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RESEARCH ARTICLE

Genetic mapping and utilization analysis of stripe rust resistance genes in a Tibetan wheat (*Triticum aestivum* L.) landrace Qubaichun

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Abstract Stripe rust, which is caused by *Puccinia striiformis* f. sp. *Tritici* (*Pst*), is one of the most destructive wheat (*Triticum aestivum* L.) diseases worldwide. To control stripe rust, the best strategy is breeding and growing novel resistant cultivars. Qubaichun (QBC), a Tibetan wheat landrace, displays near-immune resistance to wheat stripe rust in western China. Previously, our studies have shown that the stripe rust resistance of QBC is controlled by a dominant gene at the seedling stage and two independent genes at the adult-plant stage. These two genes comprise an all-stage resistance (ASR) gene and a durable adult resistance gene, which was identified as *Yr18*. The unknown ASR gene is temporarily named *Yrqbc*. To map this gene, a segregating population of QBC × Chinese Spring (CS) was generated. SSR analysis, BSR-seq and Infinium 660 K iSelect SNP genotyping were successively performed. The results show that *Yrqbc* finely mapped to a 5.1 cM genetic interval between molecular markers A009200 and A009192, and the genetic distance to the marker A009200 was 0.1 cM. Furthermore, *Yrqbc* was confirmed to be *Yr5* by sequencing. Diagnostic markers are used for detection *Yr5* and *Yr18* in 323 new cultivars (lines) worldwide. The results show that 27 cultivars (lines) carry the durable adult resistance gene *Yr18*, and only one material carrying the ASR resistance gene *Yr5*. In order to analyse the effectiveness and practicality of transferring the pyramided of *Yr18* and *Yr5*, these two stripe rust resistance genes were introduced into elite cultivars which lost resistance. Field test results verified that the combination of *Yr5* and *Yr18* could provide effective resistance to stripe rust for the whole life of wheat, which provided a genetic foundation for the near immunity of elite cultivars. As a result, this Tibetan landrace could be used for developing high-level, durable resistant wheat cultivars.

Keywords Stripe rust · Landrace · All-stage resistance · High-temperature adult plant resistance · Pyramided · Durable resistant

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Introduction

Common wheat (*Triticum aestivum* L.), one of the most important crops worldwide, provides staple food for 35–40% of the global population (https://www.fao.org/faostat/zh/?#data). However, its yield has been strongly restricted by *Pst*, which is one of the most severe wheat diseases worldwide (Chen 2005). This disease can cause up to 100% of crop yield losses, although the losses are typically in the range of 10–70% (Chen 2005). China has the largest stripe rust epidemic region in the world in terms of wheat acreage affected by the disease (Wan et al. 2007). Sichuan Province is the inoculum base, the centre of diversity, and a major over-summering area of the pathogen (Zeng and Luo 2006; Wan et al. 2007). For example, CYR34, first monitored in this area, has now become the first major epidemic race in China (Liu et al. 2010). CYR34 presents a high frequency of 17–65% and has led to severe yield losses. It has expanded to nine provinces in China and exhibits a further expansion trend, which poses a great threat to wheat safety, production, and disease resistance breeding (Jin et al. 2018). Therefore, the prevention and control of stripe rust races are imminently needed. The most effective, economical and environmentally friendly control strategy is to plant resistant varieties (Chen 2005). However, breeding a new high-yield, high-resistance cultivated variety takes a long time, and most resistance genes have lost their resistance to the new races of stripe rust because of the monoculture deployment of resistant cultivars in widespread areas (Ellis et al. 2014). Therefore, it is imperative to discover novel germplasms for their efficient incorporation into cultivars to design a "pyramiding strategy" of resistance (Elisabeth et al. 2017; Zhang et al. 2001).

To develop high-level, durable resistant cultivars, two types of resistant genes are combined by breeders when using a "pyramiding strategy" (Johnson 1981). Generally, one is the all-stage resistance (ASR) gene, and the other is a durable resistance gene (Chen 2013). The ASR gene protects wheat from infection at the seedling stage and can also play an important role in the HTAP gene at the adult stage via different molecular mechanisms (Chen et al. 2013). No studies have reported the effectiveness of the natural pyramiding of ASR and HTAP or slowing rust resistance gene using known genes except for those parts of QTLs (Guo et al. 2008). Previously, the winter wheat cultivar Druchamp was reported to contain both HTAP QTLs and ASR genes and exhibits durable resistance since it was introduced from France to the United States in the late 1940s (Hou et al. 2015).

