		193	09N988	12	182	193	09N1519	21	182	193
09N370	3		193	09N989	12	182	193	09N1520	21	182	193
09N371	34		193	09N990	21	182	193	09N1521	1-	182	193
09N372	34		193	09N991	4	182	193	09N1522	12	182	193
09N373	43		193	09N992	1	182	193	09N1523	12	182	193
09N374	1		193	09N993	12	182	193	09N1524	12	182	193
09N375	34		193	09N994	1-	182	193	09N1525	21	182	193
09N376	34		193	09N997	21	182	193	09N1526	34	182	193
09N377	43		193	09N998	1	182	193	09N1527	21	182	193
09N378	34		193	09N999	4	182	193	09N1528	21	182	193
09N379	1		193	09N1000	21	182	193	09N1529	12	182	193
09N380	34		193	09N1001	21	182	193	09N1530	34	182	193
09N381	34		193	09N1002	12	182	193	09N1531	21	182	193
09N382	43		193	09N1003	4	182	193	09N1532	1-	182	193
09N383	1		193	09N1004	32	182	193	09N1533	12	182	193
09N384	43		193	09N1005	12	182	193	09N1534	21	182	193
09N385	34		193	09N1006	32	182	193	09N1535	21	182	193
09N386	1	182	193	09N1007	21	182	193	09N1536	43	182	193
09N387	43		193	09N1008	21	182	193	09N1537	43	182	193
09N388	1	182	193	09N1009	21	182	193	09N1538	21	182	193
09N390	1	182	193	09N1010	1	182	193	09N1539	12	182	193
09N391	4		193	09N1012	34		193	09N1540	12	182	193
09N392	34		193	09N1013	1	182	193	09N1541	21	182	193
09N393	1	182	193	09N1014	12	182	193	09N1542	21	182	193
09N394	12	182	193	09N1015	1		193	09N1543	21	182	193
09N395	12	182	193	09N1016	12	182	193	09N1544	21	182	193
09N396	21	182	193	09N1017	21	182	193	09N1545	21	182	193
09N397	12	182	193	09N1018	1	182	193	09N1546	12	182	193
09N398	4		193	09N1020	21	182	193	09N1548	21	182	193
09N399	12	182	193	09N1021	1	182	193	09N1549	21	182	193

09N400	1	182	193	09N1022	4	182	193	09N1550	43	182	193
09N401	34		193	09N1023	21	182	193	09N1551	1	182	193
09N402	34		193	09N1024	1	182	193	09N1552	12	182	193
09N403	4		193	09N1025	2-	182	193	09N1553	21	182	193
09N404	21	182	193	09N1026	2	182	193	09N1554	34	182	193
09N405	43		193	09N1028	21	182	193	09N1555	21	182	193
09N406	1	182	193	09N1029	43	182	193	09N1556	32	182	193
09N407	1	182	193	09N1030	21	182	193	09N1557	12	182	193
09N408	1	182	193	09N1031	12	182	193	09N1558	43	182	193
09N409	1	182	193	09N1032	12	182	193	09N1559	12	182	193
09N410	2	182	193	09N1033	1	182	193	09N1560	12	182	193
09N411	3	182	193	09N1034	1	182	193	09N1561	12	182	193
09N412	21	182	193	09N1035	21	182	193	09N1562	34	182	193
09N413	34	182	193	09N1036	21	182	193	09N1563	12	182	193
09N414	1	182	193	09N1037	21	182	193	09N1564	21	182	193
09N415	12	182	193	09N1038	1	182	193	09N1565	21	182	193
09N416	34		193	09N1039	23	182	193	09N1567	21	182	193
09N417	21	182	193	09N1040	1	182	193	09N1568	12	182	193
09N418	1	182	193	09N1041	12	182	193	09N1570	21	182	193
09N419	1	182	193	09N1042	21	182	193	09N1571	21	182	193
09N420	34		193	09N1044	21	182	193	09N1572	21	182	193
09N422	1	182	193	09N1045	21	182	193	09N1574	21	182	193
09N423	34		193	09N1046	21	182	193	09N1575	34	182	193
09N424	12	182	193	09N1047	2	182	193	09N1576	21	182	193
09N425	34		193	09N1048	12	182	193	09N1577	21	182	193
09N426	1	182	193	09N1049	12	182	193	09N1578	21	182	193
09N427	1	182	193	09N1050	21	182	193	09N1579	12	182	193
09N428	1	182	193	09N1068	4	182	193	09N1580	21	182	193
09N429	1	182	193	09N1070	43	182	193	09N1581	4	182	193
09N430	43		193	09N1071	4	182	193	09N1582	1	182	193
09N431	34		193	09N1072	43	182	193	09N1583	21	182	193
09N432	34		193	09N1073	43	182	193	09N1584	21	182	193
09N433	1	182	193	09N1074	43	182	193	09N1585	21	182	193
09N434	43		193	09N1075	21	182	193	09N1690	43	182	193
09N435	1	182	193	09N1076	4	182	193	09N1691	34	182	193
09N504	34		193	09N1077	43	182	193	09N1692	34	182	193
09N505	1	182	193	09N1078	4		193	09N1693	34	182	193
09N506	12	182	193	09N1079	43	182	193	09N1694	43	182	193
09N507	43		193	09N1080	34	182	193	09N1695	21	182	193
09N508	4		193	09N1081	34	182	193	09N1696	43	182	193
09N509	1	182	193	09N1082	4	182	193	09N1697	43	182	193
09N510	34		193	09N1083	43	182	193	09N1698	32	182	193
09N511	1	182	193	09N1084	4	182	193	09N1699	21	182	193
09N512	1	182	193	09N1085	43	182	193	09N1700	32	182	193

