QTL Mapping for Adult Plant Resistance to Stripe Rust in Wheat line "Guixie 3"

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Research Article

Keywords: common wheat, stripe rust, adult plant resistance, SNP array, quantitative trait loci (QTL)

Posted Date: February 18th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-207823/v1>

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Abstract

Development of cultivars with multiple resistances has proven to be an effective way to prevent diseases in wheat breeding. The Guixie 3 variety (GX3) has shown excellent performance in resistance to stripe rust in field for many years. The purpose of this study was to detect quantitative trait loci (QTL) associated with resistance to stripe rust in the adult plant stage and determine closely linked molecular markers. A population of recombinant inbred lines (n=228) was derived from a cross between the susceptible landrace Mian 96-5 (M96-5) and GX3 variety and evaluated in multiple field studies to elucidate genetic resistance by identifying QTL specifically for stripe rust resistance. A total of 19 QTL located on 12 chromosomes showed resistance to wheat stripe rust when studying phenotypic data from multiple field tests over the course of six years. These chromosomes included 1B (2), 1D (2), 2A (2), 2B (2), 2D (1), 4B (2), 4D (1), 5A (3), 5B (1), 6A (1), 6B (1), and 7B (1). Two stable QTL on chromosomes 2AS (*Qyr.gaas.2AS*) and 6AS (*Qyr.gaas.6AS*) were detected in six and five different environments, respectively; both QTL were derived from the GX3 variety. *Qyr.gaas.2AS* was found to be crucial for increasing adult plant resistance, which may explain the large phenotypic variation of 45.52%. Our results provide theoretical and molecular insight for wheat breeding and suggest the cloning of genes associated with the GX3 variety may be beneficial in future studies.

Introduction

Wheat stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is one of the most damaging diseases associated with global wheat production (Wellings 2011). Since 1949, there have been four epidemics of wheat stripe rust in China (1950, 1964, 1990, and 2002), resulting in a loss of more than one million tons of wheat per year (Kang et al. 2015). The effective use of disease-resistant varieties is crucial for the control of wheat stripe rust. There are two main types of genetic wheat resistance to stripe rust. One is a resistance that presents itself at the seedling stage (or all-stage resistance [ASR]); this form of resistance is generally effective during the whole growth period. The second is adult plant resistance (APR), which usually provides partial resistance to all races at post-seedling stages.

Epidemics are caused by a loss of effective resistance genes against stripe rust in wheat production (Han et al. 2015). Currently, wheat varieties carrying the stripe rust resistance gene *Yr24/Yr26* that were once widely used in China (Hu et al. 2014), such as Chuanmai 42 (Liu et al. 2010) and Guinong 22 (He et al. 2011), have lost their resistance due to the emergence of a pathogenic group (V26). At the National Wheat Rust and Powdery Mildew Research Collaborative Group Meeting, which took place in China in 2016, the pathogenic group (V26) of Guinong 22 was officially named Chinese Yellow Rust 34 (CYR34). At present, among the 83 (*Yr1–Yr78*) officially designated resistance genes and 47 proposed resistance genes (Maccaferri et al. 2015; McIntosh et al. 2017), only a few seedling disease resistance (ASR) genes (*Yr5*, *Yr15*, *Yr53*, *Yr61*, *Yr64*, *Yr65*, and *Yr69*) and adult plant disease resistance genes (APR) (*Yr18*, *Yr30*, *Yr32*, *Yr36*, *Yr39*, *Yr52*, *Yr54*, *Yr59*, and *Yr62*) still maintain effective resistance to wheat stripe rust (Hou et al. 2016; Lu et al. 2014; Zeng et al. 2015; Zhou et al. 2015). Therefore, to ensure the sustainable management of wheat stripe rust in southwest China, it is imperative to discover new stripe rust resistance genes, identify resistance-associated molecular markers, and to ultimately breed new disease resistant wheat varieties.

In recent years, with the development of high-throughput genotyping technology, single nucleotide polymorphism (SNP) arrays have been widely used in wheat. An example includes the construction of a high-density genetic map with stripe rust resistance gene/quantitative trait locus (QTL) mapping (Chen et al. 2016a; Gao et al. 2016; Jighly et al. 2015; Liu et al. 2015; Winfield et al. 2015; Wu et al. 2018a; Wu et al. 2018b) and genome wide association analysis (Kertho et al. 2015; Liu et al. 2017; Naruoka et al. 2015; Zegeye et al. 2014). The wheat 55K SNP array is an economical medium-density SNP chip developed from the wheat 660K SNP array (Jia and Zhao 2016) and has been used in many different studies. The 660K SNP array has been used to provide a genetic map of the *P* genome of *Agropyron* (Zhou et al. 2018),

to identify the gene for grain weight using an integrated genetic map with > 100,000 SNPs (Cui et al. 2017), and to map QTL for stripe rust resistance in adult stage of wheat (Wu et al. 2017).

