Epistatic Interaction Effect Between Chromosome 1BL (*Yr29*) and A Novel Locus on 2AL Facilitating Resistance to Stripe Rust in Chinese Wheat Changwu 357-9

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Abstract

Stripe rust (yellow rust) is a serious disease of bread wheat (*Triticum aestivum* L.) worldwide. Genetic resistance is considered the most economical, effective and environmentally friendly method to control the disease. The current study focused on characterizing the components of stripe rust resistance and understanding the interactions in Changwu 357-9 (CW357-9)/Avocet S RIL population. A genetic linkage map constructed using a new GenoBaits Wheat 16K Panel and the 660K SNP array had 5,104 polymorphic SNP markers spanning 3,533.11 cM. Four stable QTL were detected on chromosome arms 1BL, 2AL, 3DS, and 6BS across all environments in Chngwu357-9. The most effective QTL Q YrCW357-1BL was Yr29 and the 6BS QTL was identified as Yr78. The novel QTL on 2AL with moderate effect showed significant epistatic interaction with Yr29. The QTL on 3DL should be same as QYrsn.nwafu-3DL and enriches the overall stripe rust resistance gene pool for breeding. Polymorphisms of flanking AQP markers AX-110020417 (for QYrCW357-1BL), AX-110020417 (for QYrCW357-2AL), AX-109466386 (for QYrCW357-3DL), and AX-109995005 (for QYrCW357-6BS), were evaluated in a diversity panel including 225 wheat cultivars and breeding lines. The results suggested that these high-throughput markers could be used to introduce QYrCW357-1BL (Yr29), QYrCW357-2AL, QYrCW357-3DL, and QYrCW357-6BS into commercial wheat cultivars. Combinations of these genes with other APR QTL should lead to higher levels of stripe rust resistance along with the beneficial effects of multi-disease resistance gene Yr29 on improving resistance to other diseases.

Key Message

Four stable QTL for adult plant resistance were identified in wheat line Changwu 357-9, including a new QTL on 2AL showing significant interaction with *Yr29* to reduce stripe rust severity.

Introduction

Wheat (*Triticum aestivum* L.) is a major cereal crop consumed widely throughout the world and production is often constrained by diseases and pests causing substantial yield losses. Stripe rust (yellow rust) caused by *Puccinia striiformis* Westend. f. sp. *tritici* Erikss. occurs in almost all wheat-producing regions. This disease causes significant economic losses in terms of reduced grain production and additional costs associated with disease management (Hovmøller et al. 2010; McIntosh et al. 1995). The most profitable and environmentally friendly strategy for farmers to control wheat rusts in both developing and developed countries is to grow genetically resistant wheat varieties (Krattinger et al. 2009).

The application of genomic tools and development of genotyping platforms for wheat improvement have lagged behind other cereals such as rice and maize for a long period largely due to its allohexaploid nature (AABBDD genome), huge genome size (~17 Gb) and highly repetitive elements (>80%). Recent advances in sequencing technology, however, radically changed the landscape and provided opportunities to overcome these difficulties. Over the past few years, great progress was made in developing the reference

genome assembly of polyploid wheat and its progenitors, including *T. urartu* (AA genome) (Ling et al. 2018), *Aegilops tauschii* (DD genome) (Jia et al. 2013; Luo et al. 2017; Luo et al. 2013; Zhao et al. 2017), and wild emmer wheat (*T. turgidum* ssp. *dicoccoides*) (AABB genome) (Avni et al.). Subsequent exon capture sequencing and resequencing technologies now accelerated marker development and establish haplotypes associated with resistant and susceptible lines (Ksenia et al. 2017; Cobo et al. 2018; Krasileva et al. 2017; Hao et al. 2020). Single nucleotide polymorphisms (SNPs) as the most abundant and important type of DNA variation were used to develop several high-throughput SNP genotyping platforms such as the 9K, 16K, 35K, 55K, 90K, and 660K high-density SNP chips (Cavanagh et al. 2013; Wang et al. 2014; Jia and Zhao 2016). A target sequencing (GBS) system with capture-in-solution (liquid chip) technology known as the wheat 16K SNP array with the advantage of greater power for detection of genetic diversity by linkage disequilibrium decay analysis and genome-wide association studies than the one-amplicon-one-SNP system was developed by a multiple single-nucleotide polymorphism (mSNP) approach (Guo et al. 2021).

