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Rapid mapping of a stripe rust resistance gene *YrZl31* using bulked segregant analysis combined with high-throughput single-nucleotide polymorphism genotyping arrays

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ABSTRACT

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is one of the most important diseases of wheat. Identification and utilization of new resistance genes is one of the most effective way for achieving durable disease control. Zhongliang 31, a cultivar of common wheat (*Triticum aestivum* L.), is highly resistant to Chinese predominant *Pst* races and has been grown widely in the Longnan region in Gansu Province, China. Inheritance analysis of F_1, F_2, and $F_{2:3}$ populations indicated that the stripe rust resistance of Zhongliang 31 was controlled by a single dominant gene, temporarily designated as *YrZl31*. Bulked segregant analysis (BSA) combined with wheat 660K single-nucleotide polymorphism (SNP) array showed the highest proportion of polymorphic SNPs located on chromosome 2B. One hundred forty-one kompetitive allele-specific PCR (KASP) and 165 simple sequence repeat (SSR) markers on chromosome 2B were used to map *YrZl31*. Linkage analysis indicated that 31 markers were linked to *YrZl31*, and the genetic distances of the two closest flanking KASP markers *AX-111650565* and *AX-110196738* were 2.8 and 4.1 cM, respectively. *YrZl31* was located on wheat chromosome 2BL. Pedigree, resistance specificity, chromosome location, molecular and allelism tests suggested that *YrZl31* may be a new gene and can be pyramided with other effective stripe rust resistance genes using the closely linked KASP markers developed in this study in wheat breeding programs.

1. Introduction

Bread wheat is an important food crop that constitutes 20% of human caloric intake, and this crop is grown in 15% of the world's cultivated area (FAOSTAT, 2015). Stripe rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici* Erikss. (*Pst*), is an economically important disease of wheat in many regions of the world (Chen et al., 2009). Stripe rust affects wheat at the early growth stages, leading to yield losses of more than 90% on susceptible cultivars when weather conditions are favorable to the disease (Sharma-Poudyal and Chen, 2011). China represents the largest epidemiological region in the world, with more than 20 million hectares of wheat affected by stripe rust (Wan et al., 2007; Chen et al., 2009). The most severe nationwide epidemics occurred in the years 1950, 1964, 1990, and 2002, which caused yield losses of wheat estimated at 6.0, 3.2, 1.8, and 1.3 million metric tons, respectively (Wan et al., 2007). Although stripe rust can be controlled by the timely application of fungicides, chemical control adds additional costs and causes environmental problems (Chen, 2014). Breeding and planting resistant cultivars has been considered as the most effective, economical, and environment-friendly strategy to control stripe rust (Chen, 2014).

To date, more than 80 stripe rust resistance genes with official *Yr* designations have been reported in wheat, and many genes and quantitative trait loci (QTL) have been temporarily named (MoIntosh et al., 2017; Pakeerathan et al., 2019). Unfortunately, because of the rapid virulence changes in pathogen populations, only very few *Yr* genes such as *Yr5* and *Yr15* are effective against all current Chinese and world's populations of *Pst* (Sharma-Poudyal et al., 2013; Zeng et al., 2015).

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Therefore, there is an urgent need to identify and use new sources of resistance for wheat breeding.

Molecular markers including restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), resistance gene-analog polymorphism (RGAP), and simple sequence repeats (SSR) have been used to identify and map new resistance genes. Owing to the advantages of a higher level of polymorphism, known map locations, accuracy, repeatability, and PCR-based amplification, SSR markers have been employed much more frequently than other markers in the past few years (Feng et al., 2015; Randhawa et al., 2015). However, because of the huge genome size (~17 Gb) and extensive stretches of repetitive DNA (>80%) in hexaploid wheat, it is very difficult to develop and identify closely linked SSR markers for stripe rust resistance genes. In recent years, high-throughput technologies such as diversity array technology (DArT arrays and DArT-GBS) (Lan et al., 2017; Ponce-Molina et al., 2018) and single-nucleotide polymorphism (SNP) (Kolmer et al., 2018) have been widely used for mapping and saturating the target regions of resistance genes. SNPs are the most abundant type of molecular markers. With the development of new sequencing technologies, an increasing number of SNPs have been discovered in wheat (Jia et al., 2013; Ling et al., 2013). Based on the BeadArray™ technology from Illumina, 9K and 90K SNP arrays have been developed and widely used to identify wheat resistance genes and in genome-wide association analysis (GWAS) (Bariana et al., 2016; Liu et al., 2017). Most recently, 660K SNP array has been designed by the Chinese Academy of Agricultural Sciences and synthesized by Affymetrix, which is currently the highest density and most efficient wheat SNP array (Cui et al., 2017).

