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

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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, multispiklet, 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*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 5RKu chromosome derived from rye Kustro (S. cereale L.) was proven to carry stripe rust resistance gene(s), and some new PCR-based and 5RKu-specific markers were developed using specific length amplified fragment sequencing (SLAF-seq) technology. These new markers were mapped to six regions on 5RKu using 5RKu dissection lines in a wheat background. Additionally, the stripe rust resistance gene(s) was also physically located on a segment of 5RKu.

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. Kusto. Some progeny was obtained by controlled backcrossing of MK with MY11 followed by strict self-seeding [18]. Seven wheat-rye monosomic addition lines, including MA1RKu, MA2RKu, MA3RKu, MA4RKu, MA5RKu, MA6RKu, and MA7RKu, were developed according to the methods described by Li et al. [18]. From the self-seeded progeny of the monosomic addition line MA5RKu, a 5RKu(5B) disomic substitution line, 5RSKu(5B) monotelosomic substitution line, 5RKL(5B) ditelosomic substitution line, 5RSKu/5BL translocation line, and four 5R deletion lines were obtained. The progeny of MA5RKu comprised a set of 5RKu 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. Kusto 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 MA5RKu 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 5RKu specific pair-end reads were obtained according to the methods described by Duan et al. [4]. Primers were designed according to 542 randomly selected 5RKu 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\textsuperscript{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\textsuperscript{Ku} but not from CS, MY11, MA1R\textsuperscript{Ku}, MA2R\textsuperscript{Ku}, MA3R\textsuperscript{Ku}, MA4R\textsuperscript{Ku}, MA6R\textsuperscript{Ku}, and MA7R\textsuperscript{Ku} were regarded as 5R\textsuperscript{Ku}-specific markers. For each of the primer pairs, PCR reactions were repeated three times. These markers combined with the 52 5R\textsuperscript{Ku}-specific markers developed by Qiu et al. [24] were mapped to specific regions of the 5R\textsuperscript{Ku} chromosome using the 5R\textsuperscript{Ku} dissection lines. Again, PCR reactions of each 5R\textsuperscript{Ku}-specific marker were repeated three times.

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

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

2.6. Stripe Rust Resistance Testing

The resistance of 5R\textsuperscript{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\textsuperscript{Ku} Dissection Lines

From the self-seeded progeny of MA5R\textsuperscript{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\textsuperscript{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 5K\textsuperscript{R} 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\textsuperscript{Ku} arm (Figure 1A,B). Line 142-77 contained a 5RS\textsuperscript{Ku} arm, therefore, it was a 5RS\textsuperscript{Ku}(5B) monotelosomic substitution line (Figure 1C,D). Two broken 5K\textsuperscript{R} chromosomes existed in line 143-9, and the breakpoints were located between the Oligo-pSc200 + 250 signal sites and the telomeres of 5RL\textsuperscript{Ku} arms (Figure 1E,F). Lines 143-61, 403-50, and 237-2 were 5K\textsuperscript{R}(5B) disomic substitution, 5RL\textsuperscript{Ku}(5B) ditelosomic substitution, and 5RS\textsuperscript{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\textsuperscript{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\textsuperscript{Ku} chromosome was observed, and the breakpoint occurred on the 5RS\textsuperscript{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\textsuperscript{Ku} chromosome could be divided into six regions (Figure 4). The broken 5R\textsuperscript{Ku} chromosomes in lines 142-30, 143-9, 444-12, and 449-4 were named Del5R\textsuperscript{Ku}L\textsuperscript{3-4}, Del5R\textsuperscript{Ku}L\textsuperscript{4}, Del5R\textsuperscript{Ku}L\textsuperscript{2-4}, and Del5R\textsuperscript{Ku}S\textsuperscript{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\textsuperscript{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 \textsuperscript{+} 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\(^{\text{Ku}}\), 39 intact wheat chromosomes and one 7BL arm               |
| 142-77 | one 5R\(^{\text{Ku}}\) arm, 40 intact wheat chromosomes                                |
| 143-9  | two broken 5R\(^{\text{Ku}}\) chromosomes, 39 intact wheat chromosomes                |
| 143-61 | two intact 5R\(^{\text{Ku}}\) chromosomes, 40 intact wheat chromosomes               |
| 237-2  | two 5R\(^{\text{Ku}}\)/5BL translocation chromosomes, 40 intact wheat chromosomes     |
| 403-50 | two 5R\(^{\text{L}}\)\(^{\text{Ku}}\) arms, 40 intact wheat chromosomes               |
| 444-12 | two broken 5R\(^{\text{Ku}}\) chromosomes, 42 intact wheat chromosomes               |
| 449-4  | one broken 5R\(^{\text{Ku}}\) 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 \( \mu \)m.