09N513	4		193	09N1086	34	182	193	09N1701	21	182	193
09N514	1	182	193	09N1087	43	182	193	09N1702	43	182	193
09N515	23	182	193	09N1088	43	182	193	09N1703	34	182	193
09N516	34		193	09N1089	4		193	09N1704	34	182	193
09N517	4		193	09N1090	34	182	193	09N1705	34	182	193
09N518	1	182	193	09N1091	21		193	09N1706	32	182	193
09N519	4		193	09N1092	34		193	09N1707	34	182	193
09N520	1	182	193	09N1093	43		193	09N1708	34	182	193
09N521	34		193	09N1094	43	182	193	09N1709	32	182	193
09N522	43		193	09N1095	43	182	193	09N1710	34		193
09N523	43		193	09N1096	34		193	09N1711	34	182	193
09N524	43		193	09N1097	1	182	193	09N1712	32	182	193
09N525	12	182	193	09N1098	43		193	09N1713	21	182	193
09N526	4		193	09N1100	4	182	193	09N1714	21	182	193
09N527	34		193	09N1101	34		193	09N1715	34	182	193
09N528	43		193	09N1102	32	182	193	09N1716	21	182	193
09N529	12	182	193	09N1103	43		193	09N1717	34	182	193
09N531	34		193	09N1105	43	182	193	09N1718	34	182	193
09N532	43		193	09N1106	43	182	193	09N1719	43		193
09N533	34		193	09N1107	34		193	09N1720	34	182	193
09N534	34	182	193	09N1108	43	182	193	09N1721	21	182	193
09N535	**1**		**193**	09N1109	32	182	193	09N1722	32	182	193
09N536	12	182	193	09N1110	21	182	193	09N1723	32	182	193
09N537	43		193	09N1111	43	182	193	09N1724	34	182	193
09N538	34		193	09N1113	43	182	193	09N1725	34	182	193
09N539	12	182	193	09N1114	4		193	09N1726	21	182	193
09N540	1	182	193	09N1115	43	182	193	09N1727	43	182	193
09N541	4		193	09N1116	34	182	193	09N1728	34	182	193
09N542	1	182	193	09N1117	43	182	193	09N1729	34	182	193
09N543	12	182	193	09N1118	21	182	193	09N1730	34	182	193
09N544	12	182	193	09N1119	21	182	193	09N1731	34	182	193
09N545	43		193	09N1121	43		193	09N1732	21	182	193
09N547	**1**		**193**	09N1122	43	182	193	09N1733	21	182	193
09N548	43		193	09N1123	12	182	193	09N1734	12	182	193
09N549	12	182	193	09N1124	21		193	09N1735	34	182	193
09N550	21	182	193	09N1125	4		193	09N1736	34	182	193
09N551	34		193	09N1126	21		193	09N1737	34	182	193
09N552	1	182	193	09N1127	43	182	193	09N1738	34	182	193
09N553	1	182	193	09N1129	2	182	193	09N1739	12	182	193
09N554	4		193	09N1130	34	182	193	09N1740	34	182	193
09N555	43		193	09N1131	43		193	09N1741	34	182	193
09N556	2	182	193	09N1132	43	182	193	09N1742	34	182	193
09N557	21	182	193	09N1133	4	182	193	09N1743	34	182	193
09N558	**12**		**193**	09N1134	21	182	193	09N1744	34		193

