agronomy

MDPI

Article

Physical Location of New Stripe Rust Resistance Gene(s) and PCR-Based Markers on Rye (*Secale cereale* L.) Chromosome 5 Using 5R Dissection Lines

Wei Xi [1,2,3,†], Zongxiang Tang [1,2,3,†], Jie Luo [1,2,3] and Shulan Fu [1,3,*]

1 College of Agronomy, Sichuan Agricultural University, Wenjiang, Chengdu 611130, China
2 Institute of Ecological Agriculture, Sichuan Agricultural University, Wenjiang, Chengdu 611130, China
3 Provincial Key Laboratory for Plant Genetics and Breeding, Wenjiang, Chengdu 611130, China
* Correspondence: fushulan@sicau.edu.cn; Tel.: +86-28-86290870
† These authors contributed equally to this work.

Received: 8 July 2019; Accepted: 26 August 2019; Published: 30 August 2019

Abstract: The rye (*Secale cereale* L.) 5R chromosome contains some elite genes that can be used to improve wheat cultivars. In this study, a set of $5R^{Ku}$ dissection lines was obtained, and 111 new PCR-based and $5R^{Ku}$-specific markers were developed using the specific length amplified fragment sequencing (SLAF-seq) method. The 111 markers were combined with the 52 $5R^{Ku}$-specific markers previously reported, and 65 *S. cereale* Lo7 scaffolds were physically mapped to six regions of the $5R^{Ku}$ chromosome using the $5R^{Ku}$ dissection lines. Additionally, the $5RL^{Ku}$ arm carried stripe rust resistance gene(s) and it was located to the region L2, the same region where 22 $5R^{Ku}$-specific markers and 11 *S. cereale* Lo7 scaffolds were mapped. The stripe rust resistance gene(s) located in the $5RL^{Ku}$ arm might be new one(s) because its source and location are different from the previously reported ones, and it enriches the resistance source of stripe rust for wheat breeding programs. The markers and the *S. cereale* Lo7 scaffolds that were mapped to the six regions of the $5R^{Ku}$ chromosome can facilitate the utilization of elite genes on the 5R chromosome in the improvement of wheat cultivars.

Keywords: wheat; rye; 5R dissection line; PCR-based markers; physical map; stripe rust

1. Introduction

Rye (*Secale cereale* L.) is a useful gene source supporting disease and insect resistance, stress resistance, and higher yield for wheat (*Triticum aestivum* L.) breeding programs [1–6]. In fact, rye 5R chromosome contains some elite genes that can be used to improve wheat cultivars. The wheat-rye 4BL/5RL translocation chromosome indicates that the 5RL arm carried loci that can increase copper efficiency [7]. The wheat-rye 5R(5A) and 5R(5D) substitution line exhibits some favorite traits, including big spike, multispikelet, high satiation seed, high protein content, and positive effects on somatic embryogenesis [8–10]. The rye 5RS arm carries the resistance gene to Russian wheat aphid (RWA) [11]. The $5R^{a}S$ arm that was derived from *S. africanum* Stapf. carries genes for stripe rust resistance, increasing spike length and reducing grain hardness [12]. The gene *Ddw1* that can reduce plant height in rye is located on the 5RL arm and its close linkage markers have been found [13]. It was also reported that the 5R chromosome harbors genes that determine its growth habit [14]. However, the elite genes on 5R chromosomes have not been successfully used in wheat cultivars because of linkage drag and the non-compensating and low recombination frequency of 5R with its wheat homologues [15,16]. Elite genes can be utilized effectively when segments of 5R chromosomes have been transferred into wheat backgrounds and been accurately identified. Rye chromosome-specific markers are conducive to

identifying rye chromatin in wheat backgrounds and help in the effective application of rye elite genes in wheat breeding programs. So far, few 5R-specific markers have been developed. Although terminal deletions of 5RL (T5RS.5RL-del) were obtained, they were not used to physically map 5R-specific markers [17]. In this study, the $5R^{Ku}$ chromosome derived from rye Kustro (*S. cereale* L.) was proven to carry stripe rust resistance gene(s), and some new PCR-based and $5R^{Ku}$-specific markers were developed using specific length amplified fragment sequencing (SLAF-seq) technology. These new markers were mapped to six regions on $5R^{Ku}$ using $5R^{Ku}$ dissection lines in a wheat background. Additionally, the stripe rust resistance gene(s) was also physically located on a segment of $5R^{Ku}$.

2. Materials and Methods

2.1. Plant Materials

The octoploid triticale line MK was derived from common wheat (*T. aestivum* L.) Mianyang 11(MY11) × *S. cereale* L. Kustro. Some progeny was obtained by controlled backcrossing of MK with MY11 followed by strict self-seeding [18]. Seven wheat-rye monosomic addition lines, including $MA1R^{Ku}$, $MA2R^{Ku}$, $MA3R^{Ku}$, $MA4R^{Ku}$, $MA5R^{Ku}$, $MA6R^{Ku}$, and $MA7R^{Ku}$, were developed according to the methods described by Li et al. [18]. From the self-seeded progeny of the monosomic addition line $MA5R^{Ku}$, a $5R^{Ku}$(5B) disomic substitution line, $5RS^{Ku}$(5B) monotelosomic substitution line, $5RL^{Ku}$(5B) ditelosomic substitution line, $5RS^{Ku}$/5BL translocation line, and four $5R^{Ku}$ deletion lines were obtained. The progeny of $MA5R^{Ku}$ comprised a set of $5R^{Ku}$ dissection lines. In addition, rye Kustro (*S. cereale* L.), common wheat (*T. aestivum* L.) Chinese Spring (CS), and MY11 were also used in this study. The materials used in this study are available upon request to interested researchers.

2.2. Non-Denaturing Fluorescence in situ Hybridization (ND-FISH) Analysis

Oligonucleotide (oligo) probes, including Oligo-Ku [19], Oligo-pSc119.2-1, Oligo-pTa535-1 [20], Oligo-pSc200, and Oligo-pSc250 [21], were used for ND-FISH analysis of the materials used in this study. Oligo-pSc119.2-1 and Oligo-pTa535-1 can be used to identify individual wheat chromosomes [20]. Oligo-Ku, Oligo-pSc200, and Oligo-pSc250 can together be used to distinguish rye chromosomes from the wheat genome [19]. In this study, the combination of Oligo-pSc200 and Oligo-pSc250 was denoted as Oligo-pSc200 + 250. Oligo probes were synthesized by Tsingke Biological Technology Co. Ltd. (Beijing, China), and they were 5′-end-labeled with 6-carboxytetramethylrhodamine (TAMRA), 6-carboxyfluorescein (6-FAM), or Cyanine Dye 5 (Cy5). The chromosome spreads of the root tips were prepared following the methods described by Han et al. [22]. Images were made using an epifluorescence Olympus BX51 microscope (Olympus Corporation, Tokyo, Japan) equipped with a cooled charge-coupled device camera and with the HCIMAGE Live software (Hamamatsu Corporation, Sewickley, PA, USA). Images were processed using Adobe Photoshop CS 3.0.

2.3. Developing PCR-Based Markers

Genomic DNA of *S. cereale* L. Kustro has already been sequenced using specific length amplified fragment sequencing (SLAF-seq) technology (Biomarker, Beijing, China) [4]. In this study, the genomic DNA of wheat-rye monosomic addition line $MA5R^{Ku}$ was also sequenced using the SLAF-seq technique. Sixty base sequences of both ends of the sequences with sizes between 450 to 500 bp were obtained. The sequencing procedure was carried out according to the methods described by Duan et al. [4]. The primary $5R^{Ku}$ specific pair-end reads were obtained according to the methods described by Duan et al. [4]. Primers were designed according to 542 randomly selected $5R^{Ku}$ specific pair-end reads using the software Primer 3 (version 4.1.0) [23], and the optimal melting temperature and size values were set to 60 °C and 20 bases, respectively. The 542 pair-end reads were deposited in the GenBank Database (GenBank accession numbers: MN325158-MN325699). A total of 542 primer pairs were designed.