However, it is not cost-efficient to a genotype a large population with SNP arrays, especially high-density SNP arrays. Bulked segregant analysis (BSA) is an effective method to identify linked markers to genes for resistance or other agronomic traits (Michelmore et al., 1991; Feng et al., 2015). Moreover, conversion of polymorphic SNP into kompetitive allele-specific PCR (KASP) could largely speed up and improve the efficiency of identification of wheat resistance genes or agronomic traits (Bariana et al., 2016), and this is also easy to use in the wheat breeding programs.

Zhongliang 31, a cultivar of common wheat (*Triticum aestivum* L.) developed by Tianshui Institute of Agricultural Sciences, is resistant to stripe rust in both the seedling and the adult plant stages. It has some other good trait such as tolerance to cold and drought conditions (Wang et al., 2012). In our previous study, Zhongliang 31 showed a high level of resistance to the Chinese predominant *Pst* races CYR31, CYR32, CYR33, and CYR34 at the seedling stage (Li et al., 2016). The objectives of this study were to quickly identify and map the stripe rust resistance gene(s) in Zhongliang 31 using BSA combined with the high-throughput 660K SNP array and KASP markers.

2. Materials and methods

2.1. Plant materials

Zhongliang 31 was crossed with stripe rust susceptible wheat cultivar Mingxian 169 using Mingxian 169 as the female parent. Spikes on F_1 plants were bagged before anthesis to prevent outcrossing. The F_2 population was derived from a single F_1 plant grown in the field during the 2014–2015 cropping season. After seedling scoring in the greenhouse, the F_2 plants were transplanted to the field to produce the $F_{2:3}$ families. F_1, F_2, and $F_{2:3}$ generations and parents were used in seedling tests in the greenhouse for determining the inheritance of the all-stage resistance in Zhongliang 31. One hundred thirty F_2 plants were used to map the resistance gene.

Five wheat lines, AvSYr5NIL (*Yr5*), AvSYr7NIL (*Yr7*), IDO377s (*Yr43*), Zak (*Yr44*), and AvSYrSPNIL (*YrSP*), each of which carried a single wheat stripe rust resistance gene on chromosome 2BL, were also used in the seedling tests and compared the responses conferred by the resistance gene in Zhongliang 31 and other resistance genes. In addition, AvSYr5NIL (*Yr5*) was used to crossed with Zhongliang 31 for allelism test. Sixty-five commercial wheat cultivars and advanced breeding lines collected from the Yellow and Huai River Valley Wheat Region were used to evaluate the polymorphisms of KASP markers closely flanking the resistance gene in Zhongliang 31.

2.2. Seedling tests

Seedling tests were conducted under controlled greenhouse conditions. Seven *Pst* races, namely, CYR29, CYR31, CYR32, CYR33, CYR34, Su11-4, and Su11-6, representing predominant Chinese races since 1985, were selected to test Zhongliang 31 and Mingxian 169 for reaction to stripe rust (Supplementary Table 1). For genetic analysis, 15–18 seeds of each parent, six seeds of F_1, 120–150 seeds of F_2, and 15–18 seeds for each of the $F_{2:3}$ families were tested with *Pst* race CYR33. Further, to determine the relationships between *YrZl31* and other stripe rust resistance genes located on chromosome 2BL, Zhongliang 31, AvSYr5NIL, AvSYr7NIL, IDO377s, Zak, and AvSYrSPNIL were inoculated with *Pst* races CYR31, CYR32, CYR33, and CYR34 at the seedling stage. For allelism test, a total of 240 Zhongliang 31/AvSYr5NIL F_2 plants was inoculated with CYR33.

Seeds were planted in 7 × 7 × 7 cm pots, with 15–18 seeds per pot except for F_1 plants. Seedlings were inoculated by dusting with a mixture of fresh urediniospores and talcum powder at a 1:20 ratio when the first leaves were fully expanded (approximately 10 days after planting). The inoculated seedlings were kept in a dark dew chamber at 10 °C for 24 h and then transferred to a greenhouse maintained with 16 h light/8 h dark at 12–17 °C. Infection types (ITs) were scored using a 0–9 scale for 16–17 days after inoculation (Line and Qayoum, 1992). Plants with ITs 0–5 were considered resistant, and those with ITs 6–9 were considered susceptible.