More than 80 permanently named stripe rust resistance (R) genes (*Yr1–Yr83*) and many QTLs have been mapped on all 21 wheat chromosomes (Li et al. 2020; McIntosh 1995). These genes/QTL can be categorized as all stage resistance (ASR) and adult plant resistance (APR) or high-temperature adult-plant resistance (HTAPR) based on the growth stage at which they can be detected. ASR is often race-specific, qualitatively inherited and controlled by a single gene, whereas APR and HTAPR is more quantitative with individual genes having minor effectiveness, but when combined there are additive effects such that agronomically acceptable levels of resistance is achieved. The added advantage of this type of resistance is durability that is based on the thesis that the genes conferring this type of resistance are non-specific or that any erosion of effectiveness will be a gradual process rather than a 'boom and bust' characteristic of widespread use of single ASR genes (Chen 2013; Chen 2005; Lagudah 2011; Chen and Line 1995), most recently evidenced in China with the emergence of the now-prevalent *Yr26*-virulent race group, including CYR34. This race group not only overcame *Yr26* but also possessed a wide array of virulence for other well-known ASR genes (Wu et al. 2020; Huang et al. 2021). Therefore, identification and characterization of APR or HTAPR genes will enrich the overall stripe rust resistance gene pool and thereby accelerate development of wheat cultivars with durable, high-level resistance that can also be combined with effective all-stage resistance (Chen 2013; Liu et al. 2019).

The wheat line Changwu 357-9 (CW357-9) with high yield and desirable end-use quality, provided by Dr. Zengji Liang, Agricultural and Rural Bureau of Changwu County, has shown a high level of resistance to stripe rust for many years in China. The objectives of this study were to: 1) investigate the genetic basis of stripe rust resistance in Changwu 357-9 using a recombinant inbred line (RIL) population tested in multiple environments, 2) identify and map QTL in CW357-9 with significant additive and epistatic effects on resistance to stripe rust using the wheat 16K SNP array, and 3) develop and validate AQP markers closely linked to three identified QTL.

Materials And Methods

Plant materials

The 167 F₆ recombinant inbred line (RIL) population was derived from across of susceptible Avocet S (AvS) and resistant Changwu 357-9 (CW357-9). A panel of 225 Chinese wheat cultivars/breeding lines and *Yr* gene carriers were evaluated for response to stripe rust across multiple field environments and the data were used to determine the prevalence of resistance genes/QTL identified in CW357-9 based on flanking SNP markers. The wheat cultivars Avocet S (AvS), Mingxian 169 (MX169), and Xiaoyan 22 (XY22) were used as susceptible controls.

Greenhouse evaluation

The stripe rust reaction of CW357-9 was tested under greenhouse conditions against *Pst* isolates identified as races PST-Lab.1, PST-Lab.2, and PST-V26 to identify the type of inheritance following the procedure described in Wu et al. (2016). The testing regime for seedlings and determination of virulence/avirulence characteristics of PST-Lab.1, PST-Lab.2, and PST-V26 were previously reported in Huang et al. (2021). Infection types (ITs) of all plants were recorded 18 to 21 days after inoculation when the disease was fully developed on the susceptible control (AvS and MX169), and based on a 0–9 scale as previously described (Line and Qayoum 1992). The records were repeated thrice to ensure reliability of the IT.

Field experiments

The 167 F₆ RILs and parents for disease assessment were grown in five environments including Jiangyou (JY) in Sichuan province and Yangling (YL) in Shaanxi province during 2017-2018 and 2018-2019, and Tianshui (TS) in Gansu province in 2018-2019. Lines carrying *Yr29* (Pavon 76, Attila, and Avocet-*Yr29*) were included as checks. The locations in Sichuan and southern Gansu experience cool, wet weather that is ideal for natural stripe rust survival and spread. At each location, 30 seeds of each line were replanted as 1 m single rows and a 30 cm row spacing with a mixture of MX169 and XY22 as susceptible spreaders sown after every 20 rows. Trials at Yangling were inoculated with a mixture of *Pst* races PST-Lab.1, PST-Lab.2 and PST-V26 suspended in a light oil (1:300) sprayed onto MX169 and XY22 at flag leaf emergence. Two replicates of the RILs were planted in each environment. Stripe rust assessments were made 5–25 April at Jiangyou (JY), 3–17 May at Yangling (YL), and 10–15 June at Tianshui (TS), when AvS and XY22 displayed 80% severity or more. Infection types (IT) using a 0 (resistant) to 9 (susceptible) scale (Line and Qayoum, 1992) and disease severities (DS) based on the modified Cobb Scale (Peterson et al. 1948) were used to evaluate the adult plant responses to YR. IT and DS of homozygous lines were recorded as single values; and for segregating lines IT and DS were recorded as two or more values, but later not used in QTL detection. Disease assessment was made at least twice and the highest IT and the maximum disease severity (MDS) for each line were used for phenotypic and QTL analyses.

Phenotypic analysis

ANOVA of the mean IT and DS for the RILs in each environment was undertaken to determine the effects of genotype (G), environment (E), and G × E interaction. Pearson's correlation coefficient (*r*) analysis and

ANOVA were conducted using the “AOV” function in QTL IciMapping software 4.1 with the default parameters (Meng et al. 2015). Broad-sense heritabilities (h^2_b) of resistance were based on the equation $h^2_b = \sigma^2_g / (\sigma^2_g + \sigma^2_{ge}/e + \sigma^2_{\epsilon}/re)$, