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Zhang Jia-qi , Kang Zhan-hai , LI Xue , LI Man , Linmiao Xue , LI Xing *

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QTL Mapping of Adult Plant Resistance to Wheat Leaf Rust in the Xinong1163-4×Thatcher RIL Population

Jiaqi Zhang [1,2], Zhanhai Kang [2], Xue Li [1,2], Man Li [1,2], Linmiao Xue [1,2] and Xing Li [1,2,*]

[1] College of Plant Protection, Hebei Agricultural University, China

[2] Technological Innovation Center for Biological Control of Crop Disease and Insect Pests of Hebei Province, Baoding, 071001, China

* Correspondence: lixing@hebau.edu.cn; Tel.:13513220265

Abstract: Wheat leaf rust (*Lr*), caused by *Puccinia triticina Eriks*. (*Pt*), is one of the most important diseases affecting wheat production worldwide. Using resistant wheat cultivars is the most economic and environmentally friendly way to control leaf rust. The Chinese wheat cultivar Xinong1163-4 has shown good resistance to *Lr* in field trials. To identify the genetic basis of *Lr* resistance in Xinong1163-4, 195 recombinant inbred lines (RILs) from the Xinong1163-4/Thatcher cross were phenotyped for *Lr* severity in three environments: the 2017/2018, 2018/2019, 2019/2020 growing seasons in Baoding, Hebei Province,. Bulked segregant analysis and simple sequence repeat markers were then used to identify the quantitative trait loci (QTLs) for *Lr* adult plant resistance (APR) in the population. As a result, six QTLs were detected, designated as *QLr.hbau-1BL.1*, *QLr.hbau-1BL.2*, and *QLr.hbau-1BL.3* which were predicted to be novel. *QLr.hbau-4BL*, *QLr.hbau-4BL.1*, and *QLr.hbau-3A*, were identified at similar physical positions to previously reported QTLs. Based on chromosome positions and molecular marker testing, *QLr.hebau-1BL* and *QLr.hbau-1BL.3* share similar flanking markers with *Lr46*, respectively. *Lr46* is race-non-specific APR gene for leaf rust, stripe rust and powdery mildew. Similarly, *QLr.hebau-4BL* showed multiple disease resistance to leaf rust, stripe rust, Fusarium head blight and powdery mildew. The QTLs identified in this study, as well as their closely linked markers, can potentially be used for marker-assisted selection in wheat breeding.

Keywords: Wheat leaf rust; Adult plant resistance; QTL; Molecular mapping; SSR marker

1. Introduction

Puccinia triticina Eriks, the causal agent of wheat leaf rust, is responsible for significant global yield and economic losses in various regions including North America, South America, Africa, Europe, Asia, and Australia [1,2]. In China, the occurrence of wheat leaf rust is primarily common in the Yangtze River valley, as well as certain parts of wheat fields in the northeast, north, and southwest regions. In typical years, wheat yield losses caused by leaf rust range from 50%, amounting to an annual reduction of approximately 3 million tonnes [3]. Currently, although wheat leaf rust is not a prominent disease in China, it often coexists with wheat stripe rust within the same wheat fields, thus hindering the collection of accurate data regarding yield losses. Regardless, severe epidemics of wheat leaf rust were recorded in China during 1969, 1973, 1975, and 1979 [4], and localized outbreaks can still occur. For instance, in 2013, leaf rust was observed in Shandong, Henan, and certain regions of Xinjiang. Moreover, in 2015, Henan suffered from the worst wheat leaf rust outbreak, affecting 1.832 million hectares and resulting in an annual yield loss of 191,000 tonnes [5].