09N559	43		193	09N1135	34	182	193	09N1745	43	182	193
09N560	43		193	09N1137	34	182	193	09N1746	34	182	193
09N561	34		193	09N1138	43	182	193	09N1747	34	182	193
09N562	34		193	09N1139	4		193	09N1748	43		193
09N563	43		193	09N1140	43		193	09N1749	34	182	193
09N564	1	182	193	09N1141	21	182	193	09N1750	34	182	193
09N565	21	182	193	09N1142	43	182	193	09N1751	32	182	193
09N566	43		193	09N1143	43	182	193	09N1752	32	182	193
09N567	12	182	193	09N1145	4	182	193	09N1753	34	182	193
09N568	1	182	193	09N1146	43	182	193	09N1754	21	182	193
09N569	12	182	193	09N1147	21	182	193	09N1755	32	182	193
09N570	12	182	193	09N1148	43	182	193	09N1756	34	182	193
09N571	2	182	193	09N1149	1	182	193	09N1758	32	182	193
09N572	12	182	193	09N1150	1	182	193	09N1759	34	182	193
09N573	34		193	09N1151	32	182	193	09N1760	21	182	193
09N574	34		193	09N1153	43	182	193	09N1761	34	182	193
09N575	34		193	09N1154	43		193	09N1762	34	182	193
09N576	43		193	09N1155	12	182	193	09N1763	32	182	193
09N577	12	182	193	09N1156	34	182	193	09N1764	34	182	193
09N579	1	182	193	09N1157	43	182	193	09N1765	12	182	193
09N580	12	182	193	09N1170	32	182	193	09N1766	21	182	193
09N581	12	182	193	09N1171	43	182	193	09N1767	34	182	193
09N582	21	182	193	09N1172	12	182	193	09N1768	34	182	193
09N583	34		193	09N1173	12	182	193	09N1769	12	182	193
09N584	12	182	193	09N1174	21	182	193	09N1770	12	182	193
09N585	12	182	193	09N1175	1	182	193	09N1771	34	182	193
09N586	1	182	193	09N1176	34	182	193	09N1772	43	182	193
09N587	12	182	193	09N1177	12	182	193	09N1773	43	182	193
09N588	34		193	09N1178	43	182	193	09N1774	34	182	193
09N589	1	182	193	09N1179	21	182	193	09N1775	34	182	193
09N590	0;1-	182	193	09N1180	34	182	193	09N1776	43	182	193
09N591	1	182	193	09N1181	21	182	193	09N1777	43	182	193
09N743	34	182	193	09N1182	1	182	193	09N1778	1-	182	193
09N744	43	182	193	09N1183	21	182	193	09N1779	34	182	193
09N746	12		193	09N1184	21	182	193				

[a] The BC_2F_1 plants in bold font including 09N12, 09N535, 09N547, and 09N558 are the parents of four wheat lines RWG1, RWG2, RWG3 and RWG4 with Sr39 on short *Ae. speltoides* chromosome segments, respectively. The plants with missing data from marker analysis were not included.

[b] IT is the infection type to TMLK.

[c] CS, Chinese Spring.

TABLE S4

Infection type (IT) of BC_2F_2 plants (pedigree: Chinese Spring//Chinese Spring *ph1bph1b2/RL6082) and parental lines to stem rust race TMLK.**