"Pyramiding strategy" has been widely implemented for disease resistance in other crops to achieve high-level or durable resistance (Elisabeth et al. 2017; Werner et al. 2005). In soybean, *Rsv1*, *Rsv3*, and *Rsv4* were pyramided for mosaic virus (SMV) resistance (Shi et al. 2009). In pea, *sym9* and *sym10* were pyramided to promote nodulation processes during rhizobial or endomycorrhizal endosymbiosis (Schneider et al. 2002). In rice, *pi21*, *pi34* and *pi35* were aggregated to enhance rice blast resistance (Nobuko et al. 2015). This "pyramiding strategy" has also been reported in wheat for other diseases (Bariana et al. 2007). For example, *Lr41*, *Lr42* and *Lr43* were transferred from *Triticum tauschii* to common wheat to improve wheat leaf rust resistance (Cox et al. 1994). For stripe rust disease, *Yr64* and *Yr15*, this two genes were estimated to be linked in repulsion and separated by 7.8 cM, both on the chromosome 1BS, were pyramided to achieve high-level and durable resistance, however, it is very difficult to pyramid genes linked in repulsion from different genotypes, but this approach may accelerate the pyramiding and selection processes in breeding programs (Yanmin et al. 2019). Moreover, two HTAP genes—even four to five HTAP genes—were aggregated to improve resistance (Yan et al. 2014).

Previously, QBC, a wheat landrace from Tibet, was collected and analysed. QBC is a landrace formed by natural for a long time due to its geographical location and growing history. Phenotypic identification has indicated that QBC displayed near-immune resistance to stripe rust in field test in Sichuan Province, where Pst is severe and naturally occurring. To analyse the resistance genes in QBC, a segregating population [QBC × Chuanmai 28 (CM 28), with CM28 being highly susceptible to stripe rust] was generated. The results suggested the presence of one dominant gene for stripe rust resistance in the test population at the seedling stage (Supplementary Table 1). However, at the adult stage, the result showed that the stripe rust resistance of QBC was controlled by two dominant genes (Zhou et al. 2015). The segregating ratio of these two populations (QBC × CM 28 direct and reciprocal crossing) was 12:3:1 (resistant:intermediate resistant:-susceptible) (Supplementary Table 2). Moreover,

QBC exhibited a phenotype of apical necrosis in field investigations, which indicated that it possibly harboured a durable adult resistance gene (Singh et al. 2005; Rosewarne et al. 2006). The durable adult resistance gene was identified as *Yr18* by using functional marker analysis (Lagudah et al. 2009).

Previous studies have confirmed that QBC resistance is controlled by two independent dominant genes. One is the durable adult resistance gene (*Yr18*), and the other is an unknown ASR gene, temporarily named *Yrqbc*. The objectives of the present study were (1) to finely map *Yrqbc* and develop co-segregating molecular markers for stripe rust resistance-assisted breeding; (2) to confirme that *Yr5* and *Yrqbc* are the same gene by using *Yr5*-specific maker and sequencing; (3) to analyse the effectiveness and practicality of the combination of two resistance genes in QBC for durable and high-level resistance breeding. This study is expected to generate information on the mechanism of "pyramiding strategy" and incorporate resistance genes from QBC into elite cultivars.

Materials and methods

Plant material and stripe rust pathogens

QBC, CS, Huixianhong (HXH, which is highly susceptible to stripe rust and was an induced cultivar) and 323 elite cultivars germplasms which included 271 Chinese wheat cultivars (lines) (32 Northern winter wheat area cultivars, 99 Huanghuai winter wheat area cultivars, 103 Southwest winter wheat area cultivars, 15 middle and lower reaches of the Yangtze River cultivars, and 5 Qinghai-Tibet spring/winter wheat area cultivars), 42 CIMMTY cultivars (lines), 10 European cultivars and Australian cultivars. All these germplasms were provided by the Chengdu Institute of Biology, Chinese Academy of Sciences. The Chinese wheat Pst races CYR32, CYR33, CYR34, Su11-4 and Su11-7 were provided by the College of Plant Protection, Northwest A&F University, for the identification of stripe rust resistance. QBC was used as a parent for the generation of two segregating populations, with each reciprocally crossed with CS (which is susceptible to stripe rust at the seedling stage but moderately resistant at the adult stage). The F_1, F_2 and $F_{2:3}$ generations derived from the reciprocal crosses of QBC × CS were planted in the field in Chengdu.

