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Broad-Spectrum Resistance to Leaf Rust in the Argentinean Wheat Cultivar "Klein Proteo" Is Controlled by *LrKP* Located on Chromosome 2BS

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Abstract: Wheat leaf rust, caused by *Puccinia triticina*, is a severe fungal disease threatening global wheat production. The rational application of genetic loci controlling wheat resistance to leaf rust in breeding practice is still the best choice for disease control. A previous study indicated that the Argentinean wheat cultivar "Klein Proteo" might carry leaf rust resistance (*Lr*) genes *Lr3a* and *Lr10*, as well as an unknown *Lr* gene. In this study, seedlings of "Klein Proteo" showed high resistance to all the 20 *Pt* pathotypes isolated in China. Using bulked segregant RNA sequencing (BSR-seq) and developed CAPS markers, the single-dominant gene *LrKP* was initially mapped to a 114–168 Mb region on chromosome 2BS. Using gene-specific primers of a previously cloned chromosome 2BS-located *Lr13* gene, we found that "Klein Proteo" also carried the *Lr13* gene. Moreover, the expression of *Lr13* in the resistant bulk was significantly higher than that in the susceptible bulk. Nevertheless, "Klein Proteo" showed a much broader and higher resistance compared with the near isogenic line and "ZhouMai 22" carrying *Lr13*. In conclusion, the wheat cultivar "Klein Proteo" showed great potential in the genetic improvement of wheat resistance to leaf rust in China and the genetic bases controlling the broad-spectrum resistance were initially revealed.

Keywords: wheat; leaf rust; resistance; BSR-seq; mapping

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1. Introduction

Leaf rust, caused by *Puccinia triticina* (*Pt*), is a severe fungal disease on common wheat (*Triticum aestivum* L.). Worldwide, it occurs more frequently than other rust diseases [1]. Under favorable conditions, the yield loss caused by leaf rust can reach as high as 40% [2]. Considering the trends of global warming during the last decade, leaf rust has expanded its epidermic area to almost all the wheat-planting regions in China [3]. The rational utilization of resistant germplasms and genetic loci in breeding practice is still the most efficient way of controlling the disease.

Generally, there are two types of resistance to leaf rust: race-specific resistance and non-race-specific resistance [4]. Race-specific resistance, following the gene-for-gene theory, provides a high resistance to specific *Pt* pathotypes throughout the seedling and adult plant stages. Most of the cloned wheat leaf rust resistance (*Lr*) genes controlling race-specific resistance encode nucleotide-binding site-leucin-rich repeat (NBS-LRR) proteins, including *Lr1*, *Lr10*, *Lr13*, *Lr21*, *Lr22a*, and *Lr42* [5–9]. As an exception, the cloned race-specific resistance *Lr14a* gene encodes a membrane-localized protein with 12 ankyrin (ANK) repeats and a Ca^{2+}-permeable non-selective cation channels-like domain [10]. The non-race-specific resistance is normally functioning at adult plant stage of wheat, also referred

as adult plant resistance (APR) or slow rusting. Currently, only two APR genes for leaf rust have been cloned, including *Lr34* encoding an adenosine triphosphate-binding cassette (ABC) transporter and *Lr67* encoding a hexose transporter [11,12].

Wheat chromosome 2BS is a hot zone carrying six formally designated *Lr* genes, including *Lr13* [8,13], *Lr16* [14], *Lr23* [15], *Lr35* [16], *Lr48* [17], and *Lr73* [18]. "Klein Proteo" (KP) is an Argentinean bread wheat cultivar. In a previous investigation, KP showed a high resistance toward most of the collected Argentinean leaf rust pathotypes (R:S = 14:3) at the seedling stage. Further gene postulation indicated that KP might carry *Lr3a*, *Lr10*, and an unknown *Lr* gene [19]. APR to wheat stripe rust was investigated in the recombinant inbred lines (RILs) developed from the cross between the "Klein Proteo" and another Argentinean cultivar "Klein Chajá". A stripe rust resistance quantitative trait locus (QTL) on chromosome 4DL (*QYr.ucw-4DL*) from "Klein Proteo" explained approximately 9.7% of phenotypic variation in infection type and severity. Using gene-specific SNPs, *QYr.ucw-4DL* was proved not to be *Lr67/Yr46*, also on chromosome 4DL [20]. This study aims to clarify the features and genetic bases of leaf rust resistance in "Klein Proteo".