Line-Plant No. [a]	IT	Line-Plant No	IT	Line-Plant No.	IT
CS-1	34	09N272-3	1	09N547-10	34
CS-2	34	09N272-4	1	09N547-11	12
CS-3	34	09N280-1	34	09N547-12	12
RL6082-1	1	09N280-2	34	09N547-13	12
RL6082-2	1	09N280-3	34	09N558-1	1
RL6082-3	;-1	09N280-5	34	09N558-2	34
RL6082-4	1	09N291-1	34	09N558-3	1
09N12-1	12	09N291-3	1	09N558-4	1
09N12-2	1	09N291-5	1	09N558-8	34
09N12-3	**1-**	09N291-6	1	09N558(5-8)-1	1
09N12-4	1	09N291-7	12-	09N558(5-8)-2	12
09N12-5	1	09N291-8	12	09N558(5-8)-3	1
09N12-6	1	09N291-9	1	09N558-9	1
09N12-7	34	09N291-10	12-	09N558(9-12)-1	34
09N12-8	1	09N291-11	34	09N558(9-12)-2	34
09N12-9	1	09N291-12	12-	09N558(9-12)-3	34
09N12-10	1	09N291-13	12	09N558-14	34
09N14-1	1	09N291-14	12-	09N558(13-16)-1	1
09N14-2	34	09N298-1	34	09N558(13-16)-2	1
09N31-1	34	09N298-2	1	09N558-13	1
09N31-2	34	09N298-3	34	09N558-19	34
09N58-2	1	09N300-3	34	09N558(17-20)-1	1
09N61-1	34	09N300-4	34	09N558(17-20)-2	12
09N61-2	1	09N300-5	1	09N558-20	1
09N74-3	1	09N301-1	34	09N558-21	1
09N74-4	34	09N301-2	34	09N558-22	1
09N74-5	1	09N302-1	1	09N558-23	34
09N74-6	1	09N302-2	34	09N558-24	12
09N87-1	2	09N302-3	34	09N558-25	34
09N87-2	2	09N302-4	1	09N558-26	1
09N87-3	2	09N306-1	1	09N558-27	1
09N87-4	12	09N306-2	1	09N558-28	34
09N87-5	1	09N306-3	1	09N558-29	1
09N229-2	34	09N313-1	12-	**09N558-30**	**1**
09N229-3	1	09N313-2	23	09N558-31	1
09N229-4	1	09N313-3	34	09N558-32	1+

09N229-5	34	09N313-5	12	09N558-33	1
09N229-6	1	09N316-1	34	09N558-34	34
09N230-2	3	09N316-2	34	09N558-35	1
09N232-1	34	09N316-3	34	09N558-36	1
09N232-2	34	09N316-4	12	09N558-38	34
09N232-3	1	09N316-5	12	09N558-39	1
09N232-4	34	09N374-2	34	09N558-40	34
09N232-5	34	09N374-3	12	09N580-2	34
09N232-6	34	09N374-4	12	09N589-2	3
09N237-2	1	09N374-5	34	09N590-1	34
09N237-3	4	09N374-6	12	09N590-2	1
09N237-4	34	09N379-1	34	09N590-3	1
09N237-5	1	09N379-2	21	09N590-4	1
09N237-6	12	09N379-3	21	09N1075-1	34
09N237-7	1	09N379-4	1	09N1075-2	1
09N237-8	4	09N379-5	34	09N1123-1	34
09N237-9	12	09N426-1	34	09N1123-2	1
09N237-10	1	09N426-2	1	09N1185-1	1
09N237-11	34	09N535-1	1	09N1185-2	1
09N237-12	12	09N535-2	34	09N1204-1	1
09N241-1	34	**09N535-3**	**1**	09N1204-2	12
09N255-3	1	**09N547-1**	**12**	09N1215-1	1
09N255-4	34	09N547-2	12	09N1215-2	1
09N255-5	1	09N547-3	34	09N1432-1	1
09N255-6	34	09N547-4	12	09N1482-1	34
09N256-1	34	09N547-5	12	09N1482-2	1
09N265-1	1	09N547-6	1	09N1766-1	1
09N265-2	34	09N547-7	1	09N1766-2	34
09N272-1	34	09N547-8	34	09N1953-1	1
09N272-2	34	09N547-9	12	09N1953-2	34

[a] CS is Chinese Spring. The BC_2F_2 plants in bold font including 09N12-3, 09N535-3, 09N547-1, 09N558-30 are homozygous for the short *Aegilops speltoides* chromosome segments carrying *Sr39* and they were designated as RWG1, RWG2, RWG3, and RWG4, respectively.

TABLE S5

Measurement and size calculation of *Aegilops speltoides* 2S chromosome segments carrying *Sr39* in RWG1, RWG2, RWG3, RWG4, and RL6082.