2.4. PCR Assay, 5R^{Ku}-Specific Markers Testing and Physical Location

The PCR amplifications and electrophoresis were carried out according to the procedure described by Duan et al. [4]. The markers that could produce bands from both rye Kustro and MA5R^{Ku} but not from CS, MY11, MA1R^{Ku}, MA2R^{Ku}, MA3R^{Ku}, MA4R^{Ku}, MA6R^{Ku}, and MA7R^{Ku} were regarded as 5R^{Ku}-specific markers. For each of the primer pairs, PCR reactions were repeated three times. These markers combined with the 52 5R^{Ku}-specific markers developed by Qiu et al. [24] were mapped to specific regions of the 5R^{Ku} chromosome using the 5R^{Ku} dissection lines. Again, PCR reactions of each 5R^{Ku}-specific marker were repeated three times.

2.5. Similarity Searches of the Pair-End Reads Against S. cereale L. Lo7 Scaffolds

The original pair-end reads for designing the 5R^{Ku}-specific markers were used for Nucleotide BLAST searching against the sequences in the *S. cereale* L. Lo7 scaffolds database in GrainGenes [25]. The *S. cereale* L. Lo7 scaffolds were reported by Bauer et al. [26].

2.6. Stripe Rust Resistance Testing

The resistance of 5R^{Ku} dissection lines and the parental wheat MY11 to stripe rust was evaluated under field conditions. Plants were grown in Qionglai, Sichuan, China. The materials were inoculated twice with the mixed stripe rust prevalent isolates CYR32, CYR33, and CYR34 in China at approximately 6 weeks and 9 weeks after sowing. Infection types (IT) were scored according to a 0–9 numerical scale as described by Wan et al. [27] in the adult stage.

3. Results

3.1. Obtaining 5R^{Ku} Dissection Lines

From the self-seeded progeny of MA5R^{Ku}, lines 142-30, 142-77, 143-9, 143-61, 237-2, 403-50, 444-12, and 449-4 were obtained (Figures 1–3; Table 1). Probe Oligo-pSc119.2-1 combined with probe Oligo-pTa535-1 can identify individual wheat chromosomes (Figures 1–3). The combination of probes, Oligo-Ku, Oligo-pSc200, Oligo-pSc250, and Oligo-pSc119.2-1, can be used to distinguish rye 5R^{Ku} chromosomes from the wheat genome and detect their structural variations (Figures 1–3). For the sake of description, the combination of Oligo-pSc200 and Oligo-pSc250 was denoted as Oligo-pSc200 + 250. Wheat 5B chromosomes disappeared from the five lines 140-30, 142-77, 143-9, 143-61, and 403-50 (Figures 1 and 2A–D). A broken 5R^{Ku} chromosome existed in line 142-30, and the breakpoint located between the interstitial Oligo-pSc119.2-1 and the Oligo-pSc200 + 250 signaled sites of the 5RL^{Ku} arm (Figure 1A,B). Line 142-77 contained a 5RS^{Ku} arm, therefore, it was a 5RS^{Ku}(5B) monotelosomic substitution line (Figure 1C,D). Two broken 5R^{Ku} chromosomes existed in line 143-9, and the breakpoints were located between the Oligo-pSc200 + 250 signal sites and the telomeres of 5RL^{Ku} arms (Figure 1E,F). Lines 143-61, 403-50, and 237-2 were 5R^{Ku}(5B) disomic substitution, 5RL^{Ku}(5B) ditelosomic substitution, and 5RS^{Ku}/5BL translocation lines, respectively (Figure 2). Additionally, a 7B chromosome in line 142-30 had no short arm (Figure 1A) and a wheat 3D chromosome disappeared from line 143-9 (Figure 1E).

Line 444-12 contained two broken $5R^{Ku}$ chromosomes, and the breakpoints were located between the centromeres and the interstitial Oligo-pSc119.2-1 signal sites (Figure 3A,B). In line 449-4, another broken $5R^{Ku}$ chromosome was observed, and the breakpoint occurred on the $5RS^{Ku}$ arm (Figure 3C,D). A wheat 3D chromosome also disappeared from line 449-4 (Figure 3C,D). The chromosome compositions of the eight lines are listed in Table 1. According to the breakpoints, the $5R^{Ku}$ chromosome could be divided into six regions (Figure 4). The broken $5R^{Ku}$ chromosomes in lines 142-30, 143-9, 444-12, and 449-4 were named $Del5R^{Ku}L^{3-4}$, $Del5R^{Ku}L^{4}$, $Del5R^{Ku}L^{2-4}$, and $Del5R^{Ku}S^{1}$, respectively (Figure 4). Therefore, 142-30, 142-77, 143-9, 143-61, 237-2, 403-50, 444-12, and 449-4 comprised a set of $5R^{Ku}$ dissection lines.

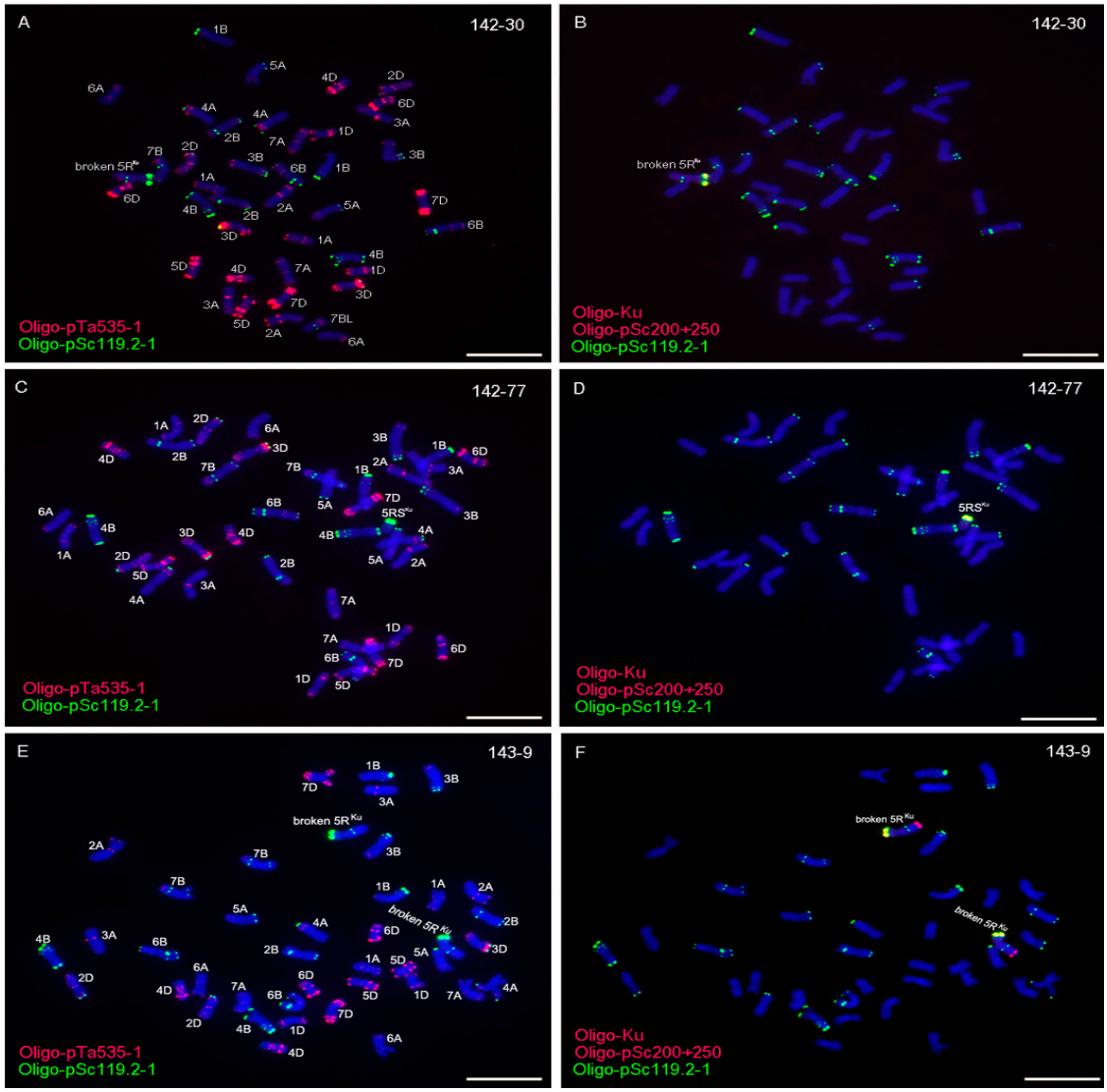

Figure 1. ND-FISH analysis of root tip metaphase chromosomes of lines 142-30, 142-77, and 143-9 using Oligo-pTa535-1, Oligo-pSc119.2-1, Oligo-Ku, and Oligo-pSc200 + 250 as probes. (**A**) and (**B**) are the same cell, (**C**) and (**D**) are the same cell, and (**E**) and (**F**) are the same cell. Chromosomes were counterstained with 4′,6′-diamidino-2-phenylindole (DAPI) (blue). Scale bar is 10 μm.

