Genetic Analysis of Adult Plant Resistance to Stripe Rust in Common Wheat Cultivar “Pascal”

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INTRODUCTION

Wheat stripe rust, also known as yellow rust, caused by the air-borne fungus Puccinia striiformis f. sp. tritici (Pst), is considered the primary biotic threat to wheat production globally (Todorovska et al., 2009). In 2017, 88% of the world’s wheat production was susceptible to stripe rust, causing yield losses estimated to be 1 billion USD (Brandt et al., 2021), and a countrywide epidemic affected about 5.5 million ha of wheat in China (Zeng et al., 2022). It shows regional characteristics and occurs frequently in the southwestern and northwestern of China, especially in the southeastern Gansu, a stripe rust hotspot in northwestern of China (Bai et al., 2012). The management of...
Genetic resistance to stripe rust in wheat can be broadly divided into two phenotypically, mechanistically, and genetically distinct categories: (a) seedling resistance (all-stage resistance, ASR), which is detected at the seedling stage but is also expressed at all developmental stages. It usually shows a major effect and is race specific (Roberts and Caldwell, 1970; Lin and Chen, 2007); (b) adult plant resistance (APR), which is expressed at the adult plant stages and appears to be durable. Some APR genes are less characterized and are difficult to select phenotypically (Ren R. et al., 2012).

To date, most identified stripe rust resistance genes conferring "seedling resistance" encode classic nucleotide-binding site leucine-rich repeat (NBS-LRR) R proteins that recognize effectors and trigger a defense response to resist disease. While it is easy to overcome the recognition of a single classical R gene via a single genetic variation in an avirulence gene (Steurnagel et al., 2016; Schwessinger, 2017). The major epidemics causing severe economic losses have occurred in various regions of the world including United States, China, and Australia (Nsabiyera et al., 2018) due to virulence on seedling resistance genes including Yr2, Yr6, Yr9, Yr17, and Yr27 (Wellings and McIntosh, 1990; Nsabiyera et al., 2018). Only a few seedling stripe rust resistance genes (i.e., Yr5 and Yr15) are still effective to some Pst races in China, which drives the demand for persistent disease-resistant cultivars.

Adult plant resistance is generally known as more durable than ASR, since a single genetic variation appears insufficient to overcome this type of resistance in the asexual stage of Pst (Schwessinger, 2017). In general, APR delays infection and production of spore, leading to slow rusting phenotypes instead of complete immunity. This type of resistance gene usually encodes allele-specific protein variants that are molecularly unrelated to NBS-LRR proteins. In the case of these genes, Yr18 and Yr46 encode two types of transporters (Krattinger et al., 2009; Moore et al., 2015). In addition, Yr36, a chloroplast-localized kinase WKS1 regulating, reactive oxygen species production (Ellis et al., 2014; Gou et al., 2017). Since the durability of R genes is usually defined by the global genetic diversity of the pathogen, the combination of APR genes with ASR genes is the best way for a wheat breeder to develop the durable stripe rust resistant wheat variety at all plant stages. To date, more than 83 Yr genes or alleles (Yr1–Yr83) have been formally named in wheat (Li et al., 2020; Draz et al., 2021). Most of them are race-specific ASR genes, and only a few are APR genes. Lr34/Yr18/Pm38/Sr57 (Dyck, 1991), Lr46/Yr29/Pm39/Sr58 (Singh et al., 1998), and Lr67/Yr46/Pm46/Sr55 (Herrera-Foessel et al., 2011) confer pleiotropic APR to stripe rust, leaf rust, powdery mildew, and stem rust. QTL analysis has been widely used to dissect complex traits through identifying the genomic location and effects of genes contributing to quantitative variation (Young, 1996). Over the past 20 years, more than 320 stripe rust resistance QTLs have been reported using different molecular markers, including diversity array technology (DArT), single sequence repeats (SSRs), and single-nucleotide polymorphisms (SNPs) (Lan et al., 2017); the genetic locations of these QTLs are continually being refined through fine mapping studies (Lan et al., 2017; Draz et al., 2021).