Wheat wild relatives can be used as a resource bank of disease resistance genes. Some genes have been officially classified for stripe rust resistance (Maccaferri et al. 2015). Wild emmer wheat (*T. dicoccoides*), a wild tetraploid ancestor of common wheat, has good resistance for wheat stripe rust and importantly shows great potential for wheat breeding. A number of resistant genes have been previously identified in wild emmer wheat, such as *YrH52*, *Yr15*, *Yr35*, and *Yr36* (Li et al. 2008; Peng et al. 1999; Uauy et al. 2005; Wang et al. 2018). Oat belongs to the *Gramineae Aveneae Dumort Avena* L. variety and has good resistance to biotic (wheat rust and scab) and abiotic (drought, cold, and barren) stress (Han et al. 2008; Sharma and Gill 1983; Zhang 1999). GX3 was obtained by distant hybridisation of wild emmer wheat (*T. dicoccoides*) with wild oat (*Avena fatua* L. var. *glabrata pat*) and then backcrossed with common wheat (Guinong 22). It has shown resistance to the current wheat stripe rust epidemic for many years. In this study, we use a wheat 55K SNP array to map QTL for APR to stripe rust in a GX3 with a recombinant inbred line (RIL) population of "M96-5/GX3", to identify tightly linked molecular markers for their use in future marker-assisted breeding.

Materials And Methods

Plant materials

The susceptible winter landrace line M96-5, and the resistant line GX3 were used as the parental lines for this study. The mapping population comprised of 228 F_{6:7} RILs from crossing M96-5×GX3. The GX3 line is a semi-winter, late maturity, long spikelet variety of common wheat and its entire growth period is an estimated 210 days. The susceptible line M96-5 has large spikelet with excellent agronomic traits and its growth period is an estimated 190 days. The RIL population was established by Dr. Zhou Qiang from the Chengdu Institute of Biology, Chinese Academy of Sciences (Chengdu, Sichuan Province). Avocet S (AVS) and SY95-71 were used as susceptible controls throughout the study.

Phenotyping

In this field trials, two parents and RIL populations were used to test resistance in mixed races of stripe rust within a natural setting. These were planted in October 2016 in Mianyang, Sichuan Province (31°23'N, 104°49'E); in October 2017, 2018, and 2019 in Guiyang, Guizhou Province (26°29'N, 106°39'E), and in November 2019 in Anshun, Guizhou Province (26°24'N, 105°96'E) and Shuangliu, Sichuan Province (30°57'N, 103°92'E). There were two rows per line, 10 lines per block, and the susceptible line AVS (or SY95-71) was planted every five lines as the control line. The first severe degree was recorded when the disease severity of AVS (or SY95-71) in the control group and the susceptible parent M96-5 reached 50% or more (i.e., the area of rust fungus accounted for more than half of the entire leaf). In accordance with the percentage of the total leaf area occupied by rust fungus, the severity of stripe rust was also recorded visually for each wheat family. Recordings were taken every other week until the susceptible control reached 100%, known as the maximum disease severity (MDS). The modified Cobb scale was referred to for the phenotypic data required for QTL analysis (i.e., 1, 5, 10, 20, 30, 50, 60, 80, and 100%) (Li and Zeng 2002).

Statistical analysis

To estimate the genetic and environmental effects in each line, we compared the environments and line×environment interactions using the AOV function in IciMapping 4.1 software (Zeng et al. 2019) and significance was measured by analysis of variance (ANOVA). The correlation between multiple field conditions was analysed by the Pearson method in SPSS v20 software.

Genotyping

Genomic DNA was extracted using the cetyl trimethyl ammonium bromide (CTAB) method (Saghai-Marooft et al. 1984). Two parents and RIL populations were genotyped using the 55K SNP array by China Golden Marker (Beijing) Co., Ltd. (<http://www.cgmb.com.cn/>). Basic quality control (QC) tests were performed on samples by measuring markers based on genotype data detection rate, minimum allele frequency (MAF), and heterozygosity. The criteria used for sample quality control were as follows: DQC > 0.82, detection rate $\geq$ 85% and heterozygosity rate $\leq$ 10%; the criteria for marking quality control were as follows: detection rate greater than or equal to 95%, and MAF of 5% or more, heterozygosity rate of 50% or less, and the number of alleles was 2.