2.3. BSA genotyping with wheat 660K SNP array

Genomic DNA was extracted from leaf tissues collected from the parents and F_2 individuals derived from the cross Mingxian 169/Zhongliang 31 using the CTAB method (Stein et al., 2001). Based on IT data of F_2 individual plants and corresponding $F_{2:3}$ families, 10 homozygous resistant and 10 homozygous susceptible F_2 plants were selected to make resistant and susceptible bulks, respectively. The resistant and susceptible bulks were constructed with an equal amount of DNA as described by Michelmore et al. (1991) and used to identify polymorphic markers between the resistant DNA bulk (RB) and susceptible DNA bulk (SB) as well as the two parents. The four samples were then genotyped with the wheat 660K SNP array (Cui et al., 2017) by the CapitalBio Corporation (Beijing, China). SNP genotype calling and clustering were performed with Genome Studio Polyploid Clustering v1.0 software (Illumina, http://www.illumina.com). Monomorphic SNPs and those with call rates less than 85%, ambiguity in calling, and minor allele frequencies below 5% were removed.

SNPs polymorphic between the resistant and susceptible bulks as well as two parents were located to chromosomes based on 660K genetic maps (Cui et al., 2017; Jizeng Jia, personal communication). Physical locations of SNP markers on chromosomes were determined using the International Wheat Genome Sequencing Consortium (IWGSC) RefSeq v1.0 (https://urgi.versailles.inra.fr/; http://www.wheatgenome.org/) as the reference database.

2.4. KASP marker assay

Based on genetic and physical positions of the polymorphic SNPs, those SNPs in the likely target region were given the priority to design KASP primers. SNP-derived KASP primers were designed using the PolyMarker tool (Ramirez-Gonzalez et al., 2015). The chromosome-specific KASP markers were first used to screen the two

bulks and parents. Only the polymorphic KASP markers were used to genotype the whole F_2 population.

KASP assays were performed in an S1000 Thermal Cycler with 384 well (Bio-Rad Laboratories, Hercules, CA). A 5.07 μL PCR reaction mixture consisted of 2.5 μL of 2 x KASP V4.0 Mastermix (LGC Genomics, Beverly, MA), which contained the universal fluorescent reporting dyes FAM™ and HEX™ and Rhodamine X (ROX) dye as the passive reference, 0.07 μL of assay primer mix (12 mM of each allele-specific primer, 30 mM of common primer), and 2.5 μL genomic DNA at 50–100 ng/μL (He et al., 2014). Thermal cycling conditions were 94 °C 15 min; followed by 10 cycles of 94 °C for 20 s, touchdown starting at 61 °C for 1 min (decreasing 0.6 °C per cycle); followed by 32 cycles of 94 °C 20 s and 55 °C 60 s (He et al., 2014). Reaction fluorescent endpoint readings were carried out with a microplate reader (FLUOstar Omega, BMG LABTECH, Germany), and the results were clustered and genotyped using KlusterCaller™ software (http://www.lgcgroup.com/).

2.5. SSR analysis

To further confirm the SNP marker location results and identify more potential markers linked to the resistance locus, a total of 165 SSR markers located on wheat chromosome 2B were used to screen the two bulks and parents before being genotyped on the entire F_2 population.

The PCR amplifications for SSR analyses were performed on a S1000 Thermo Cycler (Bio-Rad Laboratories, Hercules, CA). A 15.0 μL reaction mixture consisted of 1.5 μL 10 x PCR buffer, 1.2 μL $MgCl_2$ (25 mM), 1.5 μL each of forward and reverse primers (10 μM), 0.3 μL dNTP (10 mM), 0.15 μL Taq DNA polymerase (5U/μL), 2.1 μL DNA template (50 ng/μL), and 6.75 μL ddH_2O. The following PCR conditions were used: initial denaturation at 94 °C for 5 min, followed by 35 cycles of 30 s denaturation at 94 °C, 1 min annealing at 50–65 °C depending on primers, 45 s extension at 72 °C, and a final extension at 72 °C at 10 min. After amplification, 6 μL formamide loading buffer [98% formamide, 10 mM EDTA (pH 8.0), 0.5% (w/v) xylene cyanol and 0.5% (w/v) bromophenol blue] was added to the PCR product and denatured at 94 °C for 5 min. Five-microliter mixture of the PCR product was loaded for electrophoresis on 6% polyacrylamide gels and visualized using silver staining (Bassam et al., 1991).

2.6. Data analysis and linkage map construction

A χ^2 analysis was used to evaluate the goodness of fit of observed and expected segregation ratios in F_2 and $F_{2:3}$ progenies to establish the number of stripe rust resistance gene(s) and mode of inheritance. The "Chitest" procedure in the data analysis of Microsoft Office (2013) Excel was used to calculate χ^2 and P values. Linkage analysis was conducted with JoinMap 4.0. Map distance in centiMorgan was calculated according to the Kosambi mapping function (Kosambi, 1943), and a logarithm of odds (LOD) score of 3.0 was used as the threshold for declaration of linkage. MapChart V2.3 was used to draw the final linkage map.