Resistance to wheat leaf rust can be divided into race-specific and race-non-specific resistance. Race-specific resistance, or all-stage resistance (ASR), is effective against pathogens but is easily overcome by new virulent pathotypes due to its genetic simplicity [6,7]. The high level of mutational and virulence diversity in *P. triticina* populations allows the fungus to rapidly overcome race-specific

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resistance [8]. In contrast, race-non-specific resistance, or adult plant resistance (APR), is quantitatively inherited and genetically more complex, generally conferred by the additive effects of multiple minor genes [9]. Wheat cultivars with APR often show a susceptible response at the seedling stage, but ultimately exhibit low disease severity at the adult plant stage by delaying infection, growth and reproduction of the pathogen [10].

Currently, 83 genes have been officially named, and more than 100 leaf rust resistance genes have been documented in wheat [11]. Most of these genes are race-specific resistance genes and have a relatively short duration of action. Studies have identified only eight slow-rusting APR genes that don't target specific races, and these include *Lr34* [12], *Lr46* [13], *Lr67* [14], *Lr68* [15], *Lr74* [16–18], *Lr75* [19], *Lr77* [20] and *Lr78* [18]. *Lr34* and *Lr67* are the only two successfully cloned and characterized APR genes [21–23]. Significant attention towards the APR genes has facilitated the exploration of novel *Lr* resistance QTLs, with over 240 being discovered in wheat and dispersed throughout all 21 chromosomes [24]. It is uncommon for an individual QTL to grant sufficient resistance, especially in the presence of elevated disease pressure. Multiple QTLs or genes, comprising of 4 or 5, are necessary to develop a significant level of rust resistance [25].

Simple sequence repeat (SSR) markers are a preferable option for QTL mapping of wheat leaf rust owing to their genome-wide coverage, codominant inheritance, considerable information content, feasible detection and reproducibility. Currently, single nucleotide polymorphism (SNP) arrays are increasingly utilized to map genes and conduct genome-wide association studies (GWAS). SNP arrays offer numerous markers closely associated with agronomic traits, making a significant contribution, but they are not cost-efficient for genotyping individuals in a large, temporary segregating population, like an F2. Competitive allele-specific PCR has become a widely accepted technology for performing SNP genotyping using bioscience KASP assays and serves as a global benchmark [26]. The Chinese Academy of Agricultural Sciences and Affymetrix developed the wheat 55K SNP array specifically for this purpose (http://bioservices.capitalbio.com/index.shtml).

The wheat variety Xinong1163-4 can be traced back to 84/79 and Xinong 1376. The specific gene(s) responsible for leaf-rust resistance in this Chinese wheat cultivar from Shanxi province which is highly resistant to leaf rust, stripe rust and powdery mildew of wheat, and has good agronomic traits, are not currently understood. So far, there is no report on the genetic patterns of resistance to leaf rust in Xinong1163-4. In the present study, we used the same Xinong1163-4×Thatcher RIL population, SSR markers, and the 55K SNP array genotyping platform to identify the genetic basis of *Lr* resistance in this cultivar. The study's findings offer a theoretical and practical framework for examining and using QTLs in cultivating crop varieties resistant to diseases.

2. Materials and Methods

2.1. Plant Materials and Pathogens

The plant materials used for mapping comprised of 195 RILs from the cross between Xinong1163-4 and Thatcher were developed by single-seed descent. Xinong1163-4 is a Chinese cultivar that is sensitive to *Pt* races at the seedling stage but highly resistant at the adult-plant stage. It has excellent agronomic properties and is resistant to leaf rust, stripe rust, powdery mildew and other diseases. In turn, Thatcher is an adapted high-yield cultivar that is highly susceptible to *Pt* races at the seedling and adult-plant stages. The sensitive cultivar, Zhengzhou 5389 was used as a susceptible control. Four *Pt* races (THTT, THTQ, THTS and PHPS) were used to test the RILs in the fields. The *Pt* races were from the Biological Control Center for Plant Diseases and Plant Pests of Hebei, Hebei Agricultural University.