Identification of stripe rust resistance

In the growing season, QBC, CS, CM28, 12 elite cultivars (Chuanmai 42, Chuanmai 104, Chuanmai 107, Chuanyu 12, Chuanyu 16, Chuanyu 20, Mianyang 26, Mianyang 28, Mianmai 367, Zhongkemai 47, Zhongkemai 138 and Yangmai 158), and 323 elite cultivars (lines) as well as the F_1 and F_2 generations of the reciprocal crosses of QBC × CS were sown in the field in Chengdu. Thirteen seeds were planted in a 1.5 m row, with 10 cm spacing between rows. HXH, susceptible control or induced lines, was planted around the nursery lines every 10 rows. Standard fertilizer and cropping practices were applied for field management. Inoculation was performed with mixed races of CYR32, CYR33, CYR34, Su11-4 and Su11-7 by the field spraying method at the beginning of the stem elongation stage. Stripe rust ITs were recorded when the disease was in a sufficient infection situation. Infection types 0–3, 4–6 and 7–9 were considered resistant, intermediate and susceptible, respectively (Line and Qayoum 1992).

DNA extraction and polymerase chain reaction (PCR)

At the seedling stage, the healthy leaves of QBC, CM28, CS, the F_1 and F_2 generations of their crossing combinations, and 323 wheat cultivars (lines) were collected for extraction of genomic DNA using the improved CTAB method (Rogers and Bendich 1985).

PCR was performed on a Mastercycler Nexus SX1 PCR instrument. The reaction volume was 10 μL, which consisted of 5 μL of 2 × master mix, 0.1 μL of forward primer (10 μM), 0.1 μL of reverse primer(10 μM), 0.8 μL of DNA template and 4 μL of ddH_2O. The PCR programme consisted of initial denaturation at 94 °C for 4 min, followed by 35 cycles of 30 s of denaturation at 94 °C, 30 s to 1 min of annealing at 52–68 °C (depending on the primers) and 45 s of extension at 72 °C, with a final extension at 72 °C for 10 min. Two microlitres of PCR product was then loaded for electrophoresis on 8% polyacrylamide gels for SSR analysis (https://maswheat.ucdavis.edu).

SSR analysis

Two hundred eight $F_{2:3}$ lines from the direct cross of CS × QBC were used for mapping the resistance gene *Yrqbc*. Based on the method of bulked segregant analysis (BSA) and the F_3 disease phenotypic data, DNA from 15 homozygous resistant lines and 15 homozygous susceptible lines was used for constructing resistant and susceptible bulks, respectively. Each bulk was composed of equal amounts of DNA. A total of 371 pairs of SSR markers were screened within the two parental lines and the two bulks to detect polymorphisms (Gupta et al. 2002; Qi et al. 2004; Röder et al. 1998; Somers et al. 2004; Sourdille et al. 2004). The SSR primers were synthesized according to the published sequence of wheat SSR primers available at https://wheat.pw.usda.gov/GG3/, https://www.wheatgenome.org/, https://www.ncbi.nlm.nih.gov/ and other databases. Fragment analysis of the PCR products was carried out on 8% non-denaturing polyacrylamide gels (39 acrylamide: 1 bisacrylamide). After electrophoresis, the gels were silver stained and imaged. Mapmaker/EXP 3.0b software was used to calculate the linkage between markers and stripe rust resistance genes, and a linkage map was constructed by MapDraw 2.0 software.