To determine the genetic distances for the allelism test using the stripe rust IT data of F_2 population from the cross Zhongliang 31/AvSYr5NIL, the frequency (f) of susceptible plants in repulsion phase cross was used to estimate the recombination value (p) using the formula $f = p^2/4$. And the formula $d = -1/2 \ln (1\text{-}2p)$ was used to convert recombination value to map distances (Cheng and Chen, 2010).

3. Results

3.1. Genetic analysis of stripe rust resistance in Zhongliang 31

In the seedling tests with seven Chinese *Pst* predominant races, Zhongliang 31 was highly resistant (IT 0–1), whereas Mingxian 169 was susceptible to all races (IT 9) (Fig. 1, Supplementary Table 1). In the test with CYR33, all six F_1 plants were resistant indicating dominant resistance in Zhongliang 31. For the 130 F_2 individuals, 102 were resistant and 28 susceptible, fitting to 3:1 ratio ($\chi^2 = 0.83$, $P = 0.36$). One hundred twenty $F_{2:3}$ families were obtained from the corresponding F_2 plants, and 10 plants died during transplantation to the field. Among the 120 $F_{2:3}$ families, 31 homozygous resistant families were derived from resistant F_2 plants, 26 homozygous susceptible families were from susceptible F_2 plants, and of 63 segregating families, 62 were from resistant F_2 plants, and one were from susceptible F_2 plants. The distribution of $F_{2:3}$ families was fitting to the 1:2:1 ratio ($\chi^2 = 0.71$, $P = 0.69$). The results indicated that the stripe rust resistance of Zhongliang 31 was controlled by a single dominant gene, temporarily designated as *YrZl31*.

3.2. Genetic mapping of YrZl31 based on SNP and SSR markers

A total of 630,517 SNPs were generated based on wheat 660K SNP array genotyping (Supplementary Table 2). After SNP quality filtering, 9117 SNPs showed polymorphisms between the resistant and susceptible bulks as well as two parents. Of the 9117 polymorphic SNPs, 2050 were located on wheat chromosome 2B and the others were distributed across other chromosomes (Fig. 2A). The proportion of polymorphic SNPs on chromosome 2B was the highest, which indicated that *YrZl31* is most likely located on chromosome 2B. By locating the SNPs on chromosome 2B with reference to the IWGSC-RefSeq database, 500–800 Mb of interval on chromosome 2B had the highest SNPs distribution frequency (Fig. 2B). A total of 83 chromosome-specific SNPs in 500–800 Mb of interval and 58 in 0–500 Mb of interval were selected to be converted to KASP markers and used to genotype the two bulks and parents. Seventy KASP markers polymorphic between resistant bulk and susceptible bulk as well as two parents were then used to test the 130 F_2 individuals using the KASP method. Genotyping in some F_2 plants with two KASP markers *AX-110196738* and *AX-11165056* is shown as Fig. 3.

In addition, 165 pairs of SSR markers located on chromosome 2B were selected randomly to test two bulks and parents. Only two SSR markers, namely, *wmc317* and *wmc363*, located on wheat chromosome 2BL, showed polymorphisms between the resistant and susceptible DNA

Fig. 1. Infection types of Mingxian 169 (left, IT 9) and Zhongliang 31 (right, IT 0) inoculated with *Puccinia striiformis* f. sp. *tritici* race CYR33 at the seedling stage.