Various approaches can be used to identify genetic loci and genes in wheat. High-density SNP markers using gene-chip technology provide a superior approach for QTL mapping due to their high-throughput, efficiency, allele specificity, and high-resolution capacity (Yu et al., 2011; Draz et al., 2021). The Axiom wheat 55K SNP array with 53,063 SNP probes was selected from the wheat 660K SNP array, which is considered to be more appropriate for wheat genetic research (Ren et al., 2018; Huang et al., 2019). Based on the advantages of lower costs, higher accuracy, and its medium density, this SNP array has been widely used in QTL identification in wheat (Huang et al., 2019; Zhang et al., 2019). Also, whole-genome exome sequencing (WES) has been successfully applied for the identification of genetic loci and isolates genetics in wheat (Henry et al., 2014; Mo et al., 2018). For instance, the candidate natural variants were identified using a 110 Mb exome capture assay at the Yr6 locus responsible for stripe rust resistance (Gardiner et al., 2016). A BSA-based exome capture sequencing pipeline for rapid gene cloning has been developed by Chengdu Tcuni Technology, which is an alternative method to effectively sequence coding regions with low cost in wheat (Dong et al., 2020). Therefore, SNP chip and WES approaches can be the effective strategies to find new genetic loci and candidate genes associated with important traits in wheat.

The Italian common winter wheat cv. Pascal (Lan Yin 1), introduced to Gansu Province of China in 1995, showed a high level of APR to stripe rust in the field but was susceptible to CYR32 and CYR33 at the seedling stage. However, the seedling gene locations and inheritance of APR to stripe rust in Pascal were not clear. Thus, the objectives of this study were to (1) map seedling stripe rust resistance gene in the Huixianhong/Pascal F5 RIL population; (2) identify the APR to stripe rust in the same population; (3) determine the interaction effect between the identified resistance loci on stripe rust in the adult plant stage; and (4) develop molecular markers of new and stable resistance QTL for the wheat breeder to breed durable stripe rust-resistant wheat variety.

**MATERIALS AND METHODS**

**Parent Materials**

A population of 220 F5 RILs was developed from a cross between “Huixianhong” and “Pascal.” “Pascal” is a stripe rust-resistant parent, whereas the cultivar “Huixianhong” is highly susceptible to stripe rust in the field. Huixianhong was widely used as a susceptible spreader and negative control in the genetic analysis of wheat stripe rust resistance. A single seed descend method was used to develop the F5 RIL population.

**Seedling Test**

The parents were evaluated for their reaction to stripe rust at the seedling stage using the Pst races CYR32 and CYR33 under greenhouse conditions at Huazhong Agricultural University, and
the reaction to CYR34 was tested in the Gansu Academy of Agricultural Sciences. The races of the pathogen were inoculated by spraying urediniospores suspended in mineral oil using an atomizer when wheat seedlings reached the two-leaf stage. The inoculated plants were left in an open area for 20 min to facilitate the evaporation of the oil and then placed in the dew chamber of 7°C for 24 h and back to the greenhouse. The infection types (ITs) were recorded approximately 2 weeks post-inoculation, based on a 0–9 scale modified from McNeal et al. (1971), and ITs with the scale of “0, 1, 2, 3, 4,” “5, 6,” and “7, 8, 9” are categorized as resistant, intermediate, and susceptible groups, respectively.

**Adult Plant Stage of Field Test**

The parents and RIL population were evaluated for APR to stripe rust in Qingshui Experimental Station of Gansu Academy of Agricultural Sciences during the 2017–2018 and the 2018–2019 growing seasons (GS2018 and GS2019), and in 2018–2019 growing season in Pidu Experimental Station of Sichuan Academy of Agricultural Sciences (SC2019).