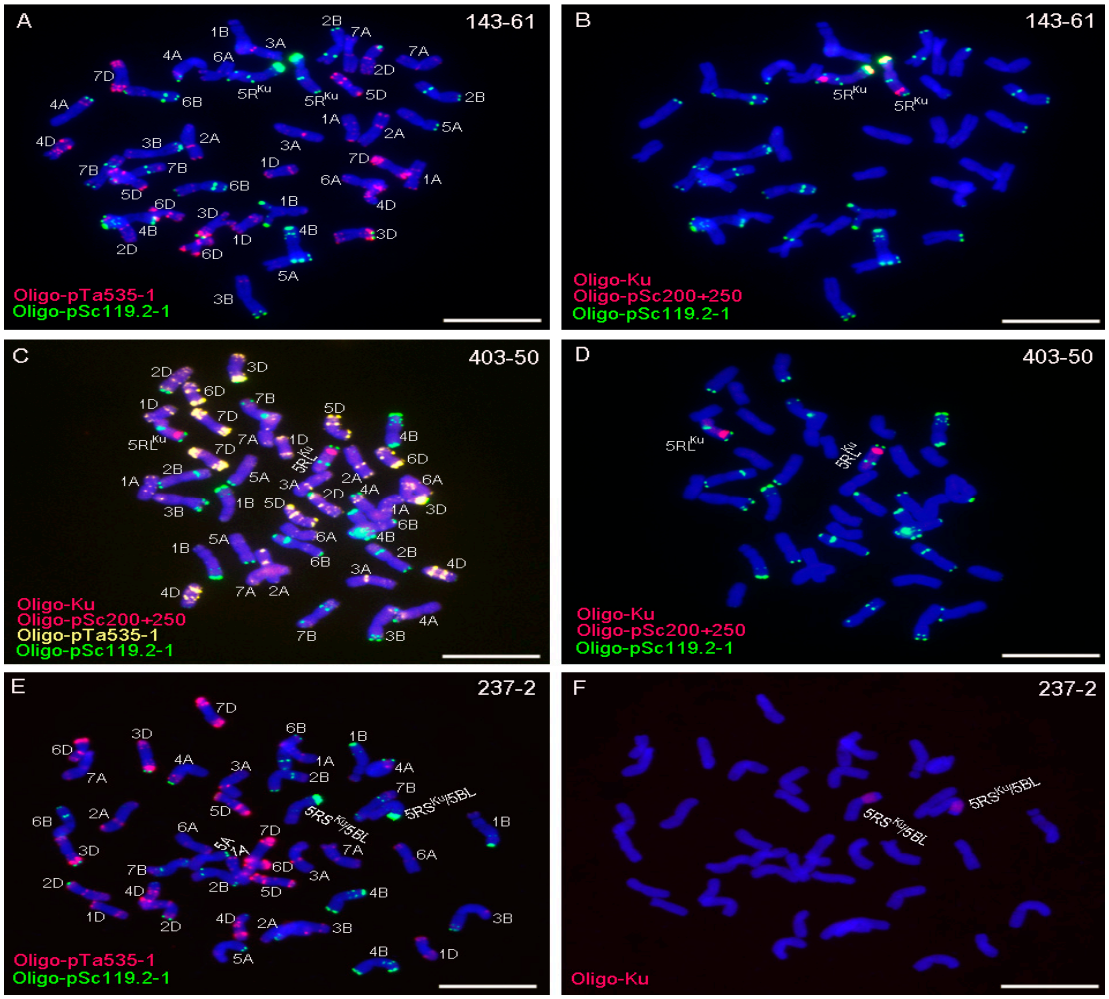

Figure 2. ND-FISH analysis of root tip metaphase chromosomes of lines 143-61, 403-50, and 237-2 using Oligo-pTa535-1, Oligo-pSc119.2-1, Oligo-Ku, and Oligo-pSc200 + 250 as probes. (**A**) and (**B**) are the same cell, (**C**) and (**D**) are the same cell, and (**E**) and (**F**) are the same cell. Chromosomes were counterstained with DAPI (blue). Scale bar is 10 μm.

Table 1. Information of the materials used in this study.

Name	Chromosome Constitution
142-30	one broken $5R^{Ku}$, 39 intact wheat chromosomes and one 7BL arm
142-77	one $5RS^{Ku}$ arm, 40 intact wheat chromosomes
143-9	two broken $5R^{Ku}$ chromosomes, 39 intact wheat chromosomes
143-61	two intact $5R^{Ku}$ chromosomes, 40 intact wheat chromosomes
237-2	two $5RS^{Ku}$/5BL translocation chromosomes, 40 intact wheat chromosomes
403-50	two $5RL^{Ku}$ arms, 40 intact wheat chromosomes
444-12	two broken $5R^{Ku}$ chromosomes, 42 intact wheat chromosomes
449-4	one broken $5R^{Ku}$ chromosome, 41 intact wheat chromosomes

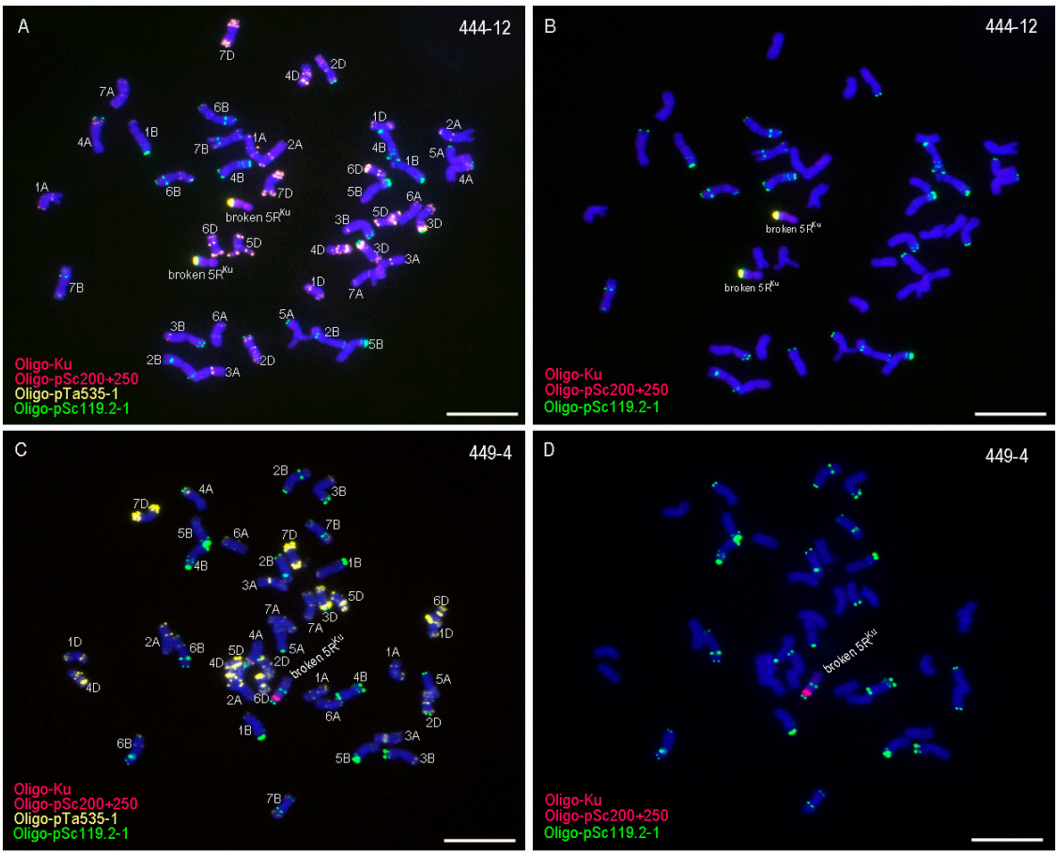

Figure 3. ND-FISH analysis of root tip metaphase chromosomes of lines 444-12 and 449-4 using Oligo-pTa535-1, Oligo-pSc119.2-1, Oligo-Ku, and Oligo-pSc200 + 250 as probes. (**A**) and (**B**) are the same cell, and (**C**) and (**D**) are the same cell. Chromosomes were counterstained with DAPI (blue). Scale bar is 10 μm.