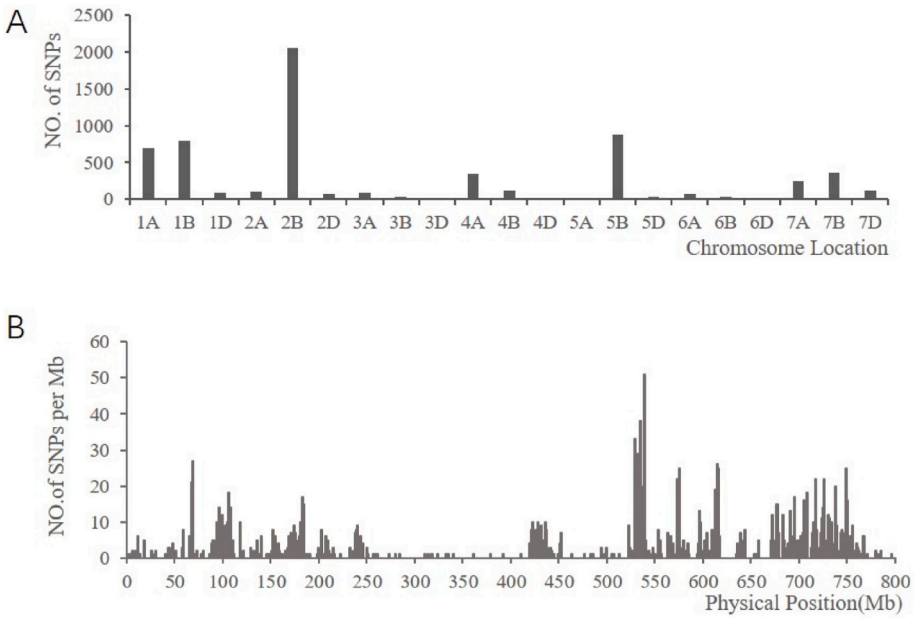

Fig. 2. Distribution of the polymorphic SNPs on each chromosome genotyped by wheat 660K SNP array (A) and physical position of SNPs on chromosome 2B (B).

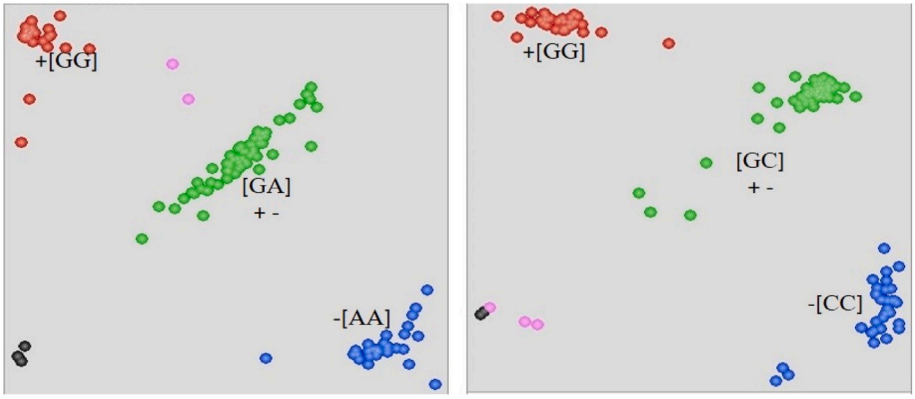

Fig. 3. Kompetitive allele-specific PCR (KASP) markers of *AX-110196738* (left) and *AX-11165056* (right) genotyped in F_2 populations showing three distinct clusters. The central cluster (green) comprised heterozygous individuals, while clusters near the axes were homozygous for either Mingxian169 (blue) or Zhongliang 31 (red). Pink dots represent missing or failed data. Black dots represent the NTC (non-template control). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

bulks as well as the parents. The 130 F_2 plants were genotyped with the two polymorphic SSR markers.

Subsequent linkage analysis, based on the phenotypic and genotypic data of the 70 KASP and two SSR polymorphic markers in the 130 F_2 individuals, showed that 30 KASP markers and one SSR marker were linked to the resistance gene *YrZl31*, and the linkage map spanned 66 cM (Fig. 4D). The two closest flanking KASP markers were *AX-111650565* and *AX-110196738* with genetic distances of 2.8 and 4.1 cM, respectively (Fig. 4D). The names and sequences of the 30 linked KASP primers and one SSR markers are listed in Supplementary Table 3.

According to the physical locations of linked SNP using IWGSC RefSeq v1.0 as the reference database, these SNP markers spanned a maximum range of 529.5 Mb–785.3 Mb; most of the markers were assigned to an 86.1 Mb physical interval (2B: 699223441-2B: 785373743) (Fig. 4E), which indicated that *YrZl31* was located on this interval.Based on the position information of polymorphic SNP markers, *YrZl31* was located in the chromosomal bin 2BL6 (0.89) close to 2BL6 (1.00) (Fig. 4C).

3.3. Relationships of YrZl31 and the other genes on chromosome 2B

Zhongliang 31 were resistant to CYR31, CYR32, CYR33, and CYR34 with ITs 0–1 (Table 1), whereas AvSYr7NIL (*Yr7*), IDO3775 (*Yr43*), and Zak (*Yr44*) were susceptible to all four *Pst* races, and AvSYrSPNIL (*YrSP*) were susceptible to CYR31, CYR32, and CYR34. Therefore, *YrZl31* should be different from *Yr7*, *Yr43*, *Yr44*, and *YrSP*. AvSYr5NIL(*Yr5*) was also highly resistant to all the tested races. To further compare *YrZl31* and *Yr5*, the *Yr5* diagnostic KASP marker (Supplementary Table 3) was used to test Zhongliang 31, AvSYr5NIL, and Mingxian 169 according to the method previously described by Marchal et al. (2018), and the result indicated that Zhongliang 31 might not contain *Yr5* gene (Fig. 5).