2. Materials and Methods

2.1. Plant Materials and Leaf Rust Inoculation

As a previous gene postulation assay indicated that KP might carry *Lr3a* and *Lr10* [19], seedlings of KP, the susceptible control "ZhengZhou 5389" (ZZ5389), near isogenic lines (NILs) carrying *Lr3a*, *Lr3bg*, *Lr3ka*, and *Lr10* in the genetic background of "Thatcher", were grown in the greenhouse and inoculated with leaf rust. On the other hand, as *Lr13*, *Lr16*, and *Lr23* are seedling resistance genes located on chromosome 2BS, NILs carrying these *Lr* genes were also included in the inoculation test. Plants were spray-inoculated with urediospores of 20 *Pt* pathotypes (PHSS-1, PHSS-2, PHQS, FHSS, PHTS-1, KHSS, PHTS-2, TGTS, FHJQ, NHJS, FRJS, FGBS, PGJS, FHSS, THSP, SHJT, PHJS, FHGS, PHTT, and FHGQ) collected in China following a previously described method [21]. After inoculation, plants were grown in growth chambers at 20 °C during the day and 18 °C during the night with an 16 h light/8 h dark photoperiod. Disease symptoms were recorded at roughly 12 days post-inoculation (dpi) using a Cobb's scale [22]. The resistant parental line KP was crossed with the susceptible parental line ZZ5389, the latter of which has been widely used in the construction of genetic populations studying wheat leaf rust resistance [8,23,24]. Segregation populations with 125 $F_{2:3}$ lines and 114 $F_{3:4}$ lines from the cross KP × ZZ5389 were inoculated with *Pt* pathotypes PHQS and PHSS-1, respectively.

2.2. Bulked Segregant RNA-Seq (BSR-Seq)

Based on the leaf rust phenotypes of 125 $F_{2:3}$ lines, plants from 20 homozygous resistant lines and 20 homozygous susceptible lines were selected and combined as resistant (R)-bulk and susceptible (S)-bulk, respectively. RNAs were extracted using the Direct-zol™ RNA kits (Zymo Research Co., Ltd., Irvine, CA, USA). RNA-sequencing was conducted using Nova-PE150 sequencing strategy on an Illumina HiSeq 2000 platform by Novogene Co., Ltd. (Beijing, China). Raw sequencing data were deposited at the NCBI (https://www.ncbi.nlm.nih.gov, accessed on 29 October 2022) under the BioProject accession PRJNA895679. Raw reads were cleaned using Fastp software to trim off adapters and remove low-quality reads. Clean reads were aligned with the common wheat "Chinese Spring" reference genome RefSeq v1.0 [25] using STAR software [26]. Then, single nucleotide polymorphism (SNP) calling was performed on the confident read alignments using the "HaplotypeCaller" module in GATK software v3.7 [27]. QTLs were detected using the R package in QTLseqr software v0.6.4 as described by Mansfeld and Grumet [28]. A modified G statistic was calculated for each SNP based on the observed and expected allele depths, and further smoothed using a tricube smoothing kernel [29]. The G' value of each SNP was calculated with a smoothing window size of 100 Mb to identify genomic regions with G' peaks as possible QTLs/genes.

2.3. Development of Molecular Markers

Based on the BSR-seq results, SNPs closely linked to the target gene were selected to develop cleaved amplified polymorphic sequence (CAPS) markers. CAPS markers were designed following previously described method [30]. The NEBcutter v2.0 tool (http://www.labtools.us/nebcutter-v2-0/, accessed on 2 September 2022) was used to detect possible restriction sites at each SNP. Primer 3 website (https://bioinfo.ut.ee/primer3-0.4.0/primer3/, accessed on 2 September 2022) was used to design genome-specific primers to amplify sequences flanking the targeted SNPs. PCR products were sequenced to determine the existence of the SNPs. Corresponding restriction enzymes (New England BioLabs Inc., Ipswich, MA, USA) were used to digest the amplified PCR products.

2.4. PCR Validation of Lr Genes in KP

Molecular markers for leaf rust resistance genes *Lr3* (*Xmwg798*) [31], *Lr10* (*Fl.2245/Lr10-6/r2*) [32], and *Lr13* (*HBAU-Lr13*) [8] were used to detect these genes in KP (Table S1). The diagnostic marker for *Lr13* (*HBAU-Lr13*) was used to genotype the 114 $F_{3:4}$ lines inoculated with *Pt* pathotype PHSS-1. ZM22 and NILs carrying *Lr3*, *Lr10*, and *Lr13* were included as positive controls, whereas ZZ5389 as a negative control. The coding regions of the *Lr13* gene were amplified from genomic DNA of KP using primers *Lr13F1/R1* and *Lr13F5/R6* (Table S1) [13].