Figure 4. Cut-pasted 5RKu dissections and the schematic diagram of 5RKu chromosome. (A) Cut-pasted 5RKu, 5RSKu, 5RLKu, and the four kinds of broken 5RKu. 5RKu chromosome was divided into six regions. (B) The schematic diagram of 5RKu chromosome. Red arrows indicate broken points. White arrow and “Cen” indicate centromere. Scale bar is 10 \( \mu \)m.

3.2. Developing 5RKu-Specific Markers and Physical Mapping

Rye Kustro, CS, MY11, and the seven wheat-rye monosomic addition lines were used to develop 5RKu-specific markers. In total, 11 of the 542 primer pairs amplified specific bands from rye Kustro and MA5RKu, but not from CS, MY11, MA1RKu, MA2RKu, MA3RKu, MA4RKu, MA6RKu, and MA7RKu (Figure 5).
and MA5RKu, but not from CS, MY11, MA1R Ku, MA2RKu, MA3RKu, MA4RKu, MA6RKu, and MA7RKu (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.
Therefore, the 22 markers were mapped to region L2 of 5RKu chromosome (Figure 7). Thirty-nine markers did not amplify their target products from lines 142-77 (5RS Ku). The target products of 34 markers only disappeared from lines 142-77 (5RS Ku), 237-2 (5RS Ku/5BL), 444-12 (Del5R KuL2-4), 142-30 (Del5R KuL3-4), and 143-9 (Del5R KuL4) but not from lines 403-50 (5RL Ku) and 449-4 (Del5R KuS1) (Figure 6A). So, the 30 markers were mapped to region L1 of the 5RKu chromosome (Figures 6B and 7). The target products of 15 markers only disappeared from line 403-50 (5RL Ku) and 449-4 (Del5R KuS1) (Figure 6D). Therefore, the 39 and 23 markers were mapped to regions L3 and L4 of 5RKu chromosome, respectively (Figure 7).

3.3. Similarity between S. cereale Lo7 Scaffolds and Pair-End Reads Used for 5RKu-Specific Primers Design

Thirty markers amplified their target bands from lines 143-16 (5R Ku), 142-77 (5R Ku), 237-2 (5R Ku/5BL), 444-12 (Del5R KuL2-4), 142-30 (Del5R KuL3-4), and 143-9 (Del5R KuL4) but not from lines 403-50 (5RL Ku) and 449-4 (Del5R KuS1) (Figure 6A). So, the 30 markers were mapped to region S1 of the 5RKu 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 5RKu chromosome (Figures 6B and 7). The target products of 34 markers only disappeared from lines 142-77 (5R Ku) and 237-2 (5R Ku/5BL), and they were mapped to region L1 of the 5RKu chromosome (Figures 6C and 7). The target products of 22 markers existed in lines 143-16 (5R Ku), 403-50 (5R Ku), 142-30 (Del5R KuL3-4), 143-9 (Del5R KuL4), and 449-4 (Del5R KuS1) but not in lines 142-77 (5R Ku), 237-2 (5R Ku/5BL), and 444-12 (Del5R KuL2-4) (Figure 6D). Therefore, the 22 markers were mapped to region L2 of 5RKu chromosome (Figure 7). Thirty-nine markers did not amplify their target products from lines 142-77 (5R Ku), 237-2 (5R Ku/5BL), 444-12 (Del5R KuL2-4), 142-30 (Del5R KuL3-4), and 143-9 (Del5R KuL4) (Figure 6E,F). Therefore, the 39 and 23 markers were mapped to regions L3 and L4 of 5RKu chromosome, respectively (Figure 7).

3.3. Similarity between S. cereale Lo7 Scaffolds and Pair-End Reads Used for 5RKu-Specific Primers Design

The original pair-end reads that were used to design 5RKu-specific primer pairs were deposited in the GenBank Database (GenBank accession numbers: MN325158-MN325268) (Table S1). The corresponding pair-end reads to 5RKu-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\textsubscript{Ku}-specific markers. The results were listed in Table S1. The 111 pair-end reads had 83% to 100% similarity with some of the \textit{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\textsubscript{Ku} chromosome because they hit single scaffolds (Figure 8).