BSR-seq and Infinium 660 K iSelect SNP genotyping

Bulked segregant analysis (BSA) combined with transcriptome sequencing (RNA-seq) was applied for enriching the makers of this gene (Ramirez-Gonzalez et al. 2015; Cheng et al. 2016). Two mixture pools were constructed by selecting individuals with extreme traits in each population. According to the phenotypic identification results, 20 homozygous resistant/susceptible individuals were screened from the F_2 population of QBC × CS. Total RNA from the mixture pools was extracted for transcriptome sequencing. The transcriptome data were ultimately analysed by the classic Bayesian algorithm, and SNP markers were developed to predict the genome segment of the target gene. RNA-seq of the bulked pools was implemented to identify SNPs associated with *Yrqbc* and then were classified based on the results from the sequenced bulks. The bulk frequency ratio (BFR) of the SNPs between the resistant and susceptible bulks was calculated, and those showing six-fold enrichment/depletion in the corresponding bulks were selected.

Furthermore, SNP genotyping of the parents of the CS × QBC population was carried out using a wheat 660 K array (Cui et al. 2017). Non-polymorphic and low-quality SNPs were excluded, which included those with more than 20% deletion value, nonsignificant clustering and less than 5% allele frequency. In addition, in reference to the version 1.0 genome sequence (IWGSC) of Chinese Spring, the different SNPs within the flanking sequences and genomes were queried via local BLAST searches. This information was then used to develop KASP markers.

Detection of *Yr5* and *Yr18* in cultivars (lines)

The *Yr5* gene-specific marker was used to detect *Yr5* in the 323 cultivars (lines) and QBC. These cultivars (lines) were the major new cultivars released in China for commercial production and planting in wheat areas. PCR amplification was conducted at 63 °C for 35 cycles. The primer combinations L34SPF/L34DINT13R2 and L34DINT9F/L34MINUSR were used for *Yr18* detection. This reaction amplified two bands of contrasting size that could easily be resolved in a 1% agarose gel: a 751 bp fragment specific for the + *Yr18* allele and a 523 bp fragment specific for the − *Yr18* allele (Lagudah et al. 2009).

Transferring of *Yr5* and *Yr18* into elite cultivars

In order to verify the validity of pyramiding *Yr5* and *Yr18*, and the feasibility of QBC breeding, we screened 12 elite cultivars (Chuanmai 42, Chuanmai 104, Chuanmai 107, Chuanyu 12, Chuanyu 16, Chuanyu 20, Mianyang 26, Mianyang 28, Mianmai 367, Zhongkemai 47, Zhongkemai 138 and Yangmai 158) were selected as receptor parents, and the IT value survey results of these cultivars (lines) were all highly susceptible. Then, using the backcross breeding method combined with the marker-aided selection (MAS) method, the resistance genes, *Yr5* and *Yr18*, in QBC were introduced into the elite cultivars (lines) selected above. Field test were record the phenotype of their offspring.

Results

Localization of *Yrqbc*

A genetic map was constructed from a set of 281 SSR markers by using bulked segregant analysis (BSA). Only one marker (*Xwmc175*) was detected to be polymorphic between the parental lines and exhibited linkage with the resistant and susceptible bulks. *Xwmc175* is located on the long arm of chromosome 2B. According to the chromosome specificity of SSR, *Xwmc175* was identified as a co-dominant marker. To enrich the location of *Yrqbc* on chromosome 2BL, 19 SSR makers (*Xcfd73*, *Xgwm501*, *Xgwm191*, *Xgwm120*, *Xgwm16*, *Xwmc332*, *Xwmc627*, *Xwmc149*, *XbarcM139*, *Xbarc1064*, *Xbarc167*, *Xbarc91*, *XbarcM147*, *Xbarc128*, *Xbarc101*, *Xgwm388*, *Xwmc435*, *Xwmc499*, *Xbarc1027*) close to *Xwmc175* were used for mapping. The results indicated that five primers (*Xwmc332*, *Xbarc167*, *Xbarc101*, *Xbarc91* and *Xgwm120)* were polymorphic.

To further confirm the genetic linkage between the six markers and *Yrqbc*, the F_2 segregating population of QBC × CS was investigated. By the chi-square test, the ratio of amplification of these markers was in accordance with the theoretical separation ratio of 1:2:1. This result suggests that these markers are reliable for genetic linkage map construction. As a result, the linkage map was constructed around *Yrqbc* by using these markers (Fig. 1; Supplementary Table 3). The results indicated that two SSR markers, *Xwmc332* and *Xwmc175*, were the closest flanking markers to *Yrqbc*, with distances of 6.7 and 10.1 cM, respectively.