Based on the preliminary results of QTL mapping, 22 pairs of simple sequence repeat (SSR) markers located on the 2AS chromosome were selected for genetic map construction (see Table S1). These markers were identified from GrainGenes (<https://wheat.pw.usda.gov/GG3/>) (Somers et al. 2004) and PCR reactions and polyacrylamide electrophoresis were performed as previously reported (Wu et al. 2018a). To distinguish the difference between *Yr17* and major QTL on chromosome 2AS identified in this study, the specific CAPS marker *URIC/LN2* for *Yr17* was used to scan wheat lines with GX3 pedigree and the carrier line of *Yr17* gene (VPMI) and followed by digesting of restriction enzyme *DpnII*. Detecting procedure for *URIC/LN2* was processed according to previously report (Helguera et al. 2003).

Map construction and QTL analysis

The BIN function in QTL IciMapping v4.1 software (<http://www.isbreeding.net/>) (Meng et al. 2015) was used for redundant marker screening. In the mapping software, Joinmap v4.0, the LOD (Likelihood of odd) value was set to 3.0 for linkage analysis and genetic map distance was calculated using the Kosambi function. QTL analysis was performed using QTL IciMapping v4.1 software and the LOD threshold was set to 2.5. Mapchart (<https://www.wur.nl/en/show/Mapchart.htm>) was used to draw images (Voorrips 2002). The R package R/qtl was used to draw the genetic map (Broman et al. 2003).

Prediction of candidate genes

According to the derivation region of the QTL on the target chromosome, the physical position of the SNP in Chinese Spring was used to search in International Wheat Genome Sequencing Consortium (IWGSC) (<https://wheat-urgi.versailles.inra.fr/Seq-Repository/Annotations>) and the corresponding gene annotation and sequence was obtained.

Results

Evaluation of resistance

In the six different field trials, phenotypic data indicated significant genetic variation in APR. M96-5 and GX3 obtained mean MDS scores of 100% and 0%, respectively. MDS of the mapping populations ranged from 0 to 100% in each field study and the phenotypic data were continuously distributed (Figure 1). Pearson's correlation among the six field trials ranged from 0.38 to 0.79 ($P < 0.001$) (Table 2). ANOVA analysis including replicates for each experiment showed that there was significant variation in MDS when comparing different locations and wheat lines. Wheat lines combined with environment interactions also differed significantly and the heritability between different locations was also found to be significantly high (0.91). These results indicated that the QTL in APR had a dramatic effect in decreasing disease severity (Table 1).

Construction of genetic linkage map

Whole genome analysis of the two parental lines and 228 RILs was performed using the wheat 55K SNP array. Within the 55, 000 SNPs, 7570 were identified as polymorphic markers to distinguish between M96-5 and GX3. A total of 589

were excluded because they had missing data (> 10%) or showed segregation distortion. The remaining 6981 SNPs fell into 1543 bins and 5438 SNPs were excluded.

Preliminary localisation was performed using IciMapping 4.1 software. These results showed that an important QTL was located at the end of chromosome 2AS with an estimated LOD value of 40. We therefore selected 22 pairs of SSR primers at the distal region of chromosome 2AS and performed PCR amplification on the parental lines (GX3 and M96-5), a disease resistant pool (B_R), and a susceptible pool (B_S). Four markers (*cf_d36*, *wmc382*, *barc124*, and *wmc296*) showed successful amplification of consistent polymorphic bands between the resistant parents and the resistant pools. The four SSR markers were then used in combination with the 1543 SNPs obtained from genotyping data to analyse the QTL for resistance to wheat stripe rust at the adult plant stage. The final genetic map included 21 linkage groups corresponding to the 21 chromosomes (Figure 2 and Table 3). The total length of the genetic map was 3371.20 cM, with a mean marker/bin interval of 0.46 cM. Chromosome 7D was the longest (255.73 cM, 0.23 markers /cM) and chromosome 6A was the shortest (49.45 cM, 0.47 markers /cM). Chromosome 2A had 91 markers with a genetic map of 125.43 cM (0.73 markers /cM).

In the wheat genome, the B genome was found to have the highest number of markers (583, 37.69%), whereas the D genome had the fewest (382, 24.69%). Among the seven homologous groups of wheat, the fifth homology group had the highest number of SNPs (318, 20.56%), while the sixth homology group had the fewest (112, 7.24%); for the 21 chromosomes in wheat, there was an average of 75 markers for each chromosome. Chromosome 5A harboured the highest number of markers (124, 8.02%), while chromosome 6A had the fewest (23, 1.49%) (Table 4).