In addition, the allelism test of Zhongliang 31/AvSYr5NIL F_2 population indicated that 236 plants were resistant with ITs 0–2, and four plants were susceptible with ITs 6–7. The frequency of resistant and susceptible plants deviated from 15:1 ratio expected for independent segregation of dominant alleles at two loci ($P < 0.05$). Assuming linkage, the recombination value was estimated at 0.26 and the genetic distance between *Yr5* and *YrZl31* was estimated as 36.70 cM.

3.4. Polymorphism of KASP markers flanking YrZl31 in various wheat genotypes

To evaluate the robustness of the markers closely flanking *YrZl31* for marker-assisted selection (MAS), a set of 65 wheat cultivars and advanced breeding lines, which were not expected to carry *YrZl31* according to their pedigrees and infection types to CYR32 and CYR33 in

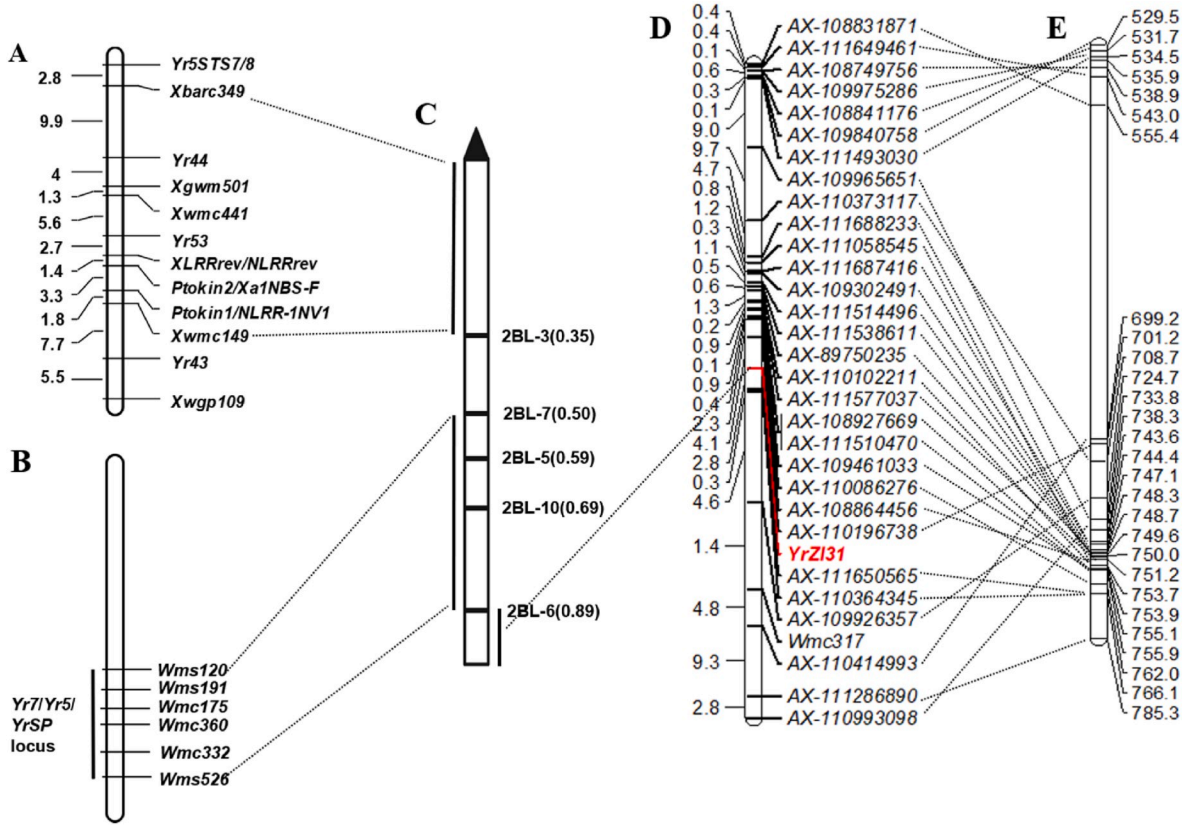

Fig. 4. Linkage maps and chromosome location of *YrZl31* and comparisons with different linkage maps of other *Yr* genes located on wheat chromosome 2BL. A, The map distances of *Yr53* to resistance genes *Yr43* and *Yr44* (Xu et al., 2013); B, linked markers of *Yr7/Yr5/YrSP* locus on wheat chromosome 2BL (Marchal et al., 2018); C, deletion map of 2BL (https://wheat.pw.usda.gov/NSF); D, genetic map for *YrZl31*; E, physical position for *YrZl31*.