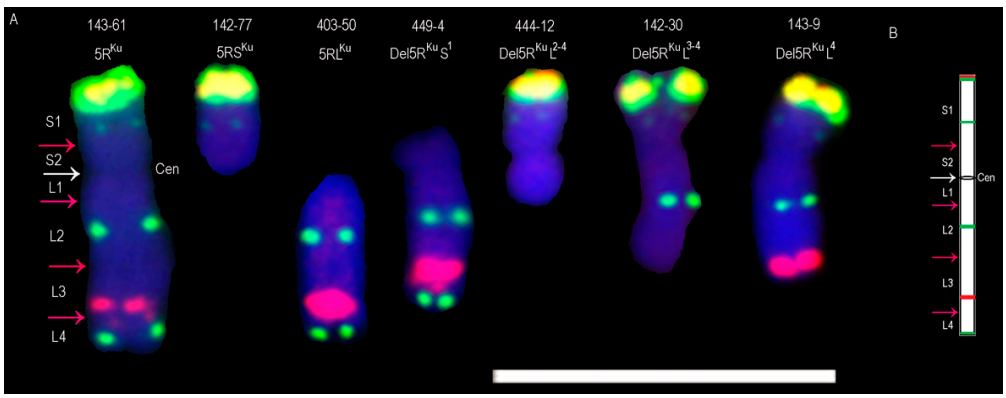

Figure 4. Cut-pasted 5R^{Ku} dissections and the schematic diagram of 5R^{Ku} chromosome. (**A**) Cut-pasted 5R^{Ku}, 5RS^{Ku}, 5RL^{Ku}, and the four kinds of broken 5R^{Ku}. 5RKu chromosome was divided into six regions. (**B**) The schematic diagram of 5R^{Ku} chromosome. Red arrows indicate broken points. White arrow and "Cen" indicate centromere. Scale bar is 10 μm.

3.2. Developing 5R^{Ku}-Specific Markers and Physical Mapping

Rye Kustro, CS, MY11, and the seven wheat-rye monosomic addition lines were used to develop 5R^{Ku}-specific markers. In total, 11 of the 542 primer pairs amplified specific bands from rye Kustro and MA5R^{Ku}, but not from CS, MY11, MA1R^{Ku}, MA2R^{Ku}, MA3R^{Ku}, MA4R^{Ku}, MA6R^{Ku}, and MA7R^{Ku} (Figure 5).

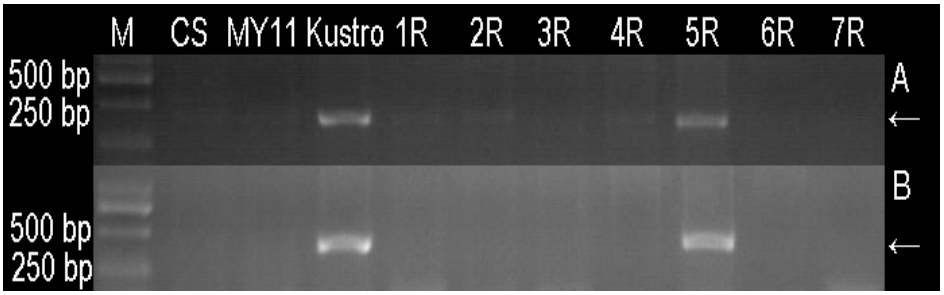

Figure 5. Developing $5R^{Ku}$-specific markers. (**A**) Products amplified by marker Ku5R-58. (**B**) Products amplified by marker Ku5R-516. M: DNA marker; CS: Chinese Spring; MY11: Mianyang 11; Kustro: rye kustro; 1R-7R: $MA1R^{Ku}$-$MA7R^{Ku}$. Arrows indicate the target bands.

Therefore, the 111 primer pairs were regarded as $5R^{Ku}$-specific markers and the information of these markers is listed in Table S1. Subsequently, the 111 markers and the 52 $5R^{Ku}$-specific markers developed by Qiu et al. [24] were physically mapped to six regions of the $5R^{Ku}$ chromosome using lines 142-30, 142-77, 143-9, 143-61, 237-2, 403-50, 444-12, and 449-4 (Figures 6 and 7; Table S2).

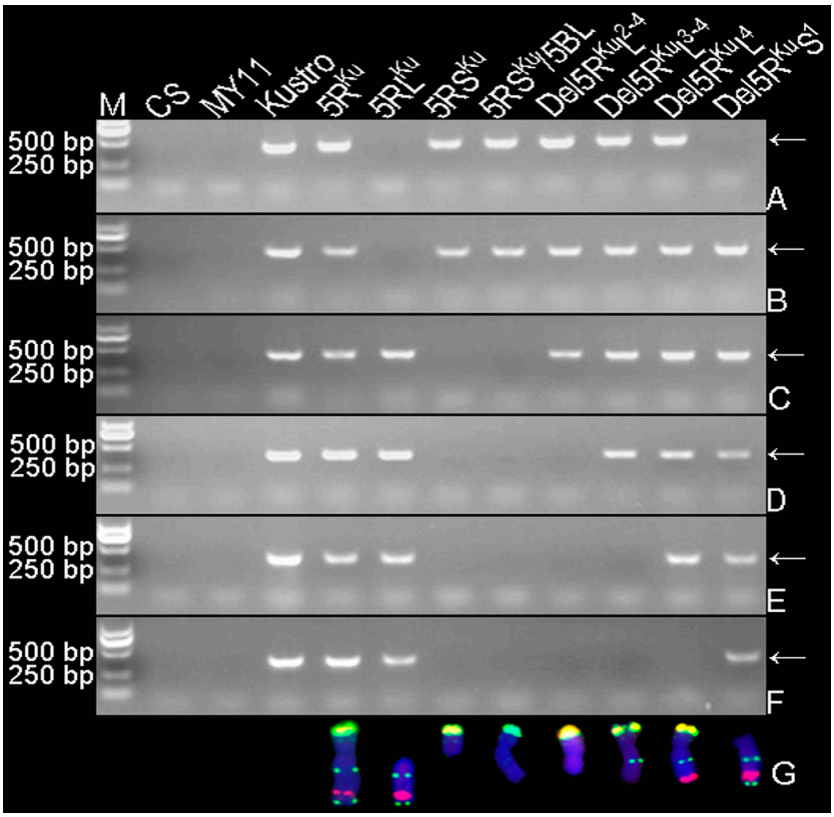

Figure 6. Physically localized $5R^{Ku}$-specific markers using $5R^{Ku}$ dissection lines. (**A**) Products amplified by primer pair Ku5R-290 representing the markers mapped to region S1. (**B**) Products amplified by primer pair Ku5R-271 representing the markers mapped to region S2. (**C**) Products amplified by primer pair Ku5R-120 representing the markers mapped to region L1. (**D**) Products amplified by primer pair Ku5R-342 representing the markers mapped to region L2. (**E**) Products amplified by primer pair Ku5R-48 representing the markers mapped to region L3. (**F**) Products amplified by primer pair Ku5R-9 representing the markers mapped to region L4. (**G**) Cut-pasted $5R^{Ku}$ chromosome, $5RS^{Ku}$ arm, $5RL^{Ku}$ arm, $5RS^{Ku}$/5BL translocation chromosome, and the four kinds of broken $5R^{Ku}$ chromosomes corresponding to their own amplified products in each electrophoresis lane. Arrows indicate the target bands amplified by each marker.