Line-Cell No.	Length (μm)		2S % [c]	Line-Cell No.	Length (μm)		2S %
	2S [a]	Total [b]			2S	Total	
RWG1-1	0.32	11.2	2.86	RWG3-1	0.41	13.21	3.10
RWG1-2	0.41	12.68	3.23	RWG3-2	0.44	9.51	4.63
RWG1-3	0.42	13.69	3.07	RWG3-3	0.41	12.11	3.39
RWG1-4	0.62	20.74	2.99	RWG3-4	0.41	12.11	3.39
RWG1-5	0.32	15.29	2.09	RWG3-5	0.29	9.23	3.14
RWG1-6	0.31	14.72	2.11	RWG3-6	0.74	20.34	3.64
RWG1-7	0.37	17.3	2.14	RWG3-7	0.58	12.07	4.81
RWG1-8	0.31	14.09	2.20	RWG3-8	0.46	14.31	3.21
RWG1-9	0.32	14.11	2.27	RWG3-9	0.51	10.8	4.72
RWG1-10	0.46	16.79	2.74	RWG3-10	0.51	17.93	2.84
RWG1-11	0.31	13.8	2.25	RWG3-11	0.32	10.15	3.15
RWG1-12	0.31	13.41	2.31	RWG3-12	0.37	10.61	3.49
RWG1-13	0.41	14.52	2.82	RWG3-13	0.55	14.8	3.72
RWG1-14	0.46	15.07	3.05	RWG3-14	0.41	12.04	3.41
RWG1-15	0.62	18.4	3.37	RWG3-15	0.51	13.04	3.91
RWG1-16	0.51	13.59	3.75	RWG3-16	0.66	15.2	4.34
RWG1-17	0.62	13.11	4.73	RWG3-17	0.37	10.29	3.60
RWG1-18	0.41	11.28	3.63	RWG3-18	0.66	20.99	3.14
				RWG3-19	0.41	11.76	3.49
				RWG3-20	0.23	7.71	2.98
Average			**2.87**				**3.60**
RWG2-1	0.55	14.61	3.76	RWG4-1	1.15	13.28	8.66
RWG2-2	0.74	11.46	6.46	RWG4-2	1.13	9.73	11.61
RWG2-3	0.52	11.14	4.67	RWG4-3	1.15	14.86	7.74
RWG2-4	0.46	13.35	3.45	RWG4-4	1.15	13.59	8.46
RWG2-5	0.62	13.02	4.76	RWG4-5	1.11	12.28	9.04
RWG2-6	0.52	10.5	4.95	RWG4-6	1.05	11.22	9.36
RWG2-7	0.51	12.74	4.00	RWG4-7	1.05	11.15	9.42
RWG2-8	0.51	12.23	4.17	RWG4-8	1.15	16.71	6.88
RWG2-9	0.51	11.78	4.33	RWG4-9	1.25	15.19	8.23
RWG2-10	0.65	13.73	4.73	RWG4-10	1.2	13.05	9.20
RWG2-11	0.74	13.27	5.58	RWG4-11	1.13	12.52	9.03
RWG2-12	0.6	13.25	4.53	RWG4-12	0.88	9.15	9.62
RWG2-13	0.6	12.23	4.91	RWG4-13	0.95	8.02	11.85
RWG2-14	0.65	10.95	5.94	RWG4-14	0.95	7.79	12.20
RWG2-15	0.51	14.43	3.53	RWG4-15	0.87	10.28	8.46
RWG2-16	0.72	11.96	6.02	RWG4-16	1.15	12.11	9.50

RWG2-17	0.58	11.42	5.08	RWG4-17	1.29	14.54	8.87
RWG2-18	0.41	8.5	4.82	RWG4-18	0.88	9.86	8.92
RWG2-19	0.51	11.14	4.58	RWG4-19	0.73	9.84	7.42
RWG2-20	0.55	13.03	4.22	RWG4-20	1.15	13.44	8.56
Average			**4.72**				**9.15**
RL6082-1	11.69	12.08	96.77	RL6082-11	9.88	10.27	96.20
RL6082-2	10.44	10.76	97.03	RL6082-12	8.31	8.64	96.18
RL6082-3	9.92	10.46	94.84	RL6082-13	10.39	11.17	93.02
RL6082-4	9.93	10.36	95.85	RL6082-14	10.32	10.86	95.03
RL6082-5	9.23	9.53	96.85	RL6082-15	8.63	9.17	94.11
RL6082-6	11.39	12.2	93.36	RL6082-16	7.09	7.38	96.07
RL6082-7	11.02	11.67	94.43	RL6082-17	7.83	8.33	94.00
RL6082-8	11.16	11.84	94.26	RL6082-18	10.23	11.13	91.91
RL6082-9	12.08	12.76	94.67	RL6082-19	8.96	9.37	95.62
RL6082-10	10.67	11.07	96.39	RL6082-20	9.03	9.6	94.06
Average							**95.03**

[a] Length of *Ae. speltoides* 2S chromosome segments carrying *Sr39*.
[b] Total length of 2B/2S translocation chromosome.
[c] Percentage of *Ae. speltoides* 2S chromosome segment carrying *Sr39* in the 2B/2S translocation chromosome.