Table 1
Infection types of wheat lines with the *Yr* gene located in the long arm of chromosome 2B tested with four *Puccinia striiformis* f. sp. *tritici* races.

Genotypes	*Yr* genes	CYR31	CYR32	CYR33	CYR34
AvSYr5NIL	*Yr5*	0	0	1	1
AvSYr7NIL	*Yr7*	9	8	9	8
IDO3775	*Yr43*	8	8	6	7
Zak	*Yr44*	8	8	6	8
AvSYrSPNIL	*YrSP*	7	8	5	8
Zhongliang 31	*YrZl31*	0	1	0	1
Mingxian 169	–	9	8	8	8

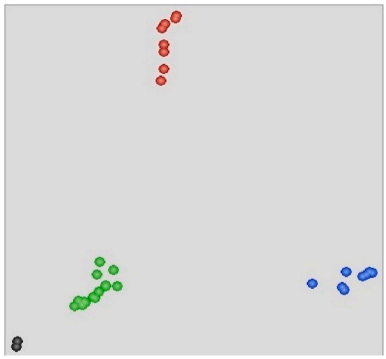

Fig. 5. *Yr5* diagnostic KASP marker (Marchal et al., 2018) genotyped in Zhongliang31 (blue), AvSYr5NIL (Red), and Mingxian 169 (Green). Black dots represented the NTC (non-template control). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the seedling tests (Li et al., 2016), were tested for polymorphism with KASP markers *AX-110196738*, *AX-110364345*, *AX-111650565*, and *AX-108864456* (Supplementary Table 4). *AX-110196738*, *AX-110364345*, and *AX-111650565* produced the *YrZl31* alleles in 25, 34, and 48 wheat genotypes, respectively, whereas *AX-108864456* carried a *YrZl31* allele in 7 wheat genotypes. However, no genotype had all the same SNP haplotypes based on the four KASP markers, indicating that these markers should be used together to detect *YrZl31* and can be used together for MAS.

4. Discussion

According to our previous studies and this study, Zhongliang 31 has high resistance to Chinese predominant *Pst* races CYR31, CYR32, CYR33, and CYR34 in the seedling tests, and the genetic analysis indicated that the resistance in Zhongliang 31 is controlled by a single dominant gene. Using BSA combined with wheat 660K SNP arrays, KASP, and SSR markers, *YrZl31* was mapped to a 6.9 cM genetic interval flanked by *AX-111650565* and *AX-110196738*. According to the marker's location information, *YrZl31* was located on wheat chromosome 2BL.

To date, five officially designated *Yr* genes, namely, *Yr5* (Macer, 1966), *Yr7* (Macer, 1966), *Yr43* (Cheng and Chen, 2010), *Yr44* (Sui et al., 2009), and *Yr53* (Xu et al., 2013), and two temporarily named genes, *YrSP* (Feng et al., 2015) and *YrS2199* (Fang et al., 2008), have been reported on wheat chromosome 2BL. *YrS2199* was further confirmed the same as *Yr5* (Zhiyong Liu, personal communication). Our seedling test results showed that AvSYr7NIL (*Yr7*), IDO3775 (*Yr43*), Zak (*Yr44*) and AvSYrSPNIL (*YrSP*) were susceptible to three or all of the tested races of CYR31, CYR32, CYR33, and CYR34. Zeng et al. (2015) also reported that AvSYr7NIL, IDO377s, Zak, and AvSYrSPNIL were

susceptible to *Pst* races CYR32 and CYR33 at the seedling stage. Therefore, *YrZl31* is obviously different from *Yr7*, *Yr43*, *Yr44*, and *YrSP*.

Xu et al. (2013) reported the relationships among *Yr53*, *Yr43*, and *Yr44*. The genetic distances from *Yr53* to *Yr43* and *Yr44* were estimated as 16.9 and 10.9 cM, respectively (Fig. 4A), and *Yr44* and *Yr53* were located in close to the centromere to 2BL-3 (0.35). Marchal et al. (2018) reported that *Yr5* is allelic to *YrSP* and paralogous to *Yr7* (Fig. 4B) and located at the bin of 2BL6-0.50–0.89, whereas *YrZl31* was mapped in the bin of 2BL6 (0.89) close to 2BL6 (1.00), more distal on 2BL (Fig. 4C). In our previous study (Li et al., 2016), Zhongliang 31 was tested with *Yr5* tightly linked markers *Xbarc167* and *Xbarc349* (Murphy et al., 2009), and the molecular tests results indicated that Zhongliang 31 might not carry *Yr5*, which is the same as the result of *Yr5* diagnostic KASP marker tested in this study (Fig. 5). To further determine if *YrZl31* and *Yr5* were at different loci, we made a cross between Zhongliang 31 and AvSYr5-NIL. The F_2 population showed clear segregation indicating that the gene in Zhongliang 31 and *Yr5* are different, but likely linked gene. Using gene postulation, Wang et al. (2015) also reported that Zhongliang 31 might not contain *Yr5*.