Field plots consisted of 1.5-m rows planted with approximately fifty seeds of each line, and we used randomized complete block design with two replicates of the RIL population in each location. “Jinmai 47” was planted at every tenth line and parents using the cetyltrimethylammonium bromide (CTAB) method (Chatterjee et al., 2002). The 55K SNP array was used to genotype the parents and the second rating about a week later when the DS of the susceptible check reached 90–100%. Combined with the natural infection, a mixture of races CYR32, CYR33, and CYR34 was used to inoculate the spreaders at the jointing stage (around 2 months after planting). The lme4 package in Rstudio was used to calculate the best linear unbiased prediction (BLUP).

**Correlation Analysis of the Phenotypic Data**

SAS 9.2 software (SAS Institute, Cary, NC) was used to calculate the correlations of final disease severity (FDS) for stripe rust in each season. The analysis of variance (ANOVA) was used to assess the significant effect of a single stripe rust resistance QTL and the interaction effect for multiple stripe rust resistance QTLs with the FDS values in each environment.

**Genetic Linkage Map Construction and Quantitative Trait Locus Mapping**

Deoxyribonucleic acid was extracted from about 20 plants of each line and parents using the cetyltrimethylammonium bromide (CTAB) method (Chatterjee et al., 2002). The 55K SNP array genotyping platform was used to genotype the parents and RIL population. A total of 55,000 SNP markers were obtained, of which 9,475 SNP markers were selected to make linkage groups by removing markers of distorted segregation ($p < 0.001$), monomorphic markers, and markers with more than 30% missing data. Genetic linkage groups were established using Joinmap 4.1 (Van, 2011) with a logarithm of odds (LODs) threshold of 10.0. MapChart (Voorrips, 2002) was used to draw genetic linkage maps. Software IciMapping 4.1 (Meng et al., 2015) was used to detect FDS-related QTL in each environment, BLUP and mean of final disease severity (MFDS) across each season to obtain the significant QTL position, LOD scores, phenotypic variance explained (PVE), and the additive effect of each locus. About one-thousand permutations were used to calculate LOD scores for each trait. The physical positions of identified QTL were compared with those previously reported on the same chromosome arm under different wheat lines, based on the wheat genome of (International Wheat Genome Sequencing Consortium [IWGSC], 2018).

**Bulked Segregant Exome Capture Sequencing**

The bulked segregant exome capture sequencing (BSE-Seq) was used for identifying causal mutations or candidate genes in this study. First, the genomic DNA of 33 extremely resistance (low FDS) lines and 43 extremely susceptible (high FDS) lines of Huixianhong and Pascal was also extracted using the same method as above. Therefore, the resistant-type and susceptible-type parent DNA were used to bulk DNA pool, resistant-type bulked DNA pool, susceptible-type bulk, respectively. Theoretically, the ED values of other loci should tend to 0 except for the target trait-related sites between bulks and evaluates the region associated with the target trait (Hill et al., 2013).

$$ ED = \sqrt{(A_{mut} - A_{wt})^2 + (T_{mut} - T_{wt})^2 + (C_{mut} - C_{wt})^2 + (G_{mut} - G_{wt})^2} $$

In the formula, $A$, $T$, $C$, and $G$ mut are the frequencies of $A$, $T$, $C$, and $G$ bases in the mutant bulk, respectively; $A$, $T$, $C$, and $G$ wt are the frequencies of $A$, $T$, $C$, and $G$ bases in the wild-type bulk, respectively. Theoretically, the ED values of other loci should tend to 0 except for the target trait-related sites between the two mixed pools.

**RESULTS**

**Phenotypic Analysis**

Both Huixianhong and Pascal were susceptible after being inoculated by CYR32, CYR33, and CYR34 at the seedling stage (IT varied from 78 to 8) (Figures 1A,B). However, the MFDS
for Huixianhong and Pascal were 100 and 3%, respectively, in the adult plant stage (Figures 1C,D) over three environments. The frequency distributions of stripe rust severity among F_5 RILs showed a normal distribution (Figure 2), indicating a typical quantitative character for controlling stripe rust resistance in the Huixianhong/Pascal population. Pearson correlation coefficients (r) of stripe rust severity ranged from 0.66 to 0.85 over the environments (Table 1). In addition, it was estimated that there were around 3–4 stripe rust resistance genes with additive effects in the Huixianhong/Pascal RIL population based on the qualitative assessment of the Mendelian genetic segregation ratios (Table 2).