2.5. Gene Expression Analysis

BSR-seq data were employed to evaluate the expressions of genes in R-bulk and S-bulk. Counts of clean reads were calculated using Kallisto software [33]. Differentially expressed genes (DEGs) were identified using edgeR software [34] following the parameters of *p*-value < 0.05 and $|\log2\text{-foldchange}| > 1$. DEGs within the 114–168 Mb interval of chromosome 2B were analyzed and a heatmap was generated. Expressions of *LrKP*-interval DEGs were further estimated using transcripts per million (TPM) values collected from previous transcriptomic studies on the wheat leaf rust resistance genes *Lr47* and *Lr57* [35,36].

3. Results

3.1. Seedlings of "Klein Proteo" Showed High Resistance to Most of the Pt Pathotypes in China

Seedlings of KP, the susceptible control ZZ5389, *Lr3a*-NIL, *Lr3bg*-NIL, *Lr3ka*-NIL, *Lr10*-NIL, *Lr13*-NIL, *Lr16*-NIL, *Lr23*-NIL, and ZhouMai 22 (*LrZH22/Lr13*) were inoculated with 20 *Pt* pathotypes collected in China (Table 1). KP showed a high resistance to all the 20 *Pt* pathotypes. By contrast, ZZ5389, *Lr3a*-NIL, and *Lr10*-NIL were susceptible to most of the tested *Pt* pathotypes. As shown in Figure 1A, KP exhibited a typical hypersensitive response (HR) to a major *Pt* pathotype PHQS, whereas ZZ5389, *Lr3*-NILs, and *Lr10*-NIL were susceptible. To verify the presence of *Lr3a* and *Lr10* genes in KP as indicated in a previous study [19], molecular markers specific for these genes were used to amplify genomic DNA of KP, ZZ5389, *Lr3a*-NIL, *Lr3bg*-NIL, *Lr3ka*-NIL, and *Lr10*-NIL. PCR results showed that KP carried *Lr3bg* (Figure 1B) and *Lr10* (Figure 1C). All these data indicate that an unknown *Lr* gene in KP, but not *Lr3* nor *Lr10*, might be responsible for the broad-spectrum resistance to leaf rust.

3.2. BSR-seq Analysis on "KP × ZZ5389" $F_{2:3}$ Lines Revealed a Resistant Locus on Chromosome 2B

A genetic population was generated by crossing the resistant parental line KP with the susceptible parental line ZZ5389. A total of 125 $F_{2:3}$ lines were inoculated with *Pt* pathotype PHQS. For the phenotypes of $F_{2:3}$ lines, we identified 37 homozygous-resistant and 60 heterozygous- and 28 homozygous-susceptible lines ($\chi^2_{1:2:1} = 1.496$). Another population consisting of 114 $F_{3:4}$ lines from one selected segregating F_3 families of "KP × ZZ5389" were challenged with the *Pt* pathotype PHSS-1. In this population, there were 30 homozygous-resistant and 55 heterozygous- and 29 homozygous-susceptible lines, fitting a ratio of 1:2:1 in *Chi*-squared test ($\chi^2_{1:2:1} = 0.158$, Table S2). A further *Pt* inoculation test on 1054 individual plants from the progeny of 55 segregating $F_{3:4}$ families resulted

in 796 resistant plants and 258 susceptible ones, which also fits a 3:1 segregation ratio (χ^2 = 0.153). These results indicate that leaf rust resistance to the *Pt* pathotypes PHQS and PHSS-1 in KP was controlled by a single dominant gene, temporally designated as *LrKP*.

Table 1. Infection types of tested wheat materials to 20 collected *Pt* pathotypes in China.

***Pt* Pathotype**	**PHSS-1**	**PHSS-2**	**PHQS**	**FHSS**	**PHTS-1**	**KHSS**	**PHTS-2**	**TGTS**	**FHJQ**	**NHJS**
Klein Proteo	1	1	2	1	1	1+	1	1	1	;
Lr3a-NIL	4	4	3	4	3+	4	4	3+	2	3
Lr3bg-NIL	3	3	3	4	3+	4	4	3	1+	3
Lr3ka-NIL	3+	3+	3	3+	3	3+	4	2	2	1
Lr10-NIL	3	3+	3	3	3+	4	4	4	3	3
ZhengZhou 5389	4	4	3+	3+	4	3+	4	4	3+	3+
Lr13-NIL	3+	4	3+	4	3+	4	3	3+	3+	3
Lr16-NIL	3+	4	4	4	3	4	4	4	4	3
Lr23-NIL	3	3+	3	3+	3+	3+	4	4	4	3
ZhouMai 22 (*LrZH22/Lr13*)	3+	2	2	3	2	1	;	2	4	3
***Pt* Pathotype**	**FRJS**	**FGBS**	**PGJS**	**FHSS**	**THSP**	**SHJT**	**PHJS**	**FHGS**	**PHTT**	**FHGQ**
Klein Proteo	;	;	;	;	1	1	;	1	;	;
Lr3a-NIL	3+	3+	3	3+	2	3	3	3	3	3
Lr3bg-NIL	3	3+	3	3	2	3	3	3	3	3+
Lr3ka-NIL	2	2	3c	3	1	1	1	2	3	2
Lr10-NIL	3	3+	3	2	3	3+	3	3	3	3
ZhengZhou 5389	3+	3+	4	3+	3+	3+	3+	3+	3+	3+
Lr13-NIL	3	3	3	3	3	3	3	3	3	3
Lr16-NIL	3	3+	3	3+	3+	3	3	3+	3	3+
Lr23-NIL	3	3+	3	2	2	1	3	3	3	3
ZhouMai 22 (*LrZH22/Lr13*)	1	2	1	3	3	3	1	2	2	1