TABLE S6

Primer pairs used to develop STS (sequence-tagged site) markers linked to *Sr39* on short *Aegilops speltoides* chromosome segments in wheat lines RWG1, RWG2, RWG3, and RWG4.

Primer [a]	Sequence (5' to 3')	Tm (50mM Na+) °C	GC content (%)
Sr39-1F	GGTGCTTGTGGTGAGAAACGA	64	52
Sr39-1R	TCAGCCGGCCCAAGAATAG	63	58
Sr39-2F	ACCATTGATGCTGGAAGGAC	60	50
Sr39-2R	AAGCCAAGGCTCATTCTTCA	60	45
Sr39-3F	TGCTAGTTTCCTGTCTCGCGT	62	52
Sr39-3R	CGACAATGATGTTGACCTGCC	64	52
Sr39-4F	CTGTCTCGCGTTTGTTACGA	60	50
Sr39-4R	GGGTGAGAAAGCATAGAACGA	59	48
Sr39-5F	GCGGCCAGAACAGCATCAT	65	58
Sr39-5R	CAGTTGCCCTCGCCATTGT	65	58
Sr39-6F	GCAGGACGTGTCCACCAT	61	61
Sr39-6R	AGTTGCCCTCGCCATTGT	62	56
Sr39-7F	CCATCGACCTCTCCAACGA	62	58
Sr39-7R	TTCCACAAGTTTGGGTTCTCG	62	48
Sr39-8F	CATCGACCTCTCCAACGAC	59	58
Sr39-8R	TTCTCGAGGTCGAGGATCTG	60	55
Sr39-9F	GTAGTATTGGCACACCGCGTT	62	52
Sr39-9R	CTTCTTCTTCCACCTCGCTCC	63	57
Sr39-10F	CCGTACTCGGTGACAACGTC	62	60
Sr39-10R	CTACAGCAGGGACCAAGCTC	60	60
Sr39-11F	CTTCCGTGAGATGGCGATTT	62	50
Sr39-11R	GAGCGGGATGAATTTGGACA	63	50
Sr39-12F	CCCAACACTGCCTAGCAATC	61	55
Sr39-12R	ATCGCAAAATACGCTCCAAT	60	40
Sr39-13F	CTTCCGTGAGATGGCGATTT	62	50
Sr39-13R	GAGCGGGATGAATTTGGACA	63	50
Sr39-14F	CCCAACACTGCCTAGCAATC	61	55
Sr39-14R	ATCGCAAAATACGCTCCAAT	60	40
Sr39-15F	TCCGCAATCCTCTCCTGGT	63	58
Sr39-15R	AGGTGTTGAGGAGGCCGAA	63	58
Sr39-16F	ATCCTTGAGAGCATGCAACC	60	50
Sr39-16R	AGCCTCGTACTTGGAGCAAA	60	50
Sr39-17F	GACTTCCTCGCATGGCAATC	63	55
Sr39-17R	TGACATGTAAGCCGGCACTG	63	55
Sr39-18F	ATGGCAATCAGATCCCAAAG	60	45
Sr39-18R	GGCATTCAACCTCATCCTGT	60	50
Sr39-19F	ATGTGCTGCCTGCTTCGAG	63	58