**Genetic Linkage Map Construction**

A genetic linkage map was constructed with a total of 9,475 markers and developed 28 linkage groups on the 21 wheat chromosomes. A total of three QTLs were identified for stripe rust resistance from “Pascal” and they were mapped on wheat chromosomes 1AL (QYr.gaas-1AL), 3DL (QYr.gaas-3DL), and 5AS (QYr.gaas-5AS) (Table 3). QYr.gaas-1AL was flanked by SNP markers AX-111218361 and AX-110577861 and located at 505.3–507.9 Mb based on IWGSC RefSeq v1.0 genome sequence information. It was stably detected in all tested environments with PVE values ranging from 11.3 to 23.1% (Table 3). QYr.gaas-3DL was closely linked to markers AX-108798599, AX-109580758, and AX-111460455. It was located around 354.6 Mb based on IWGSC RefSeq v1.0 genome sequence information and was also consistently detected in all three stripe rust field experiments explaining 16.1–20.6% total stripe rust variation (Table 3). The third QTL, QYr.gaas-5AS, was flanked by SNP markers AX-111523523 and AX-110028503 and mapped at the interval of 59.1–62.2 Mb based on IWGSC 1.0 with PVE values ranging from 11.0 to 17.3% (Figure 3C and Table 3).
Interactive Effects of Detected Resistance Loci

Based on the flanking molecular markers of detected three stripe rust resistance QTLs, we divided the F₅ RILs into 8 groups. The presence of parental alleles for each QTL was determined by markers inferred factorial combinations of the three stripe rust resistance QTLs.

There was a significantly different disease response between RILs carrying either QYr.gaas-1AL or QYr.gaas-3DL and RILs without any resistance loci (p < 0.0001), which explained 24.2 and 19.3% of the stripe rust variation, respectively (Table 4). The lines carrying QYr.gaas-5AS also showed a significantly different disease response from those without any resistance locus, but this locus only explained 8.5% of the stripe rust variation. This indicates that this third locus imparts a minor effect on stripe rust resistance than the other two loci (Table 4). A significant interaction (p < 0.0001) between QYr.gaas-1AL and QYr.gaas-3DL was observed across three environments as well as MFDS, which explained 3.3% of stripe rust variation based on MFDS. There were no significant differences among the average FDS of RILs carrying combinations of QYr.gaas-1AL and QYr.gaas-3DL and Qyr.gaas-5AS, Qyr.gaas-3DL and Qyr.gaas-5AS, and QYr.gaas-1AL and QYr.gaas-3DL and Qyr.gaas-5AS (p > 0.05); however, the average FDS of RILs carrying combinations of three resistance QTLs was significantly lower than the average FDS of RILs carrying other combinations of two resistance QTLs (p < 0.0001) (Table 4).

Bulked Segregant Exome Capture Sequencing

We filtered the data obtained by BSE-Seq with ref_Freq > 0.3, minDepth > 5 and ED = top 0.05 through the ED algorithm, and the results showed that there was a clear peak on chromosome 1AL, which is roughly around 506 Mb (Figures 4A,B). The SNPs between R bulk (same as resistance cultivar Pascal) and S bulk (same as susceptible cultivar Huixianhong) were filtered by AF < 0.2 or AF > 0.8, and the significant SNPs were found in a region of ~506 Mb on chromosome 1A (Figures 4C,D). This result is also consistent with our mapping result, which indicates significant differences between two resistant and susceptible bulks and showed a high correlation between this region and stripe rust. Unfortunately, we failed to find high confidence SNPs...
on chromosome 3D through BSE-Seq, which may be related to the collection stage of materials or different process of related gene expression.