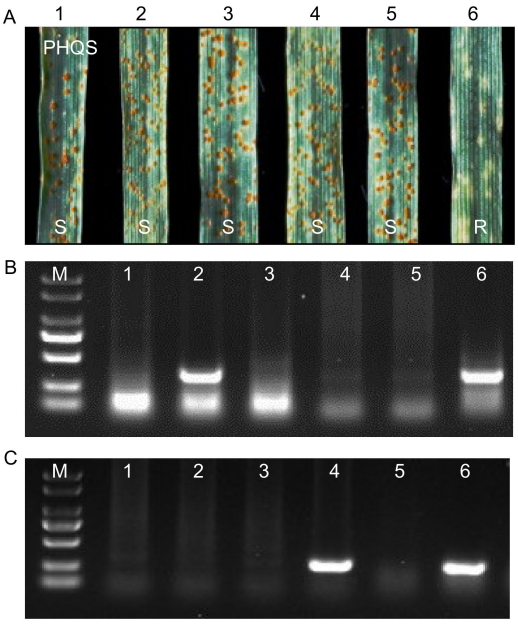

Figure 1. An unknown *Lr* gene in "Klein Proteo" conferred broad-spectrum resistance to leaf rust. (**A**) Phenotypes of wheat lines to *Pt* pathotype PHQS. R: resistance, S: susceptible. (**B**) PCR validation of *Lr3* in "Klein Proteo". (**C**) PCR validation of *Lr10* in "Klein Proteo". M: DNA marker DL2000, 1: *Lr3a*-NIL, 2: *Lr3bg*-NIL, 3: *Lr3ka*-NIL, 4: *Lr10*-NIL, 5: ZhengZhou 5389, 6: Klein Proteo.

To initially map the *LrKP* gene, RNA sequencing was applied on 20 selected homozygous resistant $F_{2:3}$ lines (R-bulk) and 20 homozygous susceptible $F_{2:3}$ lines (S-bulk). A BSR-seq analysis identified 5181 high-quality SNP variations between R-bulk and S-bulk. The G′ value for each SNP was calculated with a smoothing window size of 100 Mb and obvious G′ peaks were detected on chromosome 2B, indicating significant associations

between these SNPs and disease resistant phenotypes (Figure 2A). We speculated that a major G′ peak located in the 100–600 Mb physical interval on chromosome 2B was possibly the region carrying *LrKP*.

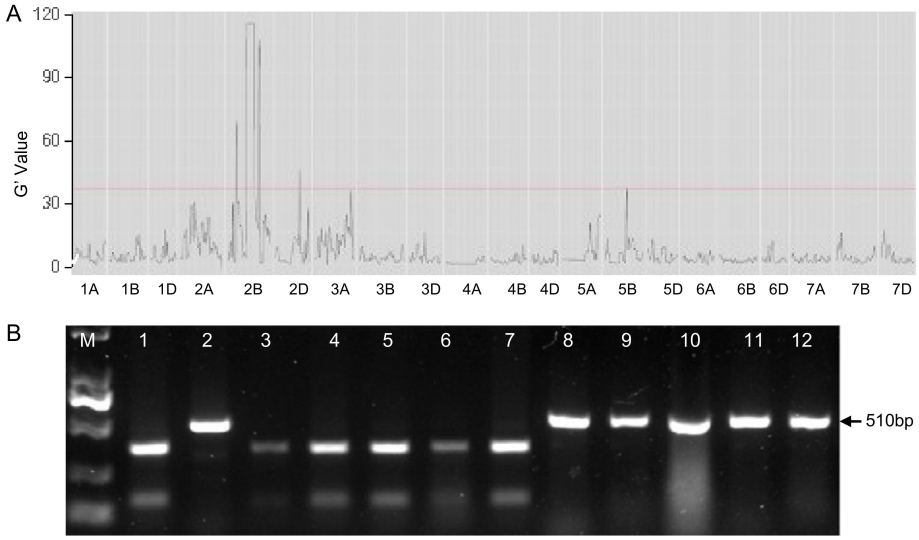

Figure 2. BSR-seq analysis revealed a leaf-rust-resistant locus on chromosome 2BS in "Klein Proteo". (**A**) Distribution of SNPs associated with leaf rust resistance was revealed by BSR-seq. (**B**) Validation of associated SNPs using the designed CAPS marker *LrkpF286R286*. M: DNA marker DL2000, 1: Klein Proteo, 2: ZhengZhou 5389, 3–7: homozygous-resistant lines, 8–12: homozygous-susceptible lines.