Mapping the QTL

A total of 19 QTL for resistance to wheat stripe rust were located on 12 chromosomes, including 1B (2), 1D (2), 2A (2), 2B (2), 2D (1), 4B (2), 4D (1), 5A (3), 5B (1), 6A (1), 6B (1), and 7B (1), of which two stable QTL on chromosome 2AS (*Qyr.gaas.2AS*) and 6AS (*Qyr.gaas.6AS*) were detected in six and five different environments, respectively (Table 5, Figure 3). An important QTL (*Qyr.gaas.2AS*) was located at the position of 5cM on chromosome 2AS. The six-year LOD values ranged from 8.01 to 44.61 with confidence intervals of *cf_d36~AX-110576889* (3.5–5.5cM), which explains the high phenotypic variation of 45.52%. *Qyr.gaas.6AS* was located at 4 cM of the end of chromosome 6AS with a confidence interval of *AX-109558600~AX-109542604* (3.5–4.5cM), accounting for a phenotypic variation of 3.27–21.73%. In addition, a QTL (*Qyr.gaas.6BL*) was identified in three different environments (Mianyang in 2017, and Guiyang in 2018 and 2019), with a confidence interval of *AX-109408478~AX-110409180* (54.5–60.5cM) and a phenotypic variation of 2.01–6.66%. The additive effect of the above three QTL was negative, indicating that their disease resistance originated from GX3. Moreover, another QTL (*Qyr.gaas.2D*) was identified in three environments (Mianyang in 2017, Guiyang in 2018, and Anshun in 2020), with a phenotypic variation of 2.56–3.55%. The additive effects were positive, indicating that its disease resistance originated from the other parental line, M96-5.

Candidate gene prediction

According to the physical locations of *Qyr.gaas.2A* (*cf_d36~AX-110576889*, 15.22Mb) and *Qyr.gaas.6A* (*AX-109558600~AX-109542604*, 5.12Mb), the sequences located within the interval of two QTL were searched in IWGSC to identify the wheat gene ID, annotation, and corresponding sequence. The results identified 620 and 61 segments at the confidence intervals of *Qyr.gaas.2A* and *Qyr.gaas.6A*, respectively (Table S2 and Table S3). The two intervals contained potentially functional genes such as nucleotide binding site-leucine rich repeat (NBS-LRR), a disease resistance protein, F-box protein, or part of the gene structure directly or indirectly involved in plant disease resistance (Table 6). Of note, 13 fragments related to disease resistance (10 NBS-LRR and 3 WRKY transcription factors) were clustered on a region of the 2AS chromosome spanning from 17,411,781bp to 17,601,016bp.

Discussion

Wheat stripe rust and phenotypic data

Until 2016, China had officially classified 34 stripe rust races (CYR1–CYR34) and more than 40 pathogenic types (Chen et al. 2014). Since the discovery of a new pathogenic type, V26 in 2009, this pathogenic group has been continuously mutating and expanding. Its toxicity profile has expanded from an initial infection of 12 to 18 Chinese identified hosts and a survey showed that the frequency of the three pathogenic groups of CYR32, CYR33, and V26 has since exceeded 70% (Zhang et al. 2015). In 2016, the pathogenic group (V26) of Guinong 22 was officially named CYR34 and this has led to an increase in its investigation within the field of wheat research in China.

The Sichuan Basin is a common source of new races of wheat stripe rust in China. Since the pathogenic group (V26) of Guinong 22 was first identified in Sichuan in 2009, it has gradually increased to become the dominant pathogenic group (Xu et al. 2016). At present, there are three main pathogenic groups of stripe rust in Guizhou; namely, the hybrid pathogenic group, the water source 11 pathogenic group, and the Guinong 22 pathogenic group. Among them, the frequencies of CYR32, CYR33, and CYR34 are 57.14%, 5.71%, and 4.29%, respectively (Chen et al. 2016b). When analysing the different field phenotypes of RILs between 2017 and 2020, significantly more susceptible varieties were found to originate in Sichuan compared to Guizhou. This difference is mainly due to the different epidemic races of stripe rust found in these two regions. Specifically, CYR32 and CYR33 are found mainly in Guizhou while CYR34 is predominantly found in Sichuan (Cheng et al. 2020).