For fine mapping QYr.gaas-1AL, we developed three Kompetitive allele-specific PCR (KASP) markers near QYr.gaas-1AL based on the SNP of BSE-Seq. QYr.gaas-1AL was 0.5–3.5 cM away from KASP marker BSE-1A-12 (Supplementary Table 1 and Figure 3A), which covered a physical interval of 505.3–506.8 Mb on chromosome 1A. There were 18 high confidence genes in this interval, whereas 13 genes have SNPs on their exon regions. *TraesCS1A02G313700* has 14 SNPs on its exon regions which encode a dentin sialophosphoprotein-like protein. *TraesCS1A02G313800* and *TraesCS1A02G314900* encode early light-induced protein and metacaspase, respectively, which have light-induced protein and metacaspase, respectively, which have

**The Distribution of QYr.gaas-1AL and QYr.gaas-3DL**

Since QYr.gaas-3DL contributed stripe rust resistance in Pascal significantly, we converted SNP marker AX-109580758 to a KASP marker and combined it with the phenotypic data for QTL mapping in 220 RIL families. The KASP marker HXPA-3D was 1.5–3.5 cm away from QYr.gaas-3DL (Figure 3B). This fact suggests that KASP marker HXPA-3D can be used as an effective marker to detect QYr.gaas-3DL for developing durable stripe rust resistant wheat varieties.

A total of two KASP markers BSE-1A-12 and HXPA-3D for two stable loci were used to genotype a collection of 153 Chinese and global germplasm resources. In the global collection, QYr.gaas-1AL appears more frequently (69%), as compared to the Chinese wheat germplasm in which QYr.gaas-1AL appeared in 62%. Similarly, QYr.gaas-3DL is very common in foreign germplasm (75%), and it is significantly lower in Chinese wheat varieties and breeding lines (60%) (Supplementary Table 2). Our results suggest that Pascal could be a useful source of effective resistance against stripe rust in China.

**DISCUSSION**

“Pascal” was conferred by two recessive genes effective against two *Pst* races CYR 31 and CYR 32, respectively, at the seedling stage (Cao et al., 2006). In this study, we found both parents, Huixianhong and Pascal, susceptible to three *Pst* races of CYR32, CYR33, and CYR34 at the seedling stage (IT varied from 7 to 8). Most of the stripe rust resistance genes conferring seedling resistance can easily lose their effect since selection pressure favors the virulence in the pathogen population, which could explain that Pascal does not provide seedling resistance to stripe rust in this study. The constant evolution of pathogens and thus the breakdown of seedling resistance make our effort to continuously identify the sources of adult plant resistance increasingly important. We mapped three adult plant resistance QTLs for stripe rust in the Huixianhong/Pascal RIL population, and Pascal showed stable resistant over the last 50 years. There were five stripe rust resistance genes on chromosome 3D, viz. Yr45 (Li et al., 2011), Yr49 (Ellis et al., 2014), Yr66 (Bariana et al., 2022), Yr71 (Bariana et al., 2016), and Yr73 (Dracatos et al., 2016). Yr45, Yr71, and Yr73 were mapped on the 3DL, and the other two genes were mapped on 3DS. Yr45 was discovered from a common spring wheat “PI181314,” flanked by RGAP markers wgp118, wgp115, and two SSR markers Xwmc656 and Xbarc6.