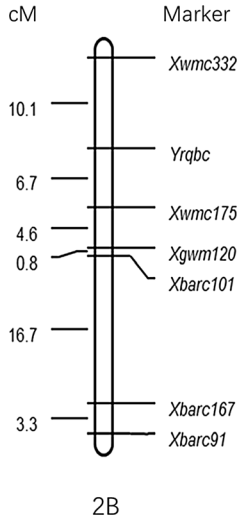

Fig. 1 Genetic linkage map of stripe rust resistance gene *Yrqbc* on chromosome 2BL based on SSR markers. *Xwmc332* and *Xwmc175* were the closest flanking marker to *Yrqbc* with distances of 6.7 and 10.1 cM, respectively

Fine mapping of *Yrqbc*

The target gene (*Yrqbc*) was mapped to the end of the 2BL chromosome by analysing the BSR-Seq data. The associated SNPs are mainly concentrated in the 633–653 Mb, 673–693 Mb and 723–763 Mb regions of 2BL (the total length of 2B is 801 Mb) (Supplementary Fig. 3). A total of 92 candidate SNPs in this region were polymorphic between the bulks. Six SNPs could be used to construct genetic linkage maps after genotyping.

A wheat 660 K array was used to map the resistance gene. Based on the above results, the genetic distance of the two flanking SSR markers (*Xwmc175 and Xwmc332*) to *Yrqbc* refer to 670–730 Mb on 2BL. In addition, 1714 SNPs in this interval were detected. With respect to the wheat genome version 1.0 (IWGSC) of Chinese Spring, the 1716 SNP flanking sequences (a total of 200–100 bp in the front and 100 bp in the back) were queried via local BLAST searches. A total of 457 specific SNPs and the flanking sequences were unique in the Chinese Spring reference genome. As a result, 14 SNPs were found to be linked to *Yrqbc* by genotyping.

Compared to Chinese Spring reference genome, the resistance gene *Yrqbc* was mapped to a 5.1 cM genetic interval between molecular markers *A009200* and *A009192* by screening the segregating population of the polymorphic markers, and the genetic distance was 0.1 cM and 5.0 cM, respectively (Fig. 2; Supplementary Table 4).

Yr5 identification

The physical location of *Yrqbc* indicates that it may be allelic to the previously reported *Yr5* in the same region. However, the associated markers of *Yr5* suggested that *Yrqbc* and *Yr5* may not be the same gene according to a previous report. For example, *Xbarc349*, *Yr5STS7/8*, *S19M93-140*, *S23M41-310*, *Xwgp19*, *Xwgp26* and other markers were not polymorphic in the F_2 segregating population of QBC × CS (Murphy et al. 2009; Yan et al. 2003). Compared to markers in previous linkage maps, the only molecular marker with polymorphism was *Xwmc175* (Supplementary Fig. 1) (Xu et al. 2013). *Yr5* was

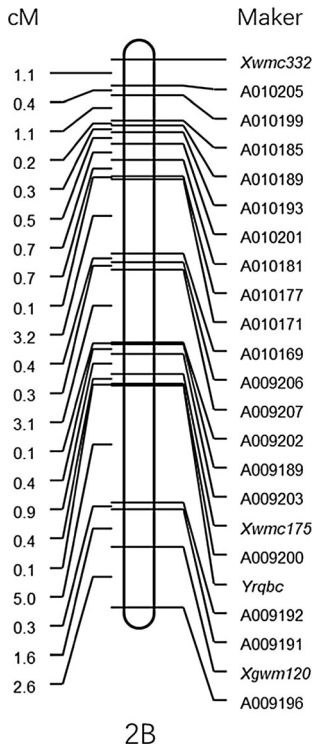

Fig. 2 Genetic linkage map of stripe rust resistance gene *Yrqbc* on chromosome 2BL based on KASP and SSR markers. *A009200* and *A009192* were the closest flanking marker to *Yrqbc* with distances of 0.1 and 5.0 cM, respectively. And the flanking SSR makers of *Yrqbc* are *Xgwm120* and *Xwmc175*, respectively

cloned and reported when we sought to further enrich the linkage map. According to the specific *Yr5* marker (Clemence et al. 2018), the results indicated that *Yrqbc* may be identical to *Yr5* (Supplementary Fig. 2). Furthermore, no difference was detected between the two sequences of *Yrqbc* and *Yr5* by sequencing using the improved primers.