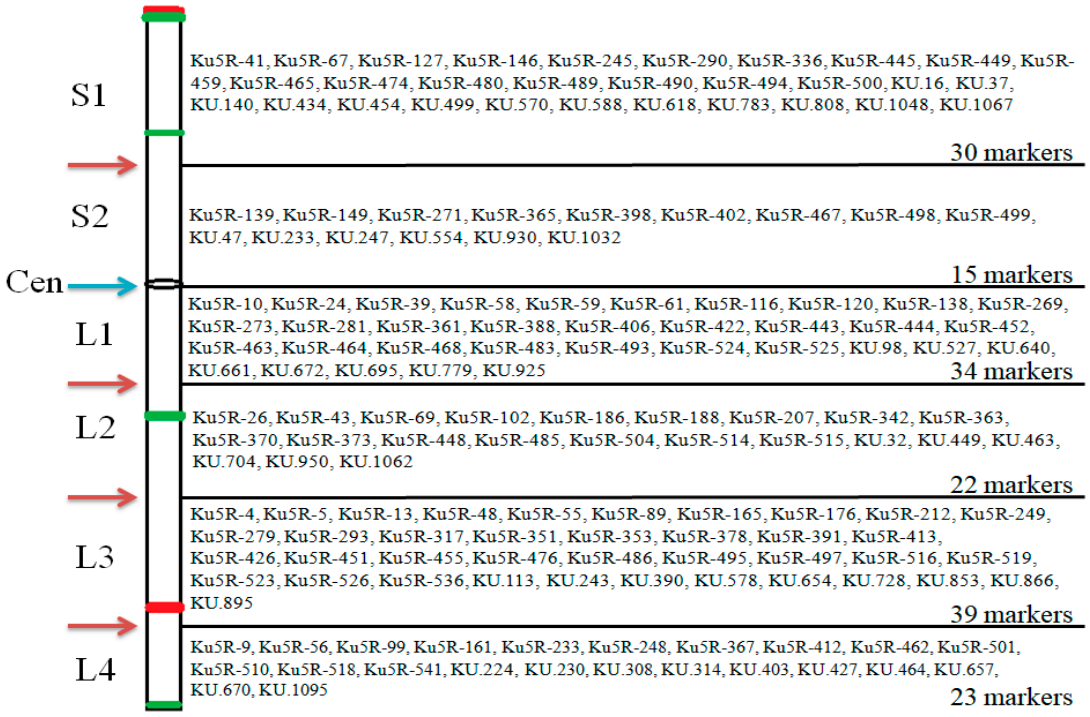

Figure 7. The schematic diagram for the physical map of $5R^{Ku}$-specific markers. These markers were mapped to six regions on $5R^{Ku}$ chromosome according to their amplicons in $5R^{Ku}$ dissection lines. The six regions are divided by five dark lines and named S1, S2, L1, L2, L3, and L4, respectively. The red arrows corresponding to each dark line indicate the breakpoints on the $5R^{Ku}$ chromosome. The blue arrow and "Cen" indicate the centromere. In each region, the names of the markers are listed on the right. In the schematic diagram, green bands represent FISH signals of Oligo-pSc119.2-1 and the red bands represent the FISH signals of Oligo-pSc200 + 250. The markers named "KU + number" were developed by Qiu et al. [24], and those named "Ku5R + number" were developed in this study.

Thirty markers amplified their target bands from lines 143-16 ($5R^{Ku}$), 142-77 ($5RS^{Ku}$), 237-2 ($5RS^{Ku}$/5BL), 444-12 ($Del5R^{Ku}L^{2-4}$), 142-30 ($Del5R^{Ku}L^{3-4}$), and 143-9 ($Del5R^{Ku}L^{4}$) but not from lines 403-50 ($5RL^{Ku}$) and 449-4 ($Del5R^{Ku}S^{1}$) (Figure 6A). So, the 30 markers were mapped to region S1 of the $5R^{Ku}$ chromosome (Figure 7). The target products of 15 markers only disappeared from line 403-50 ($5RL^{Ku}$); therefore, they were mapped to region S2 of the $5R^{Ku}$ chromosome (Figures 6B and 7). The target products of 34 markers only disappeared from lines 142-77 ($5RS^{Ku}$) and 237-2 ($5RS^{Ku}$/5BL), and they were mapped to region L1 of the $5R^{Ku}$ chromosome (Figures 6C and 7). The target products of 22 markers existed in lines 143-16 ($5R^{Ku}$), 403-50 ($5RL^{Ku}$), 142-30 ($Del5R^{Ku}L^{3-4}$), 143-9 ($Del5R^{Ku}L^{4}$), and 449-4 ($Del5R^{Ku}S^{1}$) but not in lines 142-77 ($5RS^{Ku}$), 237-2 ($5RS^{Ku}$/5BL), and 444-12 ($Del5R^{Ku}L^{2-4}$) (Figure 6D). Therefore, the 22 markers were mapped to region L2 of $5R^{Ku}$ chromosome (Figure 7). Thirty-nine markers did not amplify their target products from lines 142-77 ($5RS^{Ku}$), 237-2 ($5RS^{Ku}$/5BL), 444-12 ($Del5R^{Ku}L^{2-4}$), and 142-30 ($Del5R^{Ku}L^{3-4}$), and 23 markers did not amplify their target products from lines 142-77 ($5RS^{Ku}$), 237-2 ($5RS^{Ku}$/5BL), 444-12 ($Del5R^{Ku}L^{2-4}$), 142-30 ($Del5R^{Ku}L^{3-4}$), and 143-9 ($Del5R^{Ku}L^{4}$) (Figure 6E,F). Therefore, the 39 and 23 markers were mapped to regions L3 and L4 of $5R^{Ku}$ chromosome, respectively (Figure 7).

3.3. Similarity between S. cereale Lo7 Scaffolds and Pair-End Reads Used for $5R^{Ku}$-Specific Primers Design

The original pair-end reads that were used to design $5R^{Ku}$-specific primer pairs were deposited in the GenBank Database (GenBank accession numbers: MN325158-MN325268) (Table S1). The corresponding pair-end reads to $5R^{Ku}$-specific markers were used for nucleotide BLAST search against the *S. cereale* Lo7 scaffolds database using the blastn tool in GrainGenes [25] The *S. cereale* Lo7 scaffolds

served to validate the chromosomal localization of the majority of the $5R^{Ku}$-specific markers. The results were listed in Table S1. The 111 pair-end reads had 83% to 100% similarity with some of the *S. cereale* Lo7 scaffolds (Table S1). The left and the right 1 to 60 bp nucleotide sequences of 13 pair-end reads, respectively, hit two Lo7 scaffolds that were derived from different rye chromosomes (Table S1). Twenty-two pair-end reads only hit the Lo7 scaffolds that derived from the 0R chromosome (Table S1). Four pair-end reads respectively hit the five Lo7 scaffolds that were derived from 1R, 3R, or 4R chromosomes (Table S1). The left and the right 1 to 60 bp nucleotide sequences of seven pair-end reads hit two different Lo7 scaffolds that were derived from 5R chromosome (Table S1). Sixty-five pair-end reads hit 65 single Lo7 scaffolds that were derived from the 5R chromosome, respectively (Table S1). Although there were 72 5R-derived Lo7 scaffolds matching the pair-end reads, only 65 Lo7 scaffolds were mapped to six regions of the $5R^{Ku}$ chromosome because they hit single scaffolds (Figure 8).