| TABLE 3 | Position and effect of quantitative trait loci (QTL) that were detected for adult plant resistance (APR) to stripe rust (YR) in the test years, using each final disease severity, the mean of final disease severity (MFDS), best linear unbiased prediction (BLUP) for the Huixianhong/Pascal RIL population. |
| QTL | Traits | Chr | Pos (cM) | Marker interval | Phy pos (Mb) | LODa | PVE (%b) | Addc |
| QYr.gaas-1AL | GS2018 | 1A | 202 | AX-111218361—AX-110577861 | 505.3–507.9 | 11.2 | 23.1 | 15.3 |
| QYr.gaas-1AL | GS2019 | 1A | 202 | AX-111218361—AX-110577861 | 505.3–507.9 | 4.8 | 11.3 | 9.9 |
| QYr.gaas-1AL | SC2019 | 1A | 202 | AX-111218361—AX-110577861 | 505.3–507.9 | 19.6 | 15.3 | 22.0 |
| QYr.gaas-1AL | YRM | 1A | 202 | AX-111218361—AX-110577861 | 505.3–507.9 | 27.2 | 16.2 | 19.9 |
| QYr.gaas-1AL | BLUP | 1A | 202 | AX-111218361—AX-110577861 | 505.3–507.9 | 27.2 | 15.9 | 17.3 |
| QYr.gaas-3DL | SC2019 | 3D | 350 | AX-111460455—AX-108798599 | 354.6–360.5 | 17.4 | 16.1 | 19.0 |
| QYr.gaas-3DL | YRM | 3D | 350 | AX-111460455—AX-108798599 | 354.6–360.5 | 18.4 | 20.6 | 15.9 |
| QYr.gaas-3DL | BLUP | 3D | 350 | AX-111460455—AX-108798599 | 354.6–360.5 | 18.1 | 20.3 | 13.8 |
| QYr.gaas-3DL | GS2018 | 3D | 351 | AX-108798599—AX-109580758 | 339.7–354.6 | 13.1 | 16.4 | 15.3 |
| QYr.gaas-3DL | GS2019 | 3D | 351 | AX-108798599—AX-109580758 | 339.7–354.6 | 15.5 | 17.5 | 17.4 |
| QYr.gaas-5AL | SC2019A | 5A | 197 | AX-111523523—AX-110028503 | 59.1–62.2 | 5.4 | 11.0 | 12.8 |
| QYr.gaas-5AL | GS2019B | 5A | 198 | AX-111523523—AX-110028503 | 59.1–62.2 | 4.9 | 13.2 | 10.3 |
| QYr.gaas-5AL | GS2019B | 5A | 198 | AX-111523523—AX-110028503 | 59.1–62.2 | 6.9 | 15.7 | 12.6 |
| QYr.gaas-5AL | YRM | 5A | 198 | AX-111523523—AX-110028503 | 59.1–62.2 | 6.3 | 15.8 | 11.3 |
| QYr.gaas-5AL | BLUP | 5A | 198 | AX-111523523—AX-110028503 | 59.1–62.2 | 16.4 | 17.3 | 17.5 |

aLogarithm of odds (LOD) score of QTL peak.
bProportion of phenotypic variance explained by the QTL.
cAdditive effect of phenotypic for each QTL.
which were mapped 11.7 and 12.6 cM proximal to Yr45. We tried to test the two parents using markers wgp115, Xwmc656, and Xbarc6 (Li et al., 2011). However, none of them are polymorphic, and the physical interval of Yr45 was from 476.9 to 514.9 Mb on chromosome 3D, whereas QYr.gaas-3DL was located around 354.6 Mb on the same chromosome. Besides, Yr45 is an all-stage resistant gene, whereas QYr.gaas-3DL was an APR locus in wheat, and therefore, Yr45 could be different from QYr.gaas-3DL. Yr71 is an adult plant resistance gene mapped on 3DL in the Australian cultivar Sunco, and the physical position of its flanking
Previous studies have detected several QTLs for stripe rust on chromosome 1AL in wheat. A minor APR QTL QYr.sgi-1A was located on chromosome 1A in the population of Kariega/Avocet S, contributing 6–12% to the phenotypic variance, and flanked by markers s15m19D and s23m18E, but was inconsistently detected across environments (Ramburan et al., 2004). Unfortunately, we failed to obtain the physical location of this QTL. Whereas QYr.gas-1AL had PVE values ranging from 11.3 to 23.1%, it was stable across all environments in our studies, indicating that QYr.sgi-1A was unlikely to be the same locus as QYr.gas-1AL. Bariana et al. (2010) identified QYr.sun-1A controlling APR to stripe rust in Kukri/Janz-derived doubled haploid (DH)
population, which explained 6–7% phenotypic variation for stripe rust. This QTL was flanked by marker Xgwm164 and its physical position was around 280.6 Mb. But QYr.gaas-1AL was located in the interval of 505.3–507.9 Mb, which indicated that 9QYr.gaas-1AL was different from QYr.sun-1A.