Yr5 and *Yr18* utilization in cultivars

Afterward, 323 wheat cultivars (lines) were collected, including 42 CIMMYT cultivars (lines), 6 European cultivars (lines), 4 Australian cultivars (lines), and 271 cultivars (lines) of major wheat regions in China. The *Yr18* and *Yr5* diagnostic markers were used to detect whether these cultivars (lines) contained *Yr18* or *Yr5*. The results showed that 27 cultivars (lines) carry the *Yr18* gene and that only one cultivar (line), Zhongkemai 90, has the gene *Yr5*. According to the design principle of the marker *Yr5*-insertion, we found that 85 cultivars (lines) contain the *Yr5* allele, which has a 774 bp deletion compared to *Yr5* (Supplementary Fig. 3). Due to this 774 bp deletion, these cultivars did not have stripe rust resistance. None of the 323 cultivars (lines) had the naturally pyramided of the two genes.

Utilization analysis of QBC

12 Chinese high-yield elite cultivars were screened, which contains neither the *Yr5* nor *Yr18* gene (Supplementary Table 6). Backcross breeding and marker-aided selection (MAS) methods were used for introducing resistance gene into elite cultivars (lines). The results showed that, due to the complexity of the spontaneous structure of the stripe rust fungus and the genetic background of the parents, the resistance of the single stripe rust resistance gene (*Yr5* or *Yr18*) does not reach the immune effect (IT = 0; or 1). Only when the derived lines have two genes (*Yr5* and *Yr18*) to achieve the immune effect (IT = 0) (Fig. 3). The other agronomic traits of QBC were very close to these 12 elite cultivars, except for the plant height (Supplementary Table 7). This will greatly shorten the breeding cycle as only disease resistance and dwarf individuals are needed to screen in breeding program. Therefore, after the fourth generation, most of the derived lines have stabilized their disease resistance traits and other agronomic traits and no separation will occur (Fig. 4).

Discussion

QBC, a Tibetan wheat landrace, has been cultivated in Tibet and Sichuan for many years and displays near-immune resistance to wheat stripe rust. According to the epidemic of stripe rust in China, Sichuan is the inoculum base, the centre of diversity and a major over-summering area of this pathogen (Zeng and Luo 2006). Moreover, CYR34, the first major epidemic race, has been detected in this region, and few wheat cultivars show resistance to this species (Liu et al. 2010). As a result, it is urgent to transfer the genes in QBC to elite cultivars to improve wheat resistance to rust in this region.

To utilize the resistance genes, mapping populations were constructed. It was previously shown that there are two independent and dominant stripe rust

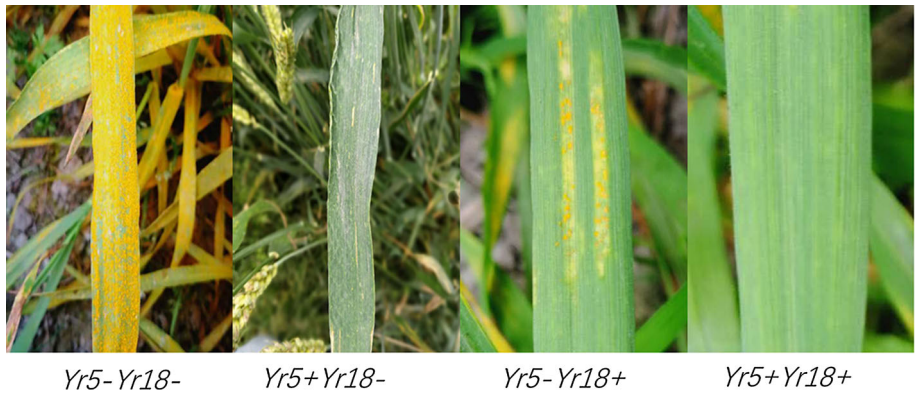

Fig. 3 The field investigation results of of QBC × elite cultivars. There were no urediospore of stripe rust on the leaves of *Yr5* + *Yr18* + plants, and its IT was recorded "0". The urediospore occurs in individual plants with only one gene; their ITs were recorded "1" or "2". The IT values of neither *Yr5* nor *Yr18* plants were recorded as 8 or 9