Sr39-19R	CAGGGTTCTTATTCAGGGCGT	63	52
Sr39-20F	GCCTTGGTGGATTTTGTGAT	60	45
Sr39-20R	GCGCTTTCAGTACAGGGTTC	60	55
Sr39-21F	CTCTTTCCTCCACGCCGTT	63	58
Sr39-21R	GCAACATAACCAGCACCAAGG	63	52
Sr39-22F	TTGGTGCTGGTTATGTTGCT	59	45
Sr39-22R	AAAGTTGACGCGAACTTCTGA	60	43
Sr39-23F	GCAGGCTAACGATGGATGGA	63	55
Sr39-23R	CCTTGTCGTCCACTTGAGCC	63	60
Sr39-24F	GTACGGGAGGAATTTGCTGA	60	50
Sr39-24R	CGATGTTCACCATCTTGTCG	60	50
Sr39-25F	TACAGGGAGATGGGCACGA	63	58
Sr39-25R	TGCAGCTGAGGATGTGAAGAA	62	48
Sr39-26F	ATACAGGGAGATGGGCACGA	63	55
Sr39-26R	TCATCATCCACCAAATCTAAACC	60	39
Sr39-27F	CGAGAAGCCCAACGTCCTCT	64	60
Sr39-27R	AACCATCCTTGGGCCCTCA	65	58
Sr39-28F	AAGAAATTCGGGAGGCAGTT	60	45
Sr39-28R	ACATCTCGAACCATCCTTGG	60	50
Sr39-29F	AGAGCCTGGGACTGTTGCTA	60	55
Sr39-29R	CAATGGCACTCTTCAAAGCA	60	45
Sr39-30F	CGGCTATTGCTCAAAGAAGG	60	50
Sr39-30R	TGTTTCTGTCAGAGGCAACG	60	50
Sr39-31F	GCCCTATTCTTGACCCATCA	60	50
Sr39-31R	CTTGCTAACAAGGCGTGACA	60	50
Sr39-32F	TCAAGTGGTGGGATTGATGA	60	45
Sr39-32R	GGTGACCTCCCCTTAACCAT	60	55
Sr39-33F	CTGCAGTTACCAGCTCCACA	60	55
Sr39-33R	TCTTTCCATGCCATCTTTCC	60	45
Sr39-34F	TCAGATCCACCAGCTCACTG	60	55
Sr39-34R	CAGCACATGCATCCATAACC	60	50
Sr39-35F	TCCAATGACAAGCATTTCCA	60	40
Sr39-35R	CAGTGAGCTGGTGGATCTGA	60	55
Sr39-36R	TTGCCAAAACTTGCTGAGTG	60	45
Sr39-37F	TGCTTTGAAGAGTGCCATTG	60	45
Sr39-37R	CCGACTAGGAGCAACAGAGG	60	60
Sr39-38F	AGCCTGGGACTGTTGCTAGA	60	55

[a] The rest of the primer pairs are Sr39-38F with Sr39-37R, Sr39-29F with Sr39-30R, Sr39-34F with Sr39-36R, Sr39-35F with Sr39-31R, and Sr39-34F with Sr39-31R. Three primer pairs (Sr39-20, Sr39-29, and Sr39-30) generated three co-dominant STS markers, which were designated as *Xrwgs27*, *Xrwgs28*, and *Xrwgs29*, respectively.

TABLE S7

Infection types (IT) to TMLK and marker genotypes of 65 BC_2F_2 plants (pedigree: Chinese Spring//Chinese Spring *ph1bph1b2/RL6082) analyzed with three markers *Xrwgs27, Xrwgs28,* and *Xrwgs29*.**

		Xrwgs27 allele (bp)		*Xrwgs28* allele (bp)		*Xrwgs29* allele (bp)	
Plant No.	IT	RL6082	CS [a]	RL6082	CS	RL6082	CS
09N12-1	12	740	710	520/450/360	350	540	550
09N12-2	1	740	710	520/450/360	350	540	550
09N12-3	1-	740		520/450/360		540	
09N12-4	1	740		520/450/360		540	
09N12-5	1	740	710	520/450/360	350	540	550
09N12-6	1	740	710	520/450/360	350	540	550
09N12-7	34		710		350		550
09N12-8	1	740	710	520/450/360	350	540	550
09N12-9	1	740	710	520/450/360	350	540	550
09N12-10	1	740	710	520/450/360	350	540	550
09N535-1	1	740	710	520/450/360	350	540	550
09N535-2	34		710		350		550
09N535-3	1	740		520/450/360		540	
09N547-1	12	740		520/450/360		540	
09N547-2	12	740	710	520/450/360	350	540	550
09N547-3	34		710		350		550
09N547-4	12	740	710	520/450/360	350	540	550
09N547-5	12	740	710	520/450/360	350	540	550
09N547-6	1	740	710	520/450/360	350	540	550
09N547-7	1	740		520/450/360		540	
09N547-8	34		710		350		550
09N547-9	12	740	710	520/450/360	350	540	550
09N547-10	34		710		350		550
09N547-11	12	740	710	520/450/360	350	540	550
09N547-12	12	740	710	520/450/360	350	540	550
09N547-13	12	740	710	520/450/360	350	540	550
09N558-1	1	740	710	520/450/360	350	540	550
09N558-2	34		710		350		550
09N558-3	1	740	710	520/450/360	350	540	550
09N558-4	1	740	710	520/450/360	350	540	550
09N558-8	34		710		350		550
09N558(5-8)-1	1	740	710	520/450/360	350	540	550
09N558(5-8)-2	12	740	710	520/450/360	350	540	550
09N558(5-8)-3	1	740		520/450/360		540	
09N558-9	1	740	710	520/450/360	350	540	550
09N558(9-12)-1	34		710		350		550
09N558(9-12)-2	34		710		350		550