In addition, different varieties carry multiple resistance genes and therefore show different degrees of resistance although none are completely immune. For each physiological race, selection pressure is greatly reduced, as the host and the pathogen are in a coexisting state and it is therefore unlikely that new mutations will develop in the pathogen. Due to this phenomenon, the resistance of slow-rust varieties is low and the resistance in specialised varieties is stronger (Yuan et al. 1995).

QTL mapping

In general, QTL that exert a larger effect on phenotype tend to be more stably expressed and are more easily detected within different environments. QTL that exert a smaller effect tend to be more influenced by genetic background and the external environment. These can therefore be more difficult to detect under certain environmental conditions (Li et al. 2010). In this study, two stable QTL were identified on the chromosomes 2AS and 6AS.

At the end of chromosome 2AS, a significant stable QTL (*Qyr.gaas.2AS*) was detected at the interval of *cfcd36~AX-110576889* (3.5–5.5cM) in all six environments assessed in this study. Five genes for the resistance of stripe rust were identified on chromosome 2AS (*Yr17* [0–20cM], *Yr56* [2.0–6.3cM], *Yr61* [1.9–16.8cM], *Yr69* [13.9–17cM], and *YrZM175* [47.8–52.1cM]). Through pedigree analysis, we found that *Yr61* was derived from the American soft red wheat Pioneer 26R61 (Hao et al. 2011), and *Yr56* from the durum wheat, Wollaro (Bansal et al. 2014). Both of the identified genes are known to promote adult resistance. *Yr69* (Hou et al. 2016) and *YrZM175* are known to be genes that promote seedling resistance in the artificial wheat introgression line, CH7086, and common wheat variety, Zhongmai 175 (Lu et al. 2016), respectively. *Yr17*, derived from *Ae. Ventricosa*, is also a seedling-resistant gene (Bariana and McIntosh 1993). The above genes have loss disease resistance to CYR34 race according to previous report (Zeng et al. 2015). However, our field investigations showed that VPML (the carrier line of *Yr17* gene) present resistant to the pathogenic group of Guinong 22 in Guiyang. Moreover, *Yr17* had the closest position to *Qyr.gaas.2AS*, the specific CAPS marker *URIC/LN2* of *Yr17* (Helguera et al. 2003) was used to further determine the differences between them. Our results showed that target bands could not be amplified in the GX3 line (Figure 4). Future experiments are required for the hybridisation of GX3 and the carrier line of *Yr17*, VPML. Furthermore, 13 QTL have been previously reported on chromosome 2AS (Bulli et al. 2016),

of which eight are adjacent to or overlapping with *Qyr.gaas.2AS*. GX3 is different from the parental lines of these disease-resistant QTL, so we therefore speculate that *Qyr.gaas.2AS* in GX3 is inconsistent with the above QTL.

On the 6AS chromosome, *Qyr.gaas.6AS* (AX-109558600~AX-109542604) is located in the interval spanning 3.5~4.5cM. According to the integration map drawn by Bulli et al. (2016), there are three adjacent or overlapping QTL. *QYr.uga-6AS_26R61* (0–7.1cM) is derived from the American soft red grain wheat Pioneer 26R61 (Hao et al. 2011). *QYr.wgp-6AS_Express* (3.8–7.1cM) is derived from the disease resistant cultivar "Express" with a high effect value (30.8–42.7%) (Lin and Chen 2007). *QYr.cim-6A_Avocet* (7.6–17.8cM) (Rosewarne et al. 2012) is derived from the CIMMYT wheat variety "Pastor" and may be associated with *Sr26* translocation genes. Due to the different sources of the identified parental QTL, *Qyr.gaas.6AS* is likely to be inconsistent with the aforementioned QTL.

Candidate gene prediction

Response to biological stress in plants can often be dependent on a variety of cell receptor proteins. Intracellular receptor proteins are encoded by NBS-LRR disease resistance genes, which have the ability to directly or indirectly recognise effector molecules (Effector) released by the pathogen into the cell and trigger a disease resistance response. This response is referred to as effector triggered immunity (Noutoshi et al. 2005) and requires mediation of the transcription factor WRKY. The NBS domain binds to ATP or GTP to play a key role in plant disease resistance. Currently, multiple wheat NBS-LRR genes have been identified and linked to disease resistance, such as *Lr1* (Qiu et al. 2007), *Lr10* (Bozkurt et al. 2007), *Lr21* and *Lr40* (Spielmeyer et al. 2000), *Sr35* (Santenac et al. 2013), *Sr33* (Pariyannan et al. 2013), *Sr60* (Chen et al. 2018), and *Yr10* (Bozkurt et al. 2007). In the present study, we identify *Qyr.gaas.2AS* (*cf36*~AX-110576889) and found a structural region (chr2A: 17411781_17601016) containing multiple NBS-LRRs and the WRKY transcription factor located near the *cf36* marker. We therefore speculate that the structural region in combination with a relatively complete NBS-LRR may represent a novel candidate gene and these 13 identified segments should be further investigated.