2.2. Field Experiments

The RIL population and parents were grown at Baoding in Hebei Province in the 2017/2018, 2018/2019 and 2019/2020 cropping seasons for evaluation of leaf rust response (environments hereinafter referred to as 18BD, 19BD, and 20BD). The field trials were conducted in randomized

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complete blocks with two replicates. Each plot consisted of a single 1 m row with 50 cm between rows. Approximately 20 seeds were sown in each plot. Every tenth row was followed by rows of the highly susceptible line Zhengzhou 5389 as a control. Leaf rust epidemics were initiated by inoculating the spreader rows with a water suspension of urediniospores of equal amounts of *Pt* races (THTT, THTQ, THTS and PHPS) with a concentration 2 to 3 mg/mL added with a few drops of Tween 20 (0.03%) at the early jointing stage. Disease severity was recorded two or three times at weekly intervals with the first scoring at 4 weeks after inoculation. Disease severity data (0 to 100%) was recorded as percentage of leaf area covered with uredinia or necrotic stripes according to the modified Cobb scale [27] where 0% = immune, and 100% = fully susceptible. Final disease severity (MDS%) was used for QTL analysis. Statistical analysis: Significant differences in phenotype and maximum disease severity (MDS) among trials (2018BD, 2019BD, 2022BD) were identified using analysis of variance (ANOVA) in Microsoft Excel.

2.3. Molecular Genotyping

The CTAB method was used to extract genomic DNA from the leaves of each of the 195 RILs and the two parental lines [28]. DNA concentrations were determined using a Thermo Scientific NanoDrop 2000. DNA samples of the 10 RILs and parents were genotyped with the Affymetrix 55K SNP Array (53,064 markers) by CapitalBio Technology Company (Beijing, China). SSR markers were also used to genotype the entire population. Linkage maps were constructed using the MAP function in IciMapping 4.1 and algorithm of groups ordered using the Kosambi map function to obtain map distances from recombination frequencies, respectively. Linkage maps were graphically visualized with MapChart 2.3 [29].

2.4. Construction of Linkage Maps and QTL Analysis

Using the genotyping data from the hybrid mapping population, QTLs associated with MDS were identified using QTL IciMapping 3.2. to make a chain diagram. A logarithm of the odds (LOD) threshold of 2.5 was used to identify significant QTL with 1000 permutations at $p < 0.01$. Stepwise regressions were used to calculate the phenotypic variance explained (PVE,R2) by each QTL. The effect orientation of each QTL was determined [30], while the gene effect was determined by calculating the DR value (the ratio of the absolute value of the dominant effect and the additive effect). The identified flanking sequences of all SNP and SSR probes were searched against the Chinese Spring wheat reference sequence (IWGSC RefSeq v1.0, https://urgi.versailles.inra.fr/blast_iwgsc/blast.php) using BLAST to identify homologous sequences.

2.5. Wheat Primers and PCR Programs

SSR primer pairs were designed from the Chinese Spring wheat genome sequence (IWGSC, http://wheat-urgi.versailles.inra.fr/) through a BLAST search. The SSR primers were designed by Batch Primer5 software. The reaction program of SSR primers used for PCR amplification was 94 °C for 5 min, 94 °C for 1 min, and 55 °C (depending on the primers, the temperature was adjusted in the range of 50–60 °C, and 55 °C was mostly used for primary screening) annealing for 45 s., 72 °C for 1 min and 72 °C for 10 min. The denaturation to extension process was 35 cycles, and the amplified samples were stored at 4 °C after the reaction was terminated. csLV46G22 PCR programs: The sequence of CAPs labelled csLV46G22 was 5′-TCGACTTTGGAATGGGAGTTGC-3′ upstream and 5′-GGCGAAGATGCCATCATCCACCAG-3′ downstream. Same as SSR PCR programs. The amplified products were digested by *BsPEI* under the reaction conditions of 37 °C for 2 h and 80 °C for 30 min, and the digested products were used for electrophoretic detection.