In addition, *Yr5* was initially identified in a *Triticum aestivum* ssp. *spelta* var. *album* accession (Macer, 1966), and *Yr53* was originally derived from durum wheat PI 480148, whereas *YrZl31* was derived from hexaploid winter wheat Zhongliang 31. The pedigree of Zhongliang 31 is Tao 157/82 (348)//AT8118/Tao 157 (Wang et al., 2012). Among these parents, Tao 157 and 82 (348) are common wheat cultivar and advanced breeding line, respectively. AT8118 is a winter wheat cultivar whose pedigree is Kangyin 655/*Elytrigia trichophora*//Jingai 21 (Wang et al., 2012) and Jingai 21 was derived from the cross of Aiganhong/Jingzhouheimai. Therefore, the origin of *YrZl31* was different from *Yr5* and *Yr53*. Based on origin, seedling resistance, chromosome location, and molecular tests, *YrZl31* should be different from the other genes located on chromosome 2BL and may be a new resistance gene.

The usefulness of a molecular marker for MAS depends on the extent of its linkage to the gene of interest and its ability to predict presence of the target gene in diverse genetic backgrounds. When tested with four closely linked markers of *YrZl31*, none of the 65 representing wheat cultivars (lines) had the same haplotype of Zhongliang 31 at all of the four markers, especially marker *AX-108864456* showed polymorphism in 89% Chinese cultivars tested. Therefore, these markers must be used together for marker-assistant incorporation of the gene into various wheat cultivars.

Because the environment and cropping systems in Longnan region provide ideal conditions for the survival, mutation, and development of races of the stripe rust pathogen, wheat stripe rust is severe almost every year and more than 80% of the new *Pst* races were first detected in this region and subsequently detected in other areas of China (Chen et al., 2009). Historically, almost every stripe rust resistance "breakdown" event occurred first in this region. Therefore, Longnan is considered a "hotspot" for the wheat stripe rust pathogen. Zhongliang 31 has been planted widely in Longnan region since 2009. According to the report of Wang et al. (2012) and our studies, Zhongliang 31 is highly resistant to the most predominant *Pst* races CYR31, CYR32, CYR33, and CYR34 in both the seedling and the adult-plant stages. Therefore, it is a desirable resistant donor for wheat breeding programs.

Elite wheat cultivars are primary genetic resources for future wheat improvement. However, because of the rapid virulence changes in stripe rust pathogen populations, wheat resistance determined by a single gene is often overcome by new virulence *Pst* races within about five years, such as the widely used resistance genes *Yr9*, *Yr3b*, *Yr4b*, and *Yr26* (Wan et al., 2007; Han et al., 2015). Therefore, pyramiding genes for different types of resistance is the best approach for durable resistance in wheat breeding programs. In China, the genes *Yr5*, *Yr15*, *Yr61*, *YrZH22* (Zeng et al., 2015; Wang et al., 2017) have effective resistance to the most predominant Chinese *Pst* races such as CYR32, CYR33, and CYR34. *YrZl31* in Zhongliang 31 can be pyramided with these genes using marker-assisted selection (MAS) in wheat breeding programs.

5. Conclusions

The common wheat cultivar Zhongliang 31 is highly resistant to Chinese predominant *Pst* races and the resistance to *Pst* race CYR33 was controlled by a single dominant gene, *YrZl31*. *YrZl31* was located on wheat chromosome 2BL by 30 KASP and one SSR markers. Pedigree, resistance, chromosome location, molecular and allelism tests suggested that *YrZl31* may be a new gene.

Ethical standards

All experiments and data analyses were conducted in Northwest A&F University, Yangling, China. All authors have contributed to the study and approved the version for submission. The manuscript has not been submitted to any other journal.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Huan Liu: Conceptualization, Investigation, Writing - original draft. **Wenwen Su:** Visualization, Investigation. **Xiaoyan Li:** Investigation. **Kaixiang Chao:** Software, Data curation. **Meinan Wang:** Writing - review & editing. **Weiyun Yue:** Resources. **Baotong Wang:** Supervision. **Qiang Li:** Conceptualization, Data curation, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cropro.2020.105174.

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