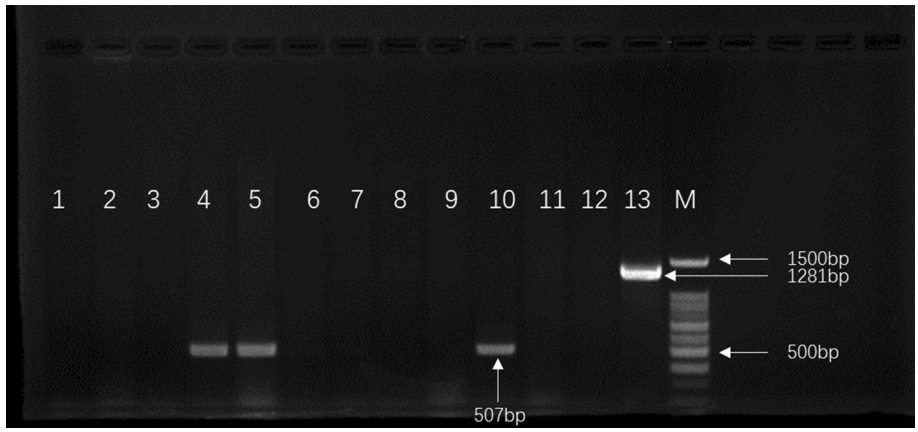

Fig. 4 The *Yr5* gene in 323 cultivars (lines) was detected using the *Yr5* functional marker. Material 13 is QBC; materials 4, 5, and 10 are Chuanmai 62, CIMMYT2015 and Fr10-11 (France), respectively. The rest of the materials are cultivars (lines) which not contain the *Yr5* gene

resistance genes in QBC. In addition, the leaves showed a phenotype of apical necrosis in the adult stage, which suggested it possibly carried a durable adult resistance gene. This gene was ultimately identified as *Yr18* by functional marker analysis. As a result, the other ASR gene needed to be identified. A combination of several biological strategies was applied to map this gene. BSA-SSR, BSR-seq, a wheat 660 K SNP array and KASP technology were used for fine mapping. The results indicated that this gene is located in the 5.1 cM genetic interval between molecular markers A009200 and A009192. In this region, several resistance genes have been reported, such as *Yr5*, *Yr7* and *YrSP*. Specifically, *Yr7* and *YrSP* provide susceptibility to epidemic races in Sichuan, especially CYR34. Furthermore, functional marker analysis and sequencing were applied, and the results indicated that *Yrqbc* was identical to *Yr5*. As a result, the two independent and dominant genes in QBC were *Yr5* and *Yr18*.

In recent years, pyramiding of high-level and durable resistance to stripe rust has been considered an effective method for controlling this disease. Cultivation of durable, resistant varieties has become the mainstream of international disease resistance breeding (Chen 2013; Hou et al. 2015). Plants with durable resistance genes are usually susceptible to disease at the seedling stage and are moderately susceptible to stripe rust at the adult stage. Yield will also decrease when the disease is severe. Furthermore,

spring wheat is very susceptible to stripe rust from the seedling stage to the milky stage, as the temperature and humidity are suitable for stripe rust growth. As a result, the ASR gene needs to be implemented. ASR genes can prevent rust infection at the early growth stage and can reduce rust inocula in epidemic regions. The presence of this gene will also slow the occurrence of new virulence combinations by genetic recombination. In general, the ideal condition of stripe rust resistance is the simultaneous presence of ASR such as *Yr5*, *Yr15*, *Yr26* and HTAP/slowing rust resistance genes such as *Yr18*, *Yr29*, *Yr36*, *Yr39* (Chen 2013).

Crop breeding generally starts from landraces which should be the basis for the improvement of varieties in various regions. They are native, long lasting and best adapted to the local natural environment and production conditions as well as the corresponding production potential. The phenotypic identification results showed that QBC provides immune resistance to all epidemic Chinese *Pst* races identified in the field and greenhouse, which indicated that QBC is an excellent landrace for wheat breeding in the winter wheat region of southwest China. This phenomenon may be explained by the combination of the ASR and durable adult resistance genes. In QBC, the ASR gene is *Yr5*, and the durable adult resistance gene is *Yr18*. *Yr5* showed a high level of resistance, and *Yr18* provides partial and durable resistance against the devastating fungal pathogens leaf rust, stripe rust, and powdery mildew. As a result, this excellent resistance material can be used as a parent to improve resistance to stripe rust. In China, resistance to stripe rust has improved in recent years. However, the disease-resistant types are relatively individual. Moreover, few cultivars show resistance to CYR34 (Liu et al. 2010). Therefore, it is necessary to further broaden the source of resistant materials and improve the diversity of disease resistance genes. Moreover, the aggregation breeding of disease resistance genes throughout the whole growth period and in the adult stage is important.