3.3. LrKP Was Mapped to a 114–168 Mb Region on Chromosome 2BS Using the Designed CAPS Markers

Based on the BSR-seq results, six CAPS markers on chromosome 2BS, including *Lrkp2B114* (located at 114 Mb), *LrkpF299R300* (168 Mb), *LrkpF286R286* (209 Mb), *Lrkp197F2R2* (210 Mb), *Lrkp2b01F4R4* (240 Mb), and *Lrkp260F1R1* (260 Mb), were designed to verify the linkage between genotype and phenotype (Table 2). The 114 $F_{3:4}$ lines were genotyped using these designed CAPS markers (Figure S1). As shown in Figure 2B, the CAPS marker *LrkpF286R286* showed good polymorphism among the parental and homozygous $F_{3:4}$ lines. A genetic map was constructed and the *LrKP* gene was initially mapped between the CAPS markers *Lrkp2B114* and *LrkpF299R300*. The genetic distance between these two flanking markers was approximately 4.8 cM (Figure 3A). Based on the common wheat "Chinese Spring" reference genome v1.0, the physical positions of these CAPS markers were collected to generate a physical map, in which the *LrKP* gene was delimited to the 114–168 Mb region on chromosome 2BS (Figure 3B).

Table 2. CAPS markers designed based on BSR-seq.

CAPS Markers	Primer Sequence (5′-3′)	SNP Location	Expected Size [a]	Annealing Temperature	Restriction Enzyme
Lrkp2B114	CAAACCCTCACCTTGGAAGC GCCCTGGAGGTTTTCACGA	114 Mb	1021 bp	58 °C	*Aat*II
LrkpF299R300	GTGCAGACGTTAGAGCGTAG TGATGTACATTTTTGTGGGGAAT	168 Mb	337 bp	55 °C	*Taq*I
LrkpF286R286	ATCCGCAGCAAGCACATAC ACACAAAGAGATTAGGGCGTGT	209 Mb	510 bp	55 °C	*Mbo*II
Lrkp197F2R2	AACTTCATGTACGCCCTGT GTTGGTCACCTAAACTGCC	210 Mb	921 bp	52 °C	*Nde*I
Lrkp2b01F4R4	ATGAACCCTCTCTGTGTTTGAGC TACTGTGCCGCAGTGTCGC	240 Mb	760 bp	60 °C	*Msp*I
Lrkp260F1R1	GACACCTTAGCAGCTCCCTA ATGTTTGCTACTTCTTCCGTC	260 Mb	679 bp	55 °C	*Hind*III

[a] The expected PCR product size before digestion.