GX3 was derived from wild crossing between wild emmer wheat (*T. dicoccoides*) and wild oat (*Avena fatua* L. *var. glabrata pat*) varieties and was subsequently obtained by backcrossing with the common wheat Guinong 22 variety. Field observations spanning many years have shown that wild emmer wheat and Guinong 22 are susceptible to CRY34, while the wild oat is immune. Therefore, we speculate that the major QTL on chromosome 2AS originated from *Avena fatua* L. *var. glabrata*. However, *in situ* hybridisation analysis showed no signal detection in the wild oat variety samples (unpublished data). These findings may be due to the fact that the introgression fragment is too small. These experiments therefore require further validation.

Conclusions

In summary, GX3 carried two stable QTL for stripe rust resistance, of which *Qyr.gaas.2AS* is a major QTL. A resistance gene on the 2AS chromosome has not been previously reported and is therefore a novel candidate for genetic resistance in wheat. GX3 could be used in future breeding of disease resistant wheat. Future investigations should focus on increasing the density of genetic maps around *Qyr.gaas.2AS* to clone the underlying gene.

Declarations

Author contribution statement BC analyzed data and wrote the manuscript. QZ, YG and XG performed resistance evaluation. NC, YD and TC scanned molecular marker. LZ and ZX conceived, designed the experiments, and revised the manuscript. All authors read and approved the final manuscript.

Acknowledgments The authors are greatly appreciate Dr. Jianhui WU, and Dr. Dejun HAN, State Key Laboratory of Crop Stress Biology for Arid Areas, Northwest A&F University, for their help in the analysis of data. We are very grateful to Professor Qiuzhen JIA, Institute of Plant Protection, Gansu Academy of Agricultural Sciences, for her help in the identification of wheat stripe rust inoculation at seedling stage. This work was funded by grants from National Natural Science Foundation of China (31660393), Natural Science Foundation of Guizhou province (QKHJC[2019]450), Natural Science Foundation of Guizhou province (QKHJC[2019]1305), and Youth Science Foundation of Guizhou Academy of Agricultural Sciences ([2018]02).

Compliance with ethical standards

Conflict of interest the authors declare no competing interests.

Ethical standard I declare on behalf of my co-authors that the work described is original, previously unpublished research, and not under consideration for publication elsewhere. The experiments in this study comply with the current laws of China.

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Tables

Table 1 Variance components of disease severity (DS) scores for the 228 RIL population derived from M96-5×GX3 across six environments

Source of variation	<i>Df</i>	Mean square	<i>F</i> value
RILs	227	8911.40	272.59**
Replicates/environment	6	23823.53	728.74**
Environments	5	56632.48	1732.34**
Lines × environment	1121	872.79	26.70**
Error	1348	32.69	
	0.91		

** Significance at *P*< 0.01

Table 2 Correlation analysis (*r*) of disease severity (DS) of the M96-5×GX3 RIL population across six environments

Environment	MY17	GY18	GY19	GY20	AS20	SL20
MY17	1					
GY18	0.75***	1				
GY19	0.58***	0.69***	1			
GY20	0.65***	0.77***	0.60***	1		
AS20	0.66***	0.67***	0.60***	0.79***	1	
SL20	0.60***	0.50***	0.38***	0.43***	0.48***	1

*** Significance at $P < 0.001$. MY17: 2017 Mianyang, GY18: 2018 Guiyang, GY19: 2019 Guiyang, GY20: 2020 Guiyang, AS20: 2020 Anshun, SL20: 2020 Shuangliu.