Breeders and researchers have been searching for durable and high-level resistance for many years (Johnson 1981). It is believed that a "pyramiding strategy" is an effective and easy way to achieve this goal (Mallick et al. 2015; Yanmin et al. 2019). However, this strategy is not easily practicable. Many studies have reported that pyramiding genes are easily lost during hybridization (Liu et al. 2018). As a result, utilizing cultivars that already contain pyramided genes is practical. QBC, a landrace from Tibet, has been planted for decades in Sichuan. It shows durable and high-level resistance to stripe rust not only in the adult stage but also in the seedling stage. The two resistance genes, *Yr5* and *Yr18*, are naturally pyramided. These pyramided two types of resistant genes (ASR and durable adult resistance) show enhanced resistance to stripe rust according to the results of the resistance of a single gene. In turn, the pyramiding of resistance genes was able to achieve durable and high-level resistance.

Elite cultivars quickly lost their resistance in China due to the emergence of new stripe rust races. *Yr9*, *Yr10*, *Yr17*, *Yr24* and other previously widely used resistance genes in production nearly lost their resistance. Even *Yr5* has been identified as a moderate resistance gene in some rust disease epidemic years. As a result, the application of additional resistance gene resources in China is urgently needed. However, few practical applications of pyramided resistance genes, such as *Yr5* and *Yr18*, have been successful in breeding so far (Singh et al. 2008; Zeng et al. 2014; Maccaferri et al. 2015; Kankwatsa et al. 2017). In 2014, 134 Sichuan wheat varieties (lines) were evaluated, and only a few genes, such as *Yr5*, *Yr10*, *Yr15* and *Yr50*, maintained resistance to rust in field identifications (Ren et al. 2014). Moreover, only 17.9% of the varieties (lines) contained adult-plant resistance genes. The occurrence of *Yr5* and *Yr18* was very low among 672 wheat accessions, and no pyramided *Yr5* and *Yr18* genes were detected (Zheng et al. 2017). Furthermore, the combination of *Yr5* and *Yr18* is seldom used in the main cultivars in major wheat-growing provinces in China (Jin et al. 2018; Miaomiao et al. 2018; Dong et al. 2012; Zhou et al. 2017; Wang et al. 2018). Our results of 323 cultivars (lines) marker analysis also demonstrate that the *Yr5* and *Yr18* resistance genes are used relatively infrequently in wheat cultivars (lines) worldwide, and no cultivars (lines) have both genes at same time. If the outbreak of stripe rust occurs at this time, it will cause immeasurable losses to production. Therefore, implementation of a "pyramiding strategy" should be accelerated. The application of QBC may accelerate the deployment of *Yr5* and *Yr18* and could improve the disadvantage of monoculture deployment of resistance genes. Many research teams have initiated research on

the pyramiding of resistance genes on chromosomes 2B and 7B.

In recent years, the large-scale use of wheat backbone parents has increased the homogenization of varieties and significantly reduced gene polymorphism, which has become one of the important bottlenecks restricting wheat breeding all over the world. QBC, a landrace from Tibet, contains the naturally pyramided *Yr5* and *Yr18* genes. *Yr5* serves as an ASR gene and *Yr18* serves as a adult-plant gene, which together provide high-level and durable resistance to all races. It is effective and practical to improve the resistance of local elite varieties by introducing pyramided genes. Therefore, this Tibetan landrace could be used as an excellent germplasm to develop high-level and durable resistant varieties in breeding program.

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Author contributions T-W coordinated the project, conceived and designed experiments. B-F conducted the bioinformatics work, generated and analyzed data, and edits the manuscript. Z-B X and F-X L collected the samples; F-W, G-S J and Q-Z performed the laboratory work. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare they have no competing interests.

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