Ren Y. et al. (2012) identified a QTL (QYr.caas-1AL) contributed APR to stripe rust in German spring wheat cultivar Naxos. It was located in marker interval XwPt-2406–Xwmc59 at 575.4 Mb, which was roughly 70 Mb away from QYr.caas-1AL. Besides, QYr.caas-1AL only explained 8.2% of the phenotypic variance in one environment, which showed lower effect and stability than QYr.gaas-1AL. QYrid.ui-1A, a minor high-temperature adult plant (HTAP) resistance QTL from the hard red winter wheat germplasm IDO444, were significant only for IT in single environment (Chen et al., 2012). QYr.gaas-1AL had high effect of stripe rust resistance and were stable across all environments. Gebrewahid et al. (2020) identified an APR QTL on chromosome 1A in spring wheat Fuyu 3 designated QYr.hebau-1AL, which was located on the interval of AX-109403007 to AX-110502416 around 286.6–289.3 Mb. This QTL should be different from QYr.gaas-1AL based on a physical distance of about 220 Mb between them. Grover et al. (2022) identified an ASR locus Yraci in the European winter wheat cultivar “Acienda” on the distal end of wheat chromosome 1A. Besides, two SNPs AX-95162217 and AX-94540853 linked with Yraci were mapped in the bin at 54.04 cM on chromosome 1A, whereas QYr.gaas-1AL covered a physical interval of 505.3–506.8 Mb on chromosome 1A was far away from Yraci. Therefore, Yraci and QYr.gaas-1AL were not the same QTL for strip rust. Further research is needed to determine the novelty of QYr.gaas-1AL from Pascal.

Combining BSA and the exome sequence strategy could accelerate gene mapping, especially in wheat with a large and complex genome. BSE-Seq is helpful for the construction of a linkage map across the whole genome, and it could be easily used to identify the linked interval regardless of the multiple gene copies and obtain most of the variations existing in the coding regions of genes (Dong et al., 2020). Using bulked segregant analysis and the exome sequence strategy, Mo et al. (2018) identified a clear peak region on chromosome 4BS associated with increased plant height. Harrington et al. (2019) identified a locus controlling an environmentally dependent chlorosis phenotype in the Durum wheat cv. Kronos. Martinez et al. (2020) finely mapped a novel TaMKK3 allele conferring the wheat ERA8 ABA-hypersensitive germination phenotype in a wheat backcross population. In our studies, BSE-Seq helped us to confirm the reality of QTL and select candidate genes in QTL QYr.hebau-1AL region. TraesCS1A02G313700, TraesCS1A02G313800, and TraesCS1A02G314900 had more than 10 SNPs on their exon regions. Of these genes, TraesCS1A02G314900 encodes metacaspase, which has been reported to modulate autophagy to confine cell death to the target cells during Arabidopsis vascular xylem differentiation (Escamez et al., 2016), which suggests that this gene may be a strong candidate related to disease resistance. Further research is needed to determine the relationship between these genes and stripe rust resistance.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

BB initiated the project, designed the experiment, and contributed to phenotype data. CL designed the experiment.
and finalized the manuscript. ZL assisted in the data analysis and contributed to drafting the manuscript. HW and XD performed the sample preparation and DNA extraction. LW and JD contributed to the part of phenotype data in the field. All authors read and approved the final manuscript.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.918437/full#supplementary-material
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