Table 3 SNPs statistics of the distribution and density on 21 chromosomes

Chromosome	Length (cM)	No. of Markers	Marker density (cM/locus)
1A	97.55	61	0.63
1B	117.13	103	0.88
1D	229.57	41	0.18
2A	125.43	91	0.73
2B	147.97	112	0.76
2D	180.82	47	0.26
3A	214.86	112	0.52
3B	149.02	77	0.52
3D	195.30	26	0.13
4A	123.25	60	0.49
4B	105.15	89	0.85
4D	110.91	61	0.55
5A	220.64	124	0.56
5B	189.42	99	0.52
5D	222.58	95	0.43
6A	49.45	23	0.47
6B	78.26	35	0.45
6D	168.03	54	0.32
7A	208.26	111	0.53
7B	181.87	68	0.37
7D	255.73	58	0.23
Total	3371.20	1547	0.46

Table 4 Distribution of polymorphic SNPs on the whole genome

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Total
A genome	61	91	112	60	124	23	111	582
B genome	103	112	77	89	99	35	68	583
D genome	41	47	26	61	95	54	58	382
Total	205	250	215	210	318	112	237	1547

Table 5 Summary of stripe rust resistance QTL detected in the M96-5×GX3 RIL population across six environments by ICIM

Environments	QTLs	Marker interval	Position/cM	Confidence interval/cM	Genetic interval/cM	Physical interval/Mb	LOD	PVE (%)	Add
2017 MY	<i>Qyr.gaas.1B</i>	AX- 111572690~AX- 94847267	1	0~1.5	0.69	3.58	3.04	1.89	-4.91
	<i>Qyr.gaas.2A</i>	<i>cfld36</i> ~AX- 110576889	5	3.5~5.5	0.46	15.22	44.61	45.52	-24.48
	<i>Qyr.gaas.2B</i>	AX- 109528193~AX- 110426897	136	132.5~141.5	7.76	6.89	2.7	1.99	-5.01
	<i>Qyr.gaas.2D</i>	AX- 110519154~AX- 111418246	32	31.5~34.5	1.39	4.91	4.66	2.97	6.09
	<i>Qyr.gaas.4B</i>	AX- 111556599~AX- 110016820	6	4.5~8.5	3.95	2.6	4.12	2.79	-5.91
	<i>Qyr.gaas.6A</i>	AX- 109558600~AX- 109542604	4	3.5~4.5	0.46	0.78	5.12	3.27	-6.4
	<i>Qyr.gaas.6B</i>	AX- 109408478~AX- 110409180	56	54.5~60.5	10.02	69.9	3.25	2.01	-5.02
2018 GY	<i>Qyr.gaas.1D</i>	AX- 110147378~AX- 109183884	54	53.5~54.5	0.69	0.43	2.94	1.69	-5.01
	<i>Qyr.gaas.2A</i>	<i>cfld36</i> ~AX- 110576889	5	3.5~5.5	0.46	15.22	41.93	38.17	-24.23
	<i>Qyr.gaas.2B.1</i>	AX- 108837623~AX- 110359903	46	44.5~46.5	0.93	7.46	3.22	1.9	-5.28
	<i>Qyr.gaas.2D</i>	AX- 111418246~AX- 110833961	33	31.5~34.5	1.85	5.09	4.34	2.59	6.18
	<i>Qyr.gaas.4B.1</i>	AX- 109410422~AX- 110036160	0	0~2.5	2.16	7.4	4.45	2.57	-6.16
	<i>Qyr.gaas.5A</i>	AX- 109471543~AX- 110616505	135	134.5~136.5	0.22	0.24	3.29	1.88	5.25
	<i>Qyr.gaas.6A</i>	AX- 109558600~AX- 109542604	4	3.5~4.5	0.46	0.78	26.27	19.11	-16.8
	<i>Qyr.gaas.6B</i>	AX- 109408478~AX- 110409180	56	54.5~60.5	10.02	69.9	4.67	2.7	-6.31
2019 GY	<i>Qyr.gaas.1D.1</i>	AX- 94444445~AX- 95126907	62	59.5~63.5	5.67	1.09	8.55	9.49	-11.03
	<i>Qyr.gaas.2A</i>	<i>cfld36</i> ~AX- 110576889	5	3.5~5.5	0.46	15.22	8.01	8.08	-10.3
	<i>Qyr.gaas.2A.1</i>	AX- 110538140~AX- 110155009	48	43.5~54.5	12.23	54.06	4.3	4.35	-7.44
	<i>Qyr.gaas.4D</i>	AX- 111688098~AX- 110768844	0	0~0.5	0.23	0.13	4.64	4.49	7.56
	<i>Qyr.gaas.6A</i>	AX- 109558600~AX- 109542604	4	3.5~4.5	0.46	0.78	19.16	21.73	-16.73
	<i>Qyr.gaas.6B</i>	AX-	56	54.5~60.5	10.02	69.9	6.72	6.66	-9.21