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3. Results

3.1. Field Testing

The mean leaf rust MDS scores of Xinong1163-4 and Thatcher were 1 and 80%, respectively, and mean leaf rust severity scores on the RILs ranged from 24.23 to 35.19% across all environments (Figure 1 and Table 1). The distribution of mean leaf rust MDS frequencies for the population was continuous, indicating quantitative inheritance. Maximum disease severity scores for leaf rust across the three environments were significantly correlated with coefficients ranging from 0.50 to 0.63 ($p < 0.001$) (Table 2). ANOVA confirmed significant variation among the genotypes, environments, and genotype × environment for leaf rust.

Table 1. Summary of MDS in the Xinong1163-4 × Thatcher RIL population phenotyped for leaf rust.

Environment[a]	Parents		195 RILs				
	Xinong1163-4	Thatcher	Mean ± SD	Range	Variance coefficient (%)	Skewness	Kurtosis
2018 BD	1	80	24.23 ± 15.58	1–80	64.31	0.77	-0.04
2019 BD	1	80	32.38 ± 23.78	1–90	73.43	0.80	-0.29
2020 BD	1	80	35.14 ± 24.05	1–90	68.44	0.54	-0.92

[a] 2018 BD, 2019 BD, and 2020BD: the MDS in 2017/2018, 2018/2019, and 2019/2020 cropping seasons in Baoding, Hebei province, respectively.

Table 2. Pearson correlation coefficients (r) for two-way comparisons of leaf rust severity data from different environments.

Environment	R
2017–2018 BD	0.495**
2018–2019 BD	0.510**
2019–2020 BD	0.629**

18BD, 19BD, and 20BD the MDS for leaf rust in 2017/2018, 2018/2019, and 2019/2020 cropping seasons at Baoding in Hebei Province, respectively. **$p < 0.001$.

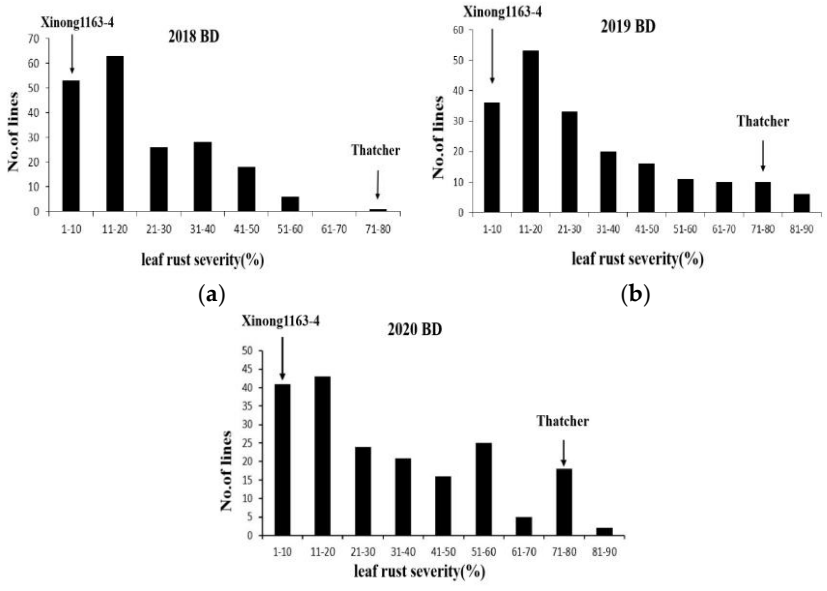

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Figure 1. All three graphs show the frequency distribution of leaf rust severity in the RIL lines of Xinong1163-4 × Thatcher, with the mean positions of Xinong1163-4 and Thatcher indicated by arrows. (**a**) In the 2018 BD graph, the number of lines of Xinong1163-4 is the highest in the 1–10% leaf rust severity range. (**b**) In the 2019 BD graph, the distribution of the number of lines in each range has changed, and the number of lines of Xinong1163-4 is still high in the 1–10% range. (**c**) In the 2020 BD graph, the number of lines of Xinong1163-4 is still prominent in the 1–10% leaf rust severity range.