09N558(9-12)-3	34		710		350		550
09N558-14	34		710		350		550
09N558(13-16)-1	1	740	710	520/450/360	350	540	550
09N558(13-16)-2	1	740	710	520/450/360	350	540	550
09N558-13	1	740	710	520/450/360	350	540	550
09N558-19	34		710		350		550
09N558(17-20)-1	1	740	710	520/450/360	350	540	550
09N558(17-20)-2	12	740	710	520/450/360	350	540	550
09N558-20	1	740	710	520/450/360	350	540	550
09N558-21	1	740	710	520/450/360	350	540	550
09N558-22	1	740		520/450/360		540	
09N558-23	34		710		350		550
09N558-24	12	740	710	520/450/360	350	540	550
09N558-25	34		710		350		550
09N558-26	1	740		520/450/360		540	
09N558-27	1	740	710	520/450/360	350	540	550
09N558-28	34		710		350		550
09N558-29	1	740	710	520/450/360	350	540	550
09N558-30	1	740		520/450/360		540	
09N558-31	1	740	710	520/450/360	350	540	550
09N558-32	1+	740	710	520/450/360	350	540	550
09N558-33	1	740		520/450/360		540	
09N558-34	34		710		350		550
09N558-35	1	740	710	520/450/360	350	540	550
09N558-36	1	740		520/450/360		540	
09N558-38	34		710		350		550
09N558-39	1	740	710	520/450/360	350	540	550
09N558-40	34		710		350		550
Thatcher	34		710		350		550
CS	43		710		350		550
RL6082	1	740		520/450/360		540	

[a] CS, Chinese Spring.

TABLE S8

Marker genotype of 29 BC_2F_1 plants (pedigree: Chinese Spring//Chinese Spring *ph1bph1b2/RL6082) exhibiting dissociation of *Sr39* from *Xgwm319* and three parental lines analyzed with *Xrwgs27*, *Xrwgs28*, and *Xrwgs29*.**

		Xrwgs27 allele (bp)		*Xrwgs28* allele (bp)		*Xrwgs29* allele (bp)	
Lines	IT [a]	RL6082	CS [b]	RL6082	CS	RL6082	CS
09N229	12	740	710	520/450/360	350	540	550
09N230	12	740	710	520/450/360	350	540	550
09N231	1	740	710	520/450/360	350	540	550
09N235	12	740	710	520/450/360	350	540	550
09N237	12	740	710	520/450/360	350	540	550
09N241	1	740	710	520/450/360	350	540	550
09N255	1	740	710	520/450/360	350	540	550
09N265	1	740	710	520/450/360	350	540	550
09N267	12	740	710	520/450/360	350	540	550
09N270	1	740	710	520/450/360	350	540	550
09N289	1	740	710	520/450/360	350	540	550
09N291	1	740	710	520/450/360	350	540	550
09N292	1	740	710	520/450/360	350	540	550
09N295	1	740	710	520/450/360	350	540	550
09N298	21	740	710	520/450/360	350	540	550
09N301	1	740	710	520/450/360	350	540	550
09N306	12	740	710	520/450/360	350	540	550
09N313	21	740	710	520/450/360	350	540	550
09N379	1	740	710	520/450/360	350	540	550
09N383	1	740	710	520/450/360	350	540	550
09N746	12	740	710	520/450/360	350	540	550
09N767	1	740	710	520/450/360	350	540	550
09N797	12	740	710	520/450/360	350	540	550
09N798	1	740	710	520/450/360	350	540	550
09N799	1	740	710	520/450/360	350	540	550
09N971	1	740	710	520/450/360	350	540	550
09N1015	1	740	710	520/450/360	350	540	550
09N1091	21	740	710	520/450/360	350	540	550
09N1124	21	740	710	520/450/360	350	540	550
Thacher	34		710		350		550
CS	43		710		350		550
RL6082	1	740		520/450/360		540	

[a] IT, infection type to TMLK.
[b] CS, Chinese Spring.