109408478~AX-
110409180

2020 GY	Qyr.gaas.2A	cfid36~AX- 110576889	5	3.5~5.5	0.46	15.22	16.53	22.36	-15.54
	Qyr.gaas.5A.1	AX- 109019768~AX- 108808620	209	204.5~209.5	0.71	1.30	2.68	3.13	-5.71
	Qyr.gaas.6A	AX- 109558600~AX- 109542604	4	3.5~4.5	0.46	0.78	21.69	31.30	-18.06
2020 AS	Qyr.gaas.1B.1	AX- 108745931~AX- 110017315	83	81.5~84.5	2.15	0.22	3.86	3.70	-7.18
	Qyr.gaas.2A	cfid36~AX- 110576889	5	3.5~5.5	0.46	15.22	17.88	19.20	-16.66
	Qyr.gaas.2D	AX- 109229475~AX- 110519154	31	29.5~32.5	1.88	7.52	3.91	3.55	7.01
	Qyr.gaas.6A	AX- 109558600~AX- 109542604	4	3.5~4.5	0.46	0.78	22.84	25.87	-18.98
	Qyr.gaas.7B	AX- 109352027~AX- 109988869	13	8.5~16	8.55	16.20	2.59	2.64	6.08
2020 SL	Qyr.gaas.1B.1	AX- 110017315~AX- 108726041	85	84.5~88.5	0.7	0.38	4.29	5.08	-8.73
	Qyr.gaas.2A	cfid36~AX- 110576889	5	3.5~5.5	0.46	15.22	15.50	20.68	-17.96
	Qyr.gaas.5A.2	AX- 110169414~AX- 111590180	73	70.5~73.5	1.42	2.20	3.58	4.25	7.97
	Qyr.gaas.5B	AX- 110689592~AX- 109479506	73	69.5~99.5	4.31	5.83	4.98	6.22	-9.66

LOD: logarithm of odds score, PVE: percentage of phenotypic variance explained by individual QTL, Add: additive effect of the resistance allele.

Table 6 The significant SNPs variations on chromosome 2AS and 6AS

ChromAnnotation of SNP		Number of SNPs
2AS	Disease resistance protein(NBS-LRR disease resistance protein)	35
	Retrotransposon protein	28
	Retrovirus-related Pol polyprotein from transposon TNT 1-94	19
	F-box protein	15
	Cysteine protease	14
	Proline-rich protein	13
	RNA-directed DNA polymerase	13
	Glycosyltransferase	11
	Receptor-kinase	11
	Protein kinase	10
	Transposon protein	10
	Protein FAR1-RELATED SEQUENCE 5	9
	Cytochrome P450	8
	Dirigent protein	8
	Leucine-rich repeat receptor-like protein kinase family protein	7
	Phosphoglycerate mutase-like protein	7
	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	6
	Serine/threonine-protein kinase	6
	Zinc finger protein	6
	FAD-binding Berberine family protein	5
6AS	Leucine-rich repeat receptor-like protein kinase family protein	10
	Receptor kinase	5
	F-box protein	4
	Retrotransposon protein	4
	Disease resistance protein(NBS-LRR disease resistance protein)	4

Figures

Figure 1

Frequency distributions of stripe rust maximum disease severities (MDS) in the 228 RIL population derived from a cross between the susceptible landrace M96-5 and the resistant variety GX3 across six environments. MY17: 2017 Mianyang, GY18: 2018 Guiyang, GY19: 2019 Guiyang, GY20: 2020 Guiyang, AS20: 2020 Anshun, SL20: 2020 Shuangliu.

Figure 2

Genetic map of the 228 RIL population obtained from M96-5×GX3

A

B

Figure 3

Graphical displays of genetic positions of major QTL for strip rust resistance across six environments for M96-5×GX3 population. Qyr.gaas.2AS on chromosomes 2AS (A) and Qyr.gaas.6AS on chromosome 6AS (B) were detected in six and five environments, respectively. Different colors represent different environments, red: 2017 Mianyang, green: 2018 Guiyang, black: 2019 Guiyang, rose: 2020 Guiyang, light blue: 2020 Anshun, brown: 2020 Shuangliu.

Figure 4

Distinguishing between Qyr.gaas.2AS and Yr17 gene by using the specific CAPS marker URIC/LN2. PCR fragments were amplified with primers URIC- LN2 (A) followed by DpnII digestion (B). "M" indicates the molecular markers (100-bp ladder). The black arrowhead indicates target band (285 bp). The gray arrowheads indicate DpnII digested fragments (166 and 109 bp).

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