3.2. Genetic Analysis of Markers and Genetic Linkage Mapping

A total of 382 SSR markers showed polymorphism between parents among 1062 SSR markers and were used to test the resistant and susceptible bulks. We found 68 primers with a recombination frequency of less than 30% in small populations, allowing genotyping of the entire population. Finally, the genotyping results of 14 markers were used to construct the linkage map for QTL detection using the IciMapping 4.1 software. Six linkage groups were constructed, and were distributed on the chromosomes 1BL, 3A and 4BL according to wheat consensus maps.

3.3. QTL Mapping

Six QTLs for leaf rust APR were identified on chromosomes 1B, 3A, and 4B (Figure 2 and Table 3). The *QLr.hbau-1BL.1* was detected in the 2019 and 2018,*QLr.hbau-1BL.2* was detected in the 2018 and 2020, and *QLr.hbau-1BL.3* was detected in the 2019 and 2020 season, and data experiments for all three traits explained 10.66%, 3.37–5.44% and 2.82–3.79% of the phenotypic variation for leaf rust, respectively (Figure 2 and Table 3). A major QTL for leaf rust resistance located on chromosome 1BL, was identified as *Lr46* based on the presence of marker *Xgwm728-Xgwm140*. The fourth QTL, *QLr.hbau-3A* was detected in the 2019 and 2020 experiments, with phenotypic variation explained (PVE) of 24.95–6.63%,for leaf rust, respectively. It was flanked by markers *Xwmc215-Xcfa2076*. A major QTL for leaf rust resistance, *QLr.hbau-4BL* was detected in all the three experiments, with a PVE of 8.13%, 5.31%, and 12.23% for leaf rust, respectively. It was flanked by markers *Xwmc657-Xgwm251*. Among the Six QTLs, the resistance were from Xinong1163-4 (Table 3).

Table 3. Quantitative trait loci for MDS to leaf rust and two potentially pleiotropic QTL by ICIM in the RIL population from Xinong1163-4×Thatcher.

Environment[b]	Chromosome	QTL[a]	Marker interval[c]	LOD[d]	PVE (%)[e]	Additive effect[f]	Dominate effect	DR
2018BD	1B	*QLr.hbau-1BL.1*	*Xgwm140-Xgpw1077*	4.20	10.66	-2.06	46.57	OD
2018BD	1B	*QLr.hbau-1BL.2*	*Xgpw1077-Xcfa2147.1*	2.82	3.37	-3.63	-5.92	OD
2018BD	4B	*QLr.hbau-4BL*	*Xwmc657-Xgwm251*	4.13	8.13	-6.23	5.06	D
2018BD	4B	*QLr.hbau-4BL.1*	*Xgwm193-Xwmc692*	3.46	12.18	-0.55	38.54	OD
2019BD	1B	*QLr.hbau-1BL.3*	*Xgwm728-Xgwm140*	6.85	21.88	-10.61	25.35	OD
2019BD	3A	*QLr.hbau-3A*	*Xwmc215-Xcfa2076*	9.57	24.95	-17.15	-3.83	PD
2019BD	4B	*QLr.hbau-4BL*	*Xwmc657-Xgwm251*	2.95	5.31	-5.60	-7.68	OD
2020BD	1B	*QLr.hbau-1BL.3*	*Xgwm728-Xgwm140*	2.68	6.16	-5.79	-9.43	OD
2020BD	1B	*QLr.hbau-1BL.2*	*Xgpw1077-Xcfa2147.1*	3.79	5.44	-7.68	-4.41	PD

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2020BD	3A	*QLr.hbau-3A*	*Xwmc215-Xcfa2076*	3.13	6.63	-7.94	-6.62	D
2020BD	4B	*QLr.hbau-4BL*	*Xwmc657-Xgwm251*	6.03	12.23	-10.36	-5.94	PD

[a] All of the resistance QTL in this table were contributed by Xinong1163-4; [b] 2018 BD, 2019 BD and 2020 BD: the MDS in 2017/2018, 2018/2019 and 2019/2020 cropping seasons in Baoding, Hebei province, respectively. [c]Peak position in centi-Morgans from the first linked marker of the relevant linkage; [d] Logarithm of odds (LOD) score; [e] Percentage of phenotypic variance explained by QTL; [F] Negative additive values indicate that relevant alleles were inherited from cultivar Xinong1163-4.

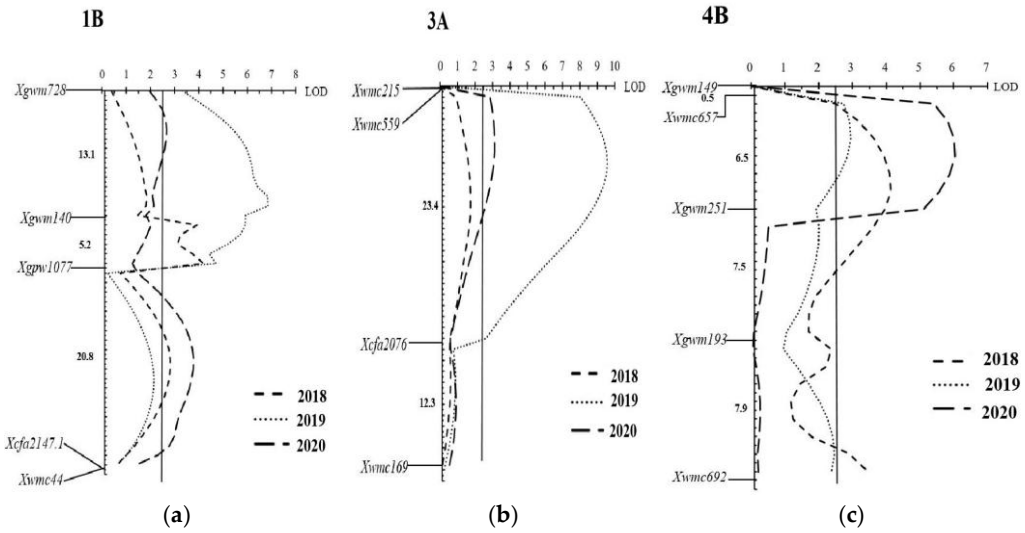

Figure 2. All three figures are genetic maps of chromosomes from the Xinong1163-4×Thatcher recombinant inbred line (RIL) population, showing the logarithm of the odds (LOD) values. The figures mark the marker names, distances between adjacent markers. Horizontal lines indicate an LOD threshold of 2.5, and small triangles on the *x*-axis represent markers used for quantitative trait locus (QTL) mapping. (**a**) 1B shows the genetic map of chromosome 1B, with the telomere of 1 BL on the left. Markers include Xgvm728, Xgvm140, etc. (**b**) 3A presents the genetic map of chromosome 3A, with the telomere of 3AL on the left. Markers are Xwmc215, Xwmc559, etc. (**c**) 4B is the genetic map of chromosome 4B, with the centromere of 4BL on the left. Markers such as Xgvm149, Xwmc657, etc.

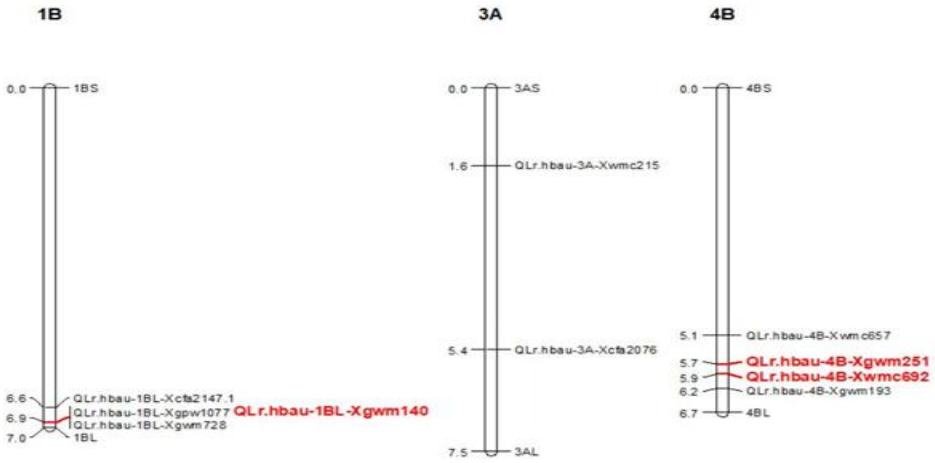

Figure 3. Distribution map of genetic loci/gene chromosomes for leaf rust resistance in wheat.

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3.4. csLV46G22 Molecular Marker Identification

The gene *Lr46*, which has now been identified at 1BL, was validated against two parents, five disease-resistant and five disease-susceptible lines using the CAPs marker *csLV46G22*, which is co-segregated with the gene (Figure 4). The near-isogenic lines containing *Lr46* could detect a 460 bp and a 120 bp DNA fragment, and the cultivars without *Lr46* could only detect a fragment of about 520 bp. In this test, both disease-resistant parents and disease-resistant subpopulations were able to detect two bands of 460 bp and 120 bp, while disease-susceptible parents and disease- susceptible subpopulations were able to detect only a single band of 520 bp, demonstrating the presence of *Lr46*.

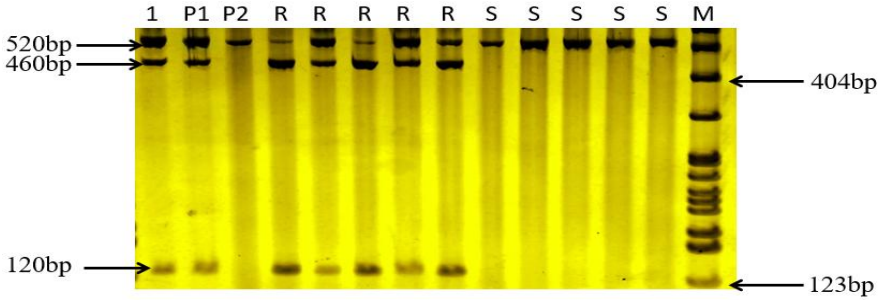

Figure 4. Electrophoresis of PCR products amplified by CAPs marker *csLV46G22* on non-denaturing polyacrylamide gels. M: PBR322/MspI Marker;1: *TCLr46*; P1: Xinong1163-4; P2: Thatcher; R: Resistant plants; S: Susceptible plants.

4. Discussion

Six QTLs for leaf rust resistance were detected in the current study. The total phenotypic variance across environments for leaf rust and 3.37–24.95% across environments for leaf rust, indicates their significant effects in reducing disease severity. The QTLs detected in the study were compared with the known genes or QTLs based on the chromosome position, molecular marker, pedigree, and resistance to rusts.