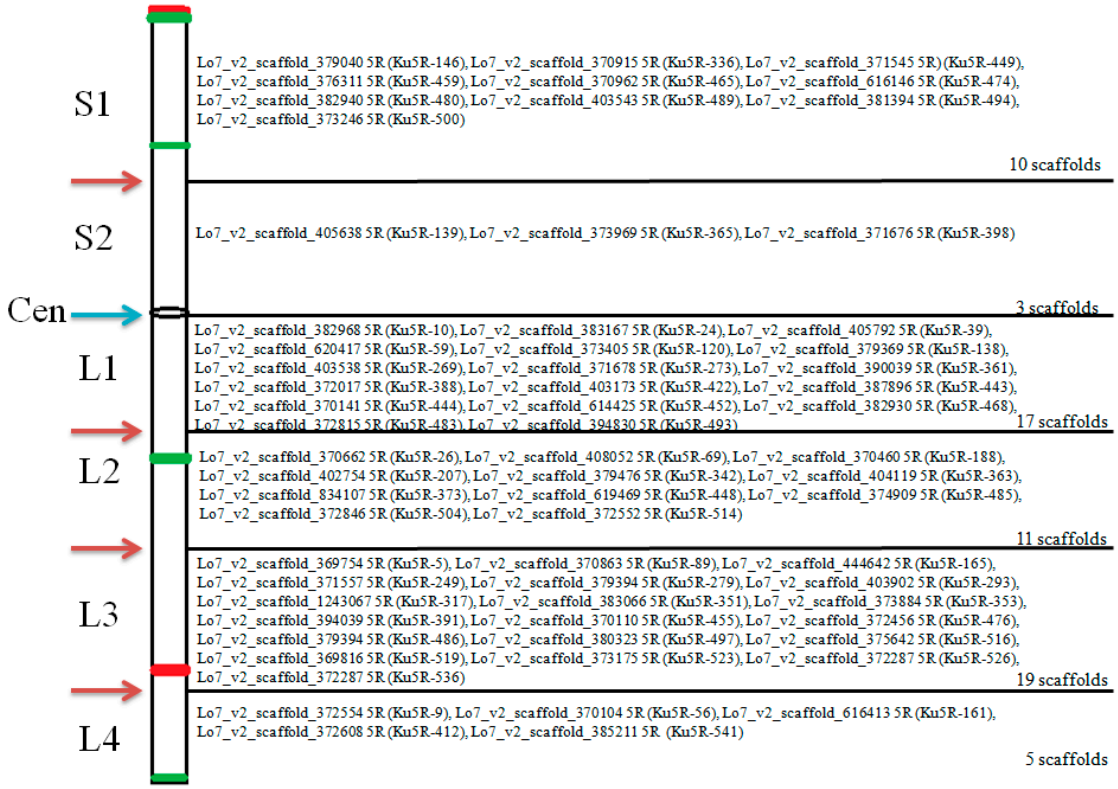

Figure 8. The schematic diagram for the physical map of *S. cereale* Lo7 scaffolds that were derived from the 5R chromosome. The names of the scaffolds are listed on the right and their corresponding markers are listed in brackets. The means of the other symbols are the same as the ones in Figure 7.

3.4. Location of Stripe Rust Gene(s) on $5R^{Ku}$ Chromosome

The resistance of lines 142-30, 142-77, 143-9, 143-61, 237-2, 403-50, 444-12, and 449-4 and parental wheat MY11 to stripe rust was tested. According to the standard scale of infection types that was described by Wan et al. [27], the infection types of lines 143-16 ($5R^{Ku}$), 403-50 ($5RL^{Ku}$), 142-30 ($Del5R^{Ku}L^{3-4}$), 143-9 ($Del5R^{Ku}L^{4}$), and 449-4 ($Del5R^{Ku}S^{1}$) were 2 to 3 (Figure 9). The infection types of MY11 and the lines 142-77 ($5RS^{Ku}$), 237-2 ($5RS^{Ku}$/5BL), and 444-12 ($Del5R^{Ku}L^{2-4}$) were 8 to 9 (Figure 9). These results indicated that the $5R^{Ku}$ chromosome carried stripe rust resistance gene(s) and it was located on the $5RL^{Ku}$ arm. Further, the resistance gene(s) was mapped to regions L2-L4 because line 444-12 ($Del5R^{Ku}L^{2-4}$) was highly susceptible to stripe rust (Figure 9). At last, the stripe rust resistance gene(s) could be mapped to region L2 of the $5R^{Ku}$ chromosome because lines 142-30 ($Del5R^{Ku}L^{3-4}$) and 143-9 ($Del5R^{Ku}L^{4}$) exhibited resistance to stripe rust (Figure 9).

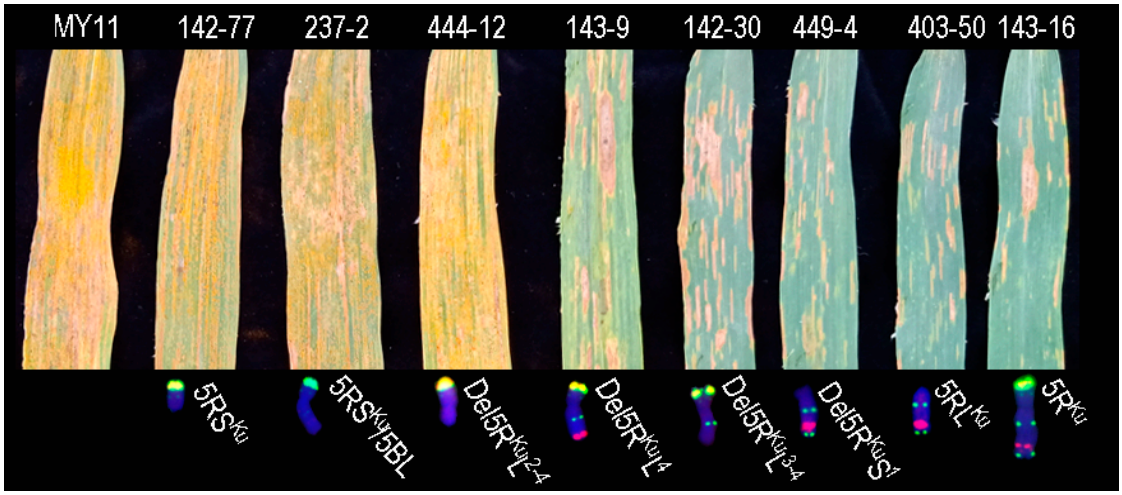

Figure 9. Stripe rust resistance testing. MY11, and lines 142-77, 237-2, and 444-12 are highly susceptible to stripe rust. Lines 143-9, 142-30, 449-4, 403-50, and 143-16 display resistance to stripe rust.

4. Discussion

4.1. 5R-Specific Markers

In our previous studies, some PCR-based and rye chromosome-specific markers have been developed using SLAF-seq technology [4,18,24]. Some of these markers have already been used to identify rye chromosomal segments in wheat backgrounds because they have easy application [5,6,28]. It has already been reported that rye 5R chromosomes carry some elite genes for wheat cultivar improvement [7–12]. Rye 5R-specific markers are contributive to identifying 5R chromatin in wheat backgrounds and help in the effective application of 5R elite genes in wheat breeding programs. So far, some PCR-based and 5R-specific markers have been reported. Two *S. cereale* inter-microsatellite (SCIM) markers were found to be 5R-specific [29]. Tomita et al. [30] reported a 5R-specific marker that was derived from the *Revolver* transposon-like sequence. A 5RS-specific and five 5RL-specific markers derived from expressed sequence tags (ESTs) were developed [31]. Sixteen PLUG markers that were developed using the PCR-based landmark unique gene system were proven to be 5R-specific [32]. Twenty-one $5R^{a}$-specific molecular markers were developed [12]. Qiu et al. [24] developed 19 $5RS^{Ku}$-specific and 33 $5RL^{Ku}$-specific markers using SLAF-seq technology. These markers mentioned above are easier to perform because they are PCR-based markers. In addition to distinguishing the 5R chromosome in wheat backgrounds, these markers can be used to construct a map of the 5R chromosome. However, more PCR-based and 5R-specific markers are needed. The 111 markers developed in this study have enriched the 5R-specific markers. In this study, a total of 163 $5R^{Ku}$-specific markers were mapped to six regions of the $5R^{Ku}$ chromosome, and this is beneficial for the identification of introgressed 5R small segments in wheat backgrounds. Additionally, the corresponding *S. cereale* Lo7 scaffolds [26] to 65 $5R^{Ku}$-specific markers were found and they were also mapped to the six regions of the $5R^{Ku}$ chromosome. This might be useful for building the physical map and the high-density genetic map of chromosome 5R. Furthermore, the *S. cereale* Lo7 scaffolds [26] mapped to the region L2 might help to further dissect the stripe resistance gene(s). Twenty-two of the 111 pair-end reads matched some scaffolds that have not yet been assigned to any rye chromosome (0R) and these pair-end reads contributed to mapping a reasonable number of *S. cereale* Lo7 scaffolds to the different regions of the 5R chromosome. The case that some $5R^{Ku}$-specific pair-end reads hit some Lo7 scaffolds derived from 1R, 3R, or 4R chromosomes might be the result from duplications, members of gene families, etc.

In addition, Silkova et al. reported some 5R deletion lines. However, their breakpoints were unclear, and these deletion lines were not used to map 5R-specific markers [17]. In this study, the signal patterns of probes Oligo-Ku, Oligo-pSc200, Oligo-pSc250, and Oligo-pSc119.2-1 on the rye

chromosomes are the same as the ones of the $5R^{Ku}$ chromosomes reported by Li et al. [18]. The rye chromosomes in this study were determined to be chromosome 5R, although the control was not used. Therefore, a new set of 5R dissection lines was developed in this study and the breakpoints on these broken 5R chromosomes were tentatively determined.