![Figure 8](image-url). The schematic diagram for the physical map of \textit{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\textsubscript{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\textsubscript{Ku}), 403-50 (5RL\textsubscript{Ku}), 142-30 (Del5R\textsubscript{Ku}L\textsuperscript{3-4}), 143-9 (Del5R\textsubscript{Ku}L\textsuperscript{1}), and 449-4 (Del5R\textsubscript{Ku}S\textsubscript{1}) were 2 to 3 (Figure 9). The infection types of MY11 and the lines 142-77 (5RS\textsubscript{Ku}), 237-2 (5RS\textsubscript{Ku}/5BL), and 444-12 (Del5R\textsubscript{Ku}L\textsuperscript{2-4}) were 8 to 9 (Figure 9). These results indicated that the 5R\textsubscript{Ku} chromosome carried stripe rust resistance gene(s) and it was located on the 5RL\textsubscript{Ku} arm. Further, the resistance gene(s) was mapped to regions L2-L4 because line 444-12 (Del5R\textsubscript{Ku}L\textsuperscript{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\textsubscript{Ku} chromosome because lines 142-30 (Del5R\textsubscript{Ku}L\textsuperscript{3-4}) and 143-9 (Del5R\textsubscript{Ku}L\textsuperscript{1}) exhibited resistance to stripe rust (Figure 9).
was derived from the Revolver Twenty-one 5R
Twenty-one 5R
signal patterns of probes Oligo-Ku, Oligo-pSc200, Oligo-pSc250, and Oligo-pSc119.2-1 on the rye
unclear, and these deletion lines were not used to map 5R-specific markers [17]. In this study, the
from 1R, 3R, or 4R chromosomes might be the result from duplications, members of gene families, etc.
The case that some 5R S. cereale reads contributed to mapping a reasonable number of
matched some sca L2 might help to further dissect the stripe resistance gene(s). Twenty-two of the 111 pair-end reads
S. cereale genetic map of chromosome 5R. Furthermore, the
of the 5R 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 5RKu-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

4. Discussion
4.1. 5R-SpecificMarkers

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 5RS-specific molecular markers were developed [12]. Qiu et al. [24] developed 19
5RSKu-specific and 33 5RLKu-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 5RKu-specific
markers were mapped to six regions of the 5RKu 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 5RKu-specific markers were found and they were also mapped to the six regions
of the 5RKu chromosome. This might be useful for building the physical map and the high-density
inter-microsatellite
S. cereale
S. cereale
S. cereale
S. cereale
SLAF-seq
SLAF-seq
SLAF-seq
SLAF-seq
SLAF-seq
SLAF-seq
SLAF-seq
SLAF-seq
chromosomes are the same as the ones of the 5R\textsuperscript{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 \textit{S. africanum} Stapf. [12,38], the 4R chromosome of \textit{rye} cultivar German White [6], and the 6R chromosome of \textit{S. cereanum} cv. Krisza [39] also carry stripe rust resistance gene(s). In this study, the 5R\textsuperscript{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\textsuperscript{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\textsuperscript{Ku} arm reported in this study enriches the resistance source of stripe rust for wheat breeding programs. Additionally, 22 markers and 11 \textit{S. cereale} Lo7 scaffolds were located to the L2 region of the 5R\textsuperscript{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\textsuperscript{Ku} dissection lines was identified, and 111 new PCR-based and 5R\textsuperscript{Ku}-specific markers were developed. The 111 markers were combined with the 52 5R\textsuperscript{Ku}-specific markers reported previously, and 65 \textit{S. cereale} Lo7 scaffolds were physically mapped to six regions of the 5R\textsuperscript{Ku} chromosome. Additionally, the 5RL\textsuperscript{Ku} arm carried stripe rust resistance gene(s) and it was mapped to region L2 of the 5R\textsuperscript{Ku} chromosome, the same region where 22 5R\textsuperscript{Ku}-specific markers and 11 \textit{S. cereale} Lo7 scaffolds were mapped. The stripe rust resistance gene(s) located in the 5RL\textsuperscript{Ku} arm enriches the resistance source of stripe rust for wheat breeding programs, and the markers and the 11 \textit{S. cereale} Lo7 scaffolds that were mapped to the L2 region of the 5R\textsuperscript{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\textsuperscript{Ku}-specific markers. Table S2: Location of 5R\textsuperscript{Ku}-specific markers on six regions of 5R\textsuperscript{Ku} chromosome using 5R\textsuperscript{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.
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