QLr.hbau-1BL

Based on the positions of closely linked markers, *QLr.hebau-1BL* appeared to be the pleiotropic gene *Lr46/Yr29/Pm39/Ltn2*. The QTLs identified on chromosome 1BL(*QLr.hbau-1BL.1,QLr.hbau-1BL.2* and *QLr.hbau-1BL.3*) were predicted to correspond to the gene *Lr46/Yr29/Pm39/Ltn2.Lr46*, a slow rust gene from common wheat that is located at the end of chromosome 1BL and confers resistance to leaf rust in adult plants. *Lr46* is widely used in CIMMYT wheat cultivars and has provided stable improvements in disease resistance for more than 30 years [31]. *Lr46* was linked to the SSR marker *Xwmc44* [32], which was detected in the 1BL linkage map in all three experiments. This suggests that the three QTLs on chromosome 1BL (*QLr.hbau-1BL.1,QLr.hbau-1BL.2,* and *QLr.hbau-1BL.3*) are equivalent to *Lr46*. Interestingly, the *Xgpw1077* marker was detected for the first time in the *Lr46* marker interval, providing a new marker reference for gene location and an additional option for molecular marker-assisted breeding. Genes associated with powdery mildew, stripe rust and stem rust resistance were also detected at this location. Therefore, Xinong1163-4 may also be resistant to powdery mildew, stripe rust and stem rust, although this possibility requires further testing.

QLr.hbau-3AL

To date, three APR QTLs have been identified on wheat chromosome 3A: *QLr.ubo-3A,QLr.sfrf-3A* and *QLr.fcu-3AL* [33]. *QLr.ubo-3A* is located at 45 cM on chromosome 3AS, near the centromere, while *QLr.sfrf-3A* and *QLr.fcu-3AL* are located at 51 cM and 82 cM, respectively, and the marker interval *Xgwm666-Xcfa2183* on chromosome 3AL. According to the Chinese spring wheat reference sequence, this QTL should be different from *QLr.hbau-3AL*, so, *QLr.hbau-3AL* may be a new QTL.

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QLr.hbau-4BL

To date, the APR genes *Lr12* [34] and *Lr49* [35]and three QTLs.*QLr.cimmyt-4BL, QLr.pbi-4BL* and *QLr.zh-4B* [20,29,36]have been identified. These genes and loci are of great significance in the study of wheat leaf rust resistance, and their specific characteristics and roles will be described in detail below The *Lr12*, has been mapped on 4BL, and is flanked by the markers *Xgwm251-Xgwm149*, and the *Lr49* flanked by the markers *Xbarc163-Xwmc349*. Both *Lr49* and *Lr12* were vulnerable to wheat leaf rust, but the slight species specificity of *Lr49* differed from that of *Lr12*. *QLr.cimmyt-4BL* flanked by the markers *Xgwm495-Xgwm368*, and the marker linked to *QLr.pbi-4BL* is wPt-1708 [37]. Kang and colleagues identified *QLr.zh-4B*, flanked by *Xwmc692* and *Xwmc657* in wheat cultivar Zhoumai 22×Chinese Spring F2:3 population [38]. The QTL was also found to be consistent with *Lr12*. *QLr.hbau-4BL* flanked by the markers *Xwmc657* and *Xgwm251* was detected in all tests. *Xgwm149* also appears in the all tested linkage maps at this locus and is only 0.5 cM away from *Xwmc657*, indicating a very close distance between them, since it closely corresponds to the reported markers in *Lr12*. *Lr12* is a significant resistant gene that does not confer disease resistance during the seedling stage but has been previously identified as highly resistant at maturity. Here, *QLr.hbau-4BL* was mapped very close to *Lr12*, thereby confirming this QTL as *Lr12*.

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Abbreviations

The following abbreviations are used in this manuscript:

Lr	Wheat leaf rust
Pt	Puccinia triticina Eriks
RILs	Recombinant Inbred L ines
QTLs	Quantitative trait loci
APR	Adult plant resistance
ASR	All-stage resistance
SSR	Simple sequence repeat
SNP	Single nucleotide polymorphism
GWAS	Genome-wide association studies
MDS	Maximum disease severity
ANOVA	Analysis of variance
LOD	Logarithm of the odds
PVE	Phenotypic variation explained
CIMMYT	International Maize and Wheat Improvement Center
MDPI	Multidisciplinary Digital Publishing Institute
DOAJ	Directory of open access journals
TLA	Three letter acronym
LD	Linear dichroism

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