4.2. Stripe Rust Resistance Gene(s) on the 5RL Arm Might be New One

It has been reported that 1R chromosomes that were derived from various rye sources carried stripe rust resistance gene(s) [33–37]. Additionally, the 2RL and 5RS arms of *S. africanum* Stapf. [12,38], the 4R chromosome of rye cultivar German White [6], and the 6R chromosome of *S. cereanum* cv. Kriszta [39] also carry stripe rust resistance gene(s). In this study, the $5RL^{Ku}$ arm that was derived from rye Kustro also carried stripe rust resistance gene(s) and the resistance gene(s) was mapped to region L2 of the $5R^{Ku}$ chromosome. Therefore, the stripe rust resistance gene(s) might be new one(s) because of its different source and location from the previously reported ones. The stripe rust resistance gene(s) on the $5RL^{Ku}$ arm reported in this study enriches the resistance source of stripe rust for wheat breeding programs. Additionally, 22 markers and 11 *S. cereale* Lo7 scaffolds were located to the L2 region of the $5R^{Ku}$ chromosome, the same region where the stripe rust resistance gene(s) was mapped, and these markers and scaffolds can facilitate the utilization of the resistance gene(s).

4.3. Variations of Wheat Chromosomes

Compared with the standard signal patterns of probes Oligo-pSc119.2-1 and Oligo-pTa535-1 on the chromosomes of common wheat MY11 [20], it can be noted that the variations of wheat chromosomes occurred in lines 142-30, 143-9, and 449-4. This indicated that wheat-rye 5R addition lines or 5R(5B) substitution lines could cause changes of the wheat genome. However, only alterations of the 7B and 3D chromosomes in the three lines were observed. Therefore, the rules and mechanisms of the alterations of the wheat genome in 5R addition and 5R(5B) substitution lines are not clear, and more attention should be paid to this issue in future research.

5. Conclusions

In the present study, a new set of $5R^{Ku}$ dissection lines was identified, and 111 new PCR-based and $5R^{Ku}$-specific markers were developed. The 111 markers were combined with the 52 $5R^{Ku}$-specific markers reported previously, and 65 *S. cereale* Lo7 scaffolds were physically mapped to six regions of the $5R^{Ku}$ chromosome. Additionally, the $5RL^{Ku}$ arm carried stripe rust resistance gene(s) and it was mapped to region L2 of the $5R^{Ku}$ chromosome, the same region where 22 $5R^{Ku}$-specific markers and 11 *S. cereale* Lo7 scaffolds were mapped. The stripe rust resistance gene(s) located in the $5RL^{Ku}$ arm enriches the resistance source of stripe rust for wheat breeding programs, and the markers and the 11 *S. cereale* Lo7 scaffolds that were mapped to the L2 region of the $5R^{Ku}$ chromosome facilitates the utilization of the stripe rust resistance gene(s) in the improvement of wheat cultivars.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/9/9/498/s1, Table S1: The information of $5R^{Ku}$-specific markers. Table S2: Location of 5RKu-specific markers on six regions of $5R^{Ku}$ chromosome using $5R^{Ku}$ dissection lines.

Author Contributions: Conceived and designed the study, S.F. and Z.T.; performed the experiments, W.X. and J.L.; data analysis, W.X.; writing-original draft preparation, Z.T.; writing-review and editing, S.F.

Funding: This manuscript is provided by the National Key Research and Development Program of China (No. 2016YFD0102001) and National Natural Science Foundation of China (No. 31770373).

Acknowledgments: We are thankful to Yanling Duan, Ruiying Liao and Shuyao Tang at the College of Agronomy, Sichuan Agricultural University for their helpful work on nucleotide blast searching and data submission to NCBI.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Dundas, I.S.; Frappell, D.E.; Crack, D.M.; Fisher, J.M. Deletion mapping of a nematode resistance gene on rye chromosome 6R in wheat. *Crop Sci.* **2001**, *41*, 1771–1778. [CrossRef]
2. Yokosho, K.; Yamaji, N.; Ma, J.F. Isolation and characterisation of two MATE genes in rye. *Funct. Plant Biol.* **2010**, *37*, 296–303. [CrossRef]
3. Howell, T.; Hale, I.; Jankuloski, L.; Bonafede, M.; Gilbert, M.; Dubcovsky, J. Mapping a region within the 1RS.1BL translocation in common wheat affecting grain yield and canopy water status. *Theor. Appl. Genet.* **2014**, *127*, 2695–2709. [CrossRef] [PubMed]
4. Duan, Q.; Wang, Y.Y.; Qiu, L.; Ren, T.H.; Li, Z.; Fu, S.L.; Tang, Z.X. Physical location of new PCR-based markers and powdery mildew resistance gene(s) on rye (*Secale cereale* L.) chromosome 4 using 4R dissection lines. *Front. Plant Sci.* **2017**, *8*, 1716. [CrossRef] [PubMed]
5. Du, H.; Tang, Z.; Duan, Q.; Tang, S.; Fu, S. Using the $6RL^{Ku}$ minichromosome of rye (*Secale cereale* L.) to create wheat-rye $6D/6RL^{Ku}$ small segment translocation lines with powdery mildew resistance. *Int. J. Mol. Sci.* **2018**, *19*, 3933. [CrossRef] [PubMed]
6. An, D.; Ma, P.; Zheng, Q.; Fu, S.; Li, L.; Han, F.; Han, G.; Wang, J.; Xu, Y.; Jin, Y.; et al. Development and molecular cytogenetic identification of a new wheat-rye 4R chromosome disomic addition line with resistances to powdery mildew, stripe rust and sharp eyespot. *Theor. Appl. Genet.* **2019**, *132*, 257–272. [CrossRef] [PubMed]
7. Schlegel, R.; Werner, T.; Hülgenhof, E. Confirmation of a 4BL/5RL wheat-rye chromosome translocation line in the wheat cultivar 'Viking' showing high copper efficiency. *Plant Breed.* **1991**, *107*, 226–234. [CrossRef]
8. Sibikeev, S.N.; Sibikeeva, Y.E.; Krupnov, V.A. Transmission of 5R chromosomes via gametes and its influence on spring bread wheat somatic embryoidogenesis in vitro. *Russ. J. Genet.* **2005**, *41*, 1650–1655. [CrossRef]
9. Li, J.L.; Wang, X.P.; Zhong, L.; Xu, X.L. Study on homoeologous chromosome pairing and translocation induced by 5A/5R × 6A/6R wheat-rye substitution lines. *Acta Genet. Sin.* **2006**, *33*, 244–250. [CrossRef]
10. Chumanova, E.V.; Efremova, T.T.; Trubacheeva, N.V.; Arbuzova, V.S.; Rosseeva, L.P. Chromosome composition of wheat-rye lines and the influence of rye chromosomes on disease resistance and agronomic traits. *Russ. J. Genet.* **2014**, *50*, 1169–1178. [CrossRef]
11. Andersson, S.C.; Johansson, E.; Baum, M.; Rihawi, F.; Bouhssini, M.E.L. New resistance sources to Russian wheat aphid (*Diuraphis noxia*) in Swedish wheat substitution and translocation lines with rye (*Secale cereale*) and *Leymus mollis*. *Czech J. Genet. Plant Breed.* **2015**, *51*, 162–165. [CrossRef]
12. Li, G.; Gao, D.; La, S.; Wang, H.; Li, J.; He, W.; Yang, E.; Yang, Z. Characterization of wheat-*Secale africanum* chromosome $5R^a$ derivatives carrying *Secale specific* genes for grain hardness. *Planta* **2016**, *243*, 1203–1212. [CrossRef] [PubMed]
13. Braun, E.M.; Tsvetkova, N.; Rotter, B.; Siekmann, D.; Schwefel, K.; Krezdorn, N.; Plieske, J.; Winter, P.; Melz, G.; Voylokov, A.V.; et al. Gene expression profiling and fine mapping identifies a gibberellin 2-Oxidase gene co-segregating with the dominant dwarfing gene *Ddw1* in rye (*Secale cereale* L.). *Front. Plant Sci.* **2019**, *10*, 857. [CrossRef] [PubMed]
14. Konovalov, A.A.; Moiseeva, E.A.; Goncharov, N.P.; Kondratenko, E.Y. The order of the *bs*, *Skdh*, and *Aadh1* genes in chromosome 5R of rye *secale cereal* L. *Russ. J. Genet.* **2010**, *46*, 666–671. [CrossRef]
15. Lukaszewski, A.J.; Porter, D.R.; Baker, C.A.; Rybka, K.; Lapinski, B. Attempts to transfer Russion wheat aphid resistance from a rye chromosome in Russian triticales to wheat. *Crop Sci.* **2001**, *41*, 1743–1749. [CrossRef]
16. Lukaszewski, A.J. *Alien Introgression in Wheat*, 1st ed.; Molnár-Láng, M., Ceoloni, C., Doležel, J., Eds.; Springer: Cham, Switzerland, 2015; Chapter 7; pp. 163–189.
17. Silkova, O.G.; Leonova, I.N.; Krasilova, N.M.; Dubovets, N.I. Preferential elimination of chromosome 5R of rye in the progeny of 5R5D dimonosomics. *Russ. J. Genet.* **2011**, *47*, 942–950. [CrossRef]
18. Li, M.; Tang, Z.; Qiu, L.; Wang, Y.; Tang, S.; Fu, S. Identification and physical mapping of new PCR-Based markers specific for the long arm of rye (*Secale cereale* L.) chromosome 6. *J. Genet. Genom.* **2016**, *43*, 209–216. [CrossRef]