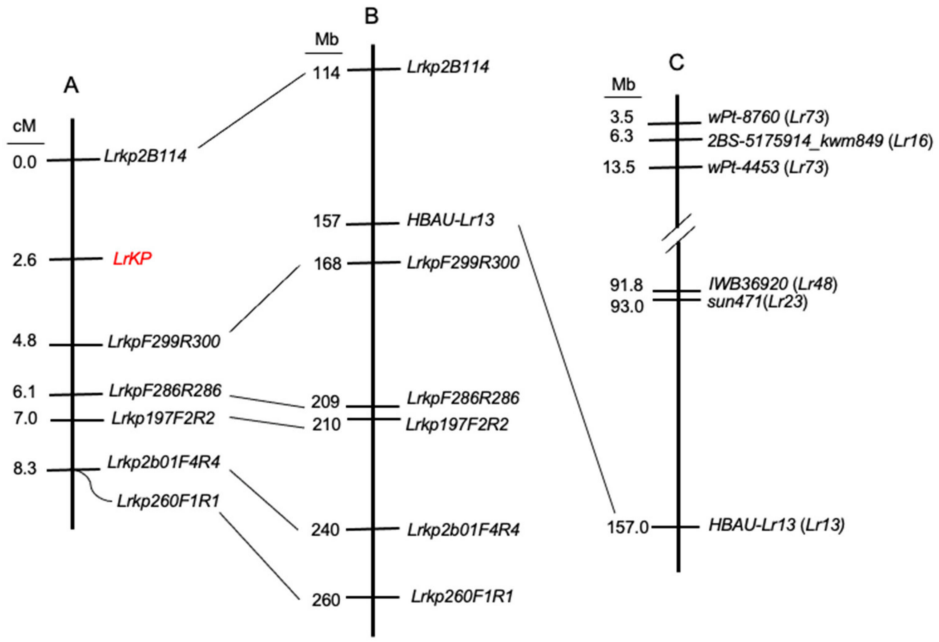

Figure 3. Genetic and physical maps of *LrKP*. (**A**) Genetic map of *LrKP* was generated based on linkage between phenotypes of 114 $F_{3:4}$ lines and genotypes using six developed CAPS markers. The *LrKP* gene was mapped between two flanking CAPS markers *Lrkp2B114* and *LrkpF299R300* on chromosome 2BS. (**B**) Physical positions of CAPS markers were collected from the "Chinese Spring" reference genome v1.0. The *LrKP* gene was mapped to the 114–168 Mb region on chromosome 2BS. (**C**) Physical positions of known *Lr* genes located on chromosome 2BS were estimated based on locations of their co-segregated or flanking markers in the "Chinese Spring" reference genome v1.0.

3.4. The Previously Cloned Lr13 Gene Located in the LrKP Interval

A total of six *Lr* genes, including *Lr13*, *Lr16*, *Lr23*, *Lr35*, *Lr48*, and *Lr73*, have been previously mapped to chromosome 2BS (Table S3). NILs carrying *Lr13*, *Lr16*, and *Lr23*, as well as ZhouMai 22 (ZM22) carrying *Lr13*, were also inoculated with those 20 *Pt* pathotypes (Table 1). Compared with *Lr13*-NIL, *Lr16*-NIL, *Lr23*-NIL, and ZM22, KP showed a much broader resistance to all the tested *Pt* pathotypes. Meanwhile, chromosome distributions of *Lr13*, *Lr16*, *Lr23*, *Lr48*, and *Lr73* were profiled based on locations of their co-segregated or flanking markers in "Chinese Spring" reference genome v1.0 (Figure 3C). Only *Lr13* (157 Mb) was located within the *LrKP* interval (114–168 Mb).

The diagnostic marker for *Lr13* (*HBAU-Lr13*) was initially employed to verify the existence of this gene in KP. PCR and restriction enzyme digest results showed good polymorphism among the parental and selected $F_{3:4}$ lines (Figure 4). When genotyping the 114 $F_{3:4}$ lines with marker *HBAU-Lr13*, we found that this marker was completely linked to the *LrKP* region. The coding regions of the *Lr13* gene were partially amplified from genomic DNA of KP using the gene-specific primers *Lr13F1/R1* and *Lr13F5/R6* (Figure S2). PCR products were sequenced, and the deduced sequences were identical to the corresponding segments of the cloned *Lr13*.

3.5. Lr13 Was Identified as a Differentially Expressed Gene (DEG) in the LrKP Interval

DEGs between R-bulk and S-bulk were analyzed using clean reads from the BSR-seq assay. A total of 907 DEGs (p-value < 0.05 and $|\log2\text{-foldchange}| > 1$) were identified, including 189 up-regulated DEGs and 718 down-regulated DEGs. There were only 12 DEGs in the *LrKP* interval (114–168 Mb) on chromosome 2BS (Table S4). Among these 12 DEGs, only *TraesCS2B02G182800*, which was an orthologue of the cloned *Lr13* gene, encoded an NBS-LRR protein. The expression of *TraesCS2B02G182800* in R-bulk was significantly higher than that in S-bulk (Figure 5). We also profiled the expressions of all these 12 *LrKP*-interval DEGs in the *Lr47*- and *Lr57*-mediated resistance (Figure S3). Certain DEGs

were also differentially expressed upon leaf rust infection in association with *Lr47* or *Lr57*, except for the *Lr13* gene. All these results indicate that *Lr13* was specifically induced and might function in KP upon rust infection.

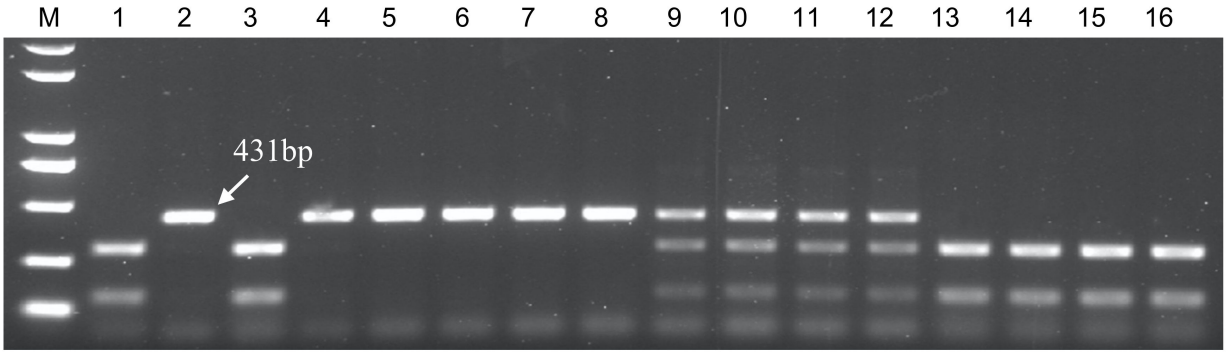

Figure 4. PCR validation of *Lr13* in "Klein Proteo". *Lr13*-gene-specific CAPS marker *HBAU-Lr13* was employed to detect *Lr13* in KP. M: DNA marker DL2000, 1: ZhouMai 22, 2: Chinese Spring, 3: Klein Proteo, 4: ZhengZhou 5389, 5–8: homozygous susceptible lines of "KP × ZZ5389" $F_{3:4}$ population; 9–12: heterozygous lines; 13–16: homozygous resistant lines.

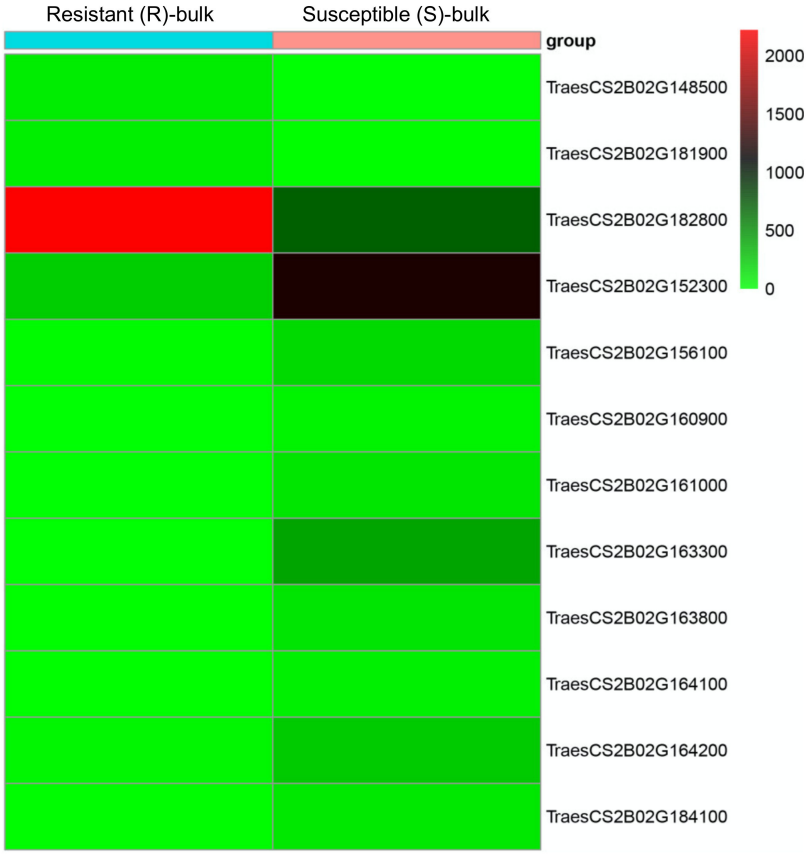

Figure 5. Expressions of DEGs located in the *LrKP* interval (114–168 Mb) on chromosome 2BS. DEGs between R-bulk and S-bulk were identified using clean reads from the BSR-seq assay. Only 12 DEGs located in the *LrKP* interval (114–168 Mb) on chromosome 2BS, including the cloned *Lr13* gene (*TraesCS2B02G182800*).

4. Discussion

In the seedling inoculation assay, KP conferred high resistance to all the 20 *Pt* pathotypes in China (Table 1). Vanzetti et al. reported that seedlings of KP showed high resistance

to 14 *Pt* pathotypes and susceptible to only three *Pt* pathotypes in Argentina [19]. All these data indicated that KP might be a valuable global germplasm for the genetic improvement of wheat resistance to leaf rust fungus. In addition, Vanzetti et al. also postulated the existence of *Lr3a*, *Lr10*, and an unknown *Lr* gene in KP.

The *Lr3* locus was located on chromosome 6BL, including three alleles as *Lr3a*, *Lr3bg*, and *Lr3ka* [37]. Since *Lr3* has not been cloned yet, it is a great challenge to distinguish different alleles of *Lr3*. The fragment length polymorphism (RFLP) marker *Xmwg798* was co-segregated with the *Lr3a* allele in the common wheat cultivar "Sinvalocho MA" [38]. Künzel et al. then converted this RFLP marker to a PCR-based STS marker with the same name of *Xmwg798* as an allele-specific one [39]. In this case, based on the amplification results in Figure 1B, we confirmed that KP was more likely to carry a *Lr3bg* allele, but not *Lr3a*. *Lr10* is a typical race-specific resistance gene encoding a nucleotide-binding site leucine-rich repeat (NBS-LRR) protein [5]. The gene-specific STS marker *Fl.2245/Lr10-6/r2* has been widely used for the identification of *Lr10* [32]. Based on the PCR validation result in Figure 1C, we proved that KP indeed carried *Lr10*. Nevertheless, NILs carrying *Lr3a*, *Lr3bg*, *Lr3ka*, and *Lr10* partially or completely lost their resistance to the 20 inoculated *Pt* pathotypes (Table 1), indicating that the broad spectrum and high resistance in KP might be controlled by unknown *Lr* genes.

Using BSR-seq analysis, *LrKP* was initially mapped to 114–168 Mb region on chromosome 2BS (Figures 2 and 3). In previous investigations, a total of six *Lr* genes, including *Lr13* [8,13], *Lr16* [14], *Lr23* [15], *Lr35* [16], *Lr48* [17], and *Lr73* [18], have been mapped to chromosome 2BS (Figure 3 and Table S3). Among them, *Lr35* was an APR gene originally introgressed from *Triticum speltoides* [16]. Using the co-segregation marker *BCD260F1/35R2* for *Lr35*, we did not detect *Lr35* in KP. NILs carrying *Lr16* and *Lr23* have lost their resistance to most of the 20 tested *Pt* pathotypes (Table 1). Moreover, the chromosome locations of *Lr16*, *Lr23*, *Lr48*, and *Lr73* (3.5–93 Mb) were different from that of the mapped *LrKP* (114–168 Mb).

On the contrary, the other chromosome 2BS-located leaf rust resistance gene *Lr13* was detected and highly expressed in KP (Figures 4, 5 and S2). The cloned *Lr13* encoded a typical NBS-LRR protein [8,13]. *Lr13* was initially considered as an APR gene and the NIL carrying *Lr13* was susceptible to most of *Pt* pathotypes in China [40]. The *Lr13*-mediated seedling resistance seemed to be associated with moderately high temperature (25 °C), which was different from the *LrKP*-mediated resistance presented at normal temperature (18–20 °C). Moreover, this gene showed a different strength in resistance in different genetic background. For instance, the donor material for the gene cloning of *Lr13*, ZM22, showed a much broader and stronger resistance to various *Pt* pathotypes than *Lr13*-NIL (Table 1). Nevertheless, in the parallel leaf rust inoculation assay, KP showed an even more broad-spectrum resistance to all the *Pt* pathotypes than ZM22 (Table 1). This phenomenon might result from the altered resistance conferred by *Lr13* in the genetic background of KP with *Lr3bg* and *Lr10*. On the other hand, *LrKP* might be a novel broad-spectrum resistance gene on chromosome 2BS closely linked with *Lr13* that did not segregate with each other in our limited sample sizes of mapping populations.

5. Conclusions

In conclusion, the Argentinean wheat cultivar "Klein Proteo" showed a high resistance to 20 collected *Puccinia triticina* pathotypes in China, including the major pathotypes of PHTT, PHTS, and PHSS. A single-dominant *LrKP* gene, but not the detected *Lr3* or *Lr10*, was responsible for the broad-spectrum resistance to leaf rust. Using BSR-seq analysis, *LrKP* was initially mapped to 114–168 Mb region on chromosome 2BS. The *Lr13* gene was detected and highly expressed in "Klein Proteo", but this Argentinean wheat cultivar showed much broader and higher resistance than the near isogenic line and "ZhouMai 22" carrying *Lr13*. "Klein Proteo" has a great potential in the genetic improvement of wheat resistance to leaf rust in China. The designated *LrKP* and its relationship with *Lr13* remain to be explored.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12111836/s1, Figure S1: CAPS markers were developed based on BSR-seq analysis and employed to initially map the *LrKP* gene; Figure S2: PCR amplification of the coding regions of the *Lr13* gene in "Klein Proteo"; Figure S3: Expression profiles of *LrKP*-interval DEGs during *Lr47*- and *Lr57*-mediated wheat resistance to leaf rust; Table S1: Primers used for identification of *Lr* genes in the wheat cultivar "Klein Proteo"; Table S2: Segregation of seedling reactions to the *Pt* pathotype PHQS in "Klein Proteo", "ZhengZhou 5389", and their selected $F_{3:4}$ lines; Table S3: Information of designated *Lr* genes on chromosome 2BS; Table S4: Information of 12 DEGs in the 114–168 Mb interval on chromosome 2B.

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