19. Xiao, Z.; Tang, S.; Qiu, L.; Tang, Z.; Fu, S. Oligonucleotides and ND-FISH displaying different arrangements of tandem repeats and identification of *Dasypyrum villosum* chromosomes in wheat backgrounds. *Molecules* **2017**, *22*, 973. [CrossRef]
20. Tang, Z.; Yang, Z.; Fu, S. Oligonucleotides replacing the roles of repetitive sequences pAs1, pSc119.2, pTa-535, pTa71, CCS1, and pAWRC.1 for FISH analysis. *J. Appl. Genet.* **2014**, *55*, 313–318. [CrossRef]
21. Fu, S.; Chen, L.; Wang, Y.; Li, M.; Yang, Z.; Qiu, L.; Yan, B.; Ren, Z.; Tang, Z. Oligonucleotide probes for ND-FISH analysis to identify rye and wheat chromosomes. *Sci. Rep.* **2015**, *5*, 10552. [CrossRef]
22. Han, F.; Lamb, J.C.; Birchler, J.A. High frequency of centromere inactivation resulting in stable dicentric chromosomes of maize. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 3238–3243. [CrossRef] [PubMed]
23. Untergasser, A.; Cutcutache, I.; Koressaar, T.; Ye, J.; Faircloth, B.C.; Remm, M.; Rozen, S.G. Primer3—New capabilities and interfaces. *Nucleic Acids Res.* **2012**, *40*, e115. Available online: http://bioinfo.ut.ee/primer3 (accessed on 11 May 2016). [CrossRef] [PubMed]
24. Qiu, L.; Tang, Z.X.; Li, M.; Fu, S.L. Development of new PCR-based markers specific for chromosome arms of rye (*Secale cereale* L.). *Genome* **2016**, *59*, 159–165. [CrossRef]
25. Blake, V.C.; Woodhouse, M.R.; Lazo, G.R.; Odell, S.G.; Wight, C.P.; Tinker, N.A.; Wang, Y.; Gu, Y.Q.; Birkett, C.L.; Jannink, J.L.; et al. GrainGenes: Centralized Small Grain Resources and Digital Platform for Geneticists and Breeders. *Database (Oxford)* **2019**, *2019*, baz065. Available online: https://wheat.pw.usda.gov/cgi-bin/seqserve/blast_rye.cgi (accessed on 16 August 2019). [PubMed]
26. Bauer, E.; Schmutzer, T.; Barilar, I.; Mascher, M.; Gundlach, H.; Martis, M.M.; Twardziok, S.O.; Hackauf, B.; Gordillo, A.; Wilde, P.; et al. Towards a whole-genome sequence for rye (*Secale cereale* L.). *Plant J.* **2017**, *89*, 853–869. [CrossRef]
27. Wan, A.; Wang, X.; Kang, Z.; Chen, X. Variability of the Stripe Rust Pathogen. In *Stripe Rust*; Chen, X., Kang, Z., Eds.; Springer: Dordrecht, The Netherlands, 2017; Chapter 2, pp. 35–154.
28. Hao, M.; Luo, J.; Fan, C.; Yi, Y.; Zhang, L.; Yuan, Z.; Ning, S.; Zheng, Y.; Liu, D. Introgression of powdery mildew resistance gene Pm56 on rye chromosome arm 6RS into wheat. *Front. Plant Sci.* **2018**, *9*, 1040. [CrossRef]
29. Camacho, M.V.; Matos, M.; Gonzales, C.; Perez-Flores, V.; Pernauta, B.; Pinto-Carnida, O.; Benito, C. Secale cereale inter-microsatellites (SCIMs): Chromosomal location and genetic inheritance. *Genetica* **2005**, *123*, 303–311. [CrossRef]
30. Tomita, M.; Okutani, A.; Beiles, A.; Nevo, E. Genomic, RNA, and ecological divergences of the Revolver transposon-like multi-gene family in Triticeae. *BMC Evol. Biol.* **2011**, *11*, 269. [CrossRef]
31. Xu, H.; Yin, D.; Li, L.; Wang, Q.; Li, X.; Yang, X.; An, D. Development and application of EST-based markers specific for chromosome arms of rye (*Secale cereale* L.). *Cytogenet. Genome Res.* **2012**, *136*, 220–228. [CrossRef]
32. Li, J.; Endo, T.R.; Saito, M.; Ishikawa, G.; Nakamura, T.; Nasuda, S. Homoeologous relationship of rye chromosome arms as detected with wheat PLUG markers. *Chromosoma* **2013**, *122*, 555–564. [CrossRef]
33. Friebe, B.; Jiang, J.; Raupp, W.J.; McIntosh, R.A.; Gill, B.S. Characterization of wheat alien translocations conferring resistance to diseases and pests: Current status. *Euphytica* **1996**, *91*, 59–87. [CrossRef]
34. Ren, T.H.; Yang, Z.J.; Yan, B.J.; Zhang, H.Q.; Fu, S.L.; Ren, Z.L. Development and characterization of a new 1BL. 1RS translocation line with resistance to stripe rust and powdery mildew of wheat. *Euphytica* **2009**, *169*, 207–213. [CrossRef]
35. Fu, S.; Tang, Z.; Ren, Z.; Zhang, H. Transfer to wheat (*Triticum aestivum*) of small chromosome segments from rye (*Secale cereale*) carrying disease resistance genes. *J. Appl. Genet.* **2010**, *51*, 115–121. [CrossRef] [PubMed]
36. Lei, M.P.; Li, G.R.; Liu, C.; Yang, Z.J. Characterization of wheat-Secale africanum introgression lines reveals evolutionary aspects of chromosome 1R in rye. *Genome* **2012**, *55*, 765–774. [CrossRef] [PubMed]
37. Li, Z.; Ren, Z.; Tan, F.; Tang, Z.; Fu, S.; Yan, B.; Ren, T. Molecular cytogenetic characterization of New Wheat-Rye 1R(1B) Substitution and Translocation Lines from a Chinese *Secale cereal* L. Aigan with resistance to stripe rust. *PLoS ONE* **2016**, *11*, e0163642. [CrossRef] [PubMed]

38. Lei, M.P.; Li, G.R.; Zhou, L.; Li, C.H.; Liu, C.; Yang, Z.J. Identification of wheat-*Secale africanum* chromosome 2Rafr introgression lines with novel disease resistance and agronomic characteristics. *Euphytica* **2013**, *194*, 197–205. [CrossRef]
39. Schneider, A.; Rakszegi, M.; Molnár-Láng, M.; Szakács, É. Production and cytomolecular identification of new wheat-perennial rye (*Secale cereanum*) disomic addition lines with yellow rust resistance (6R) and increased arabinoxylan and protein content (1R, 4R, 6R). *Theor. Appl. Genet.* **2016**, *129*, 1045–1059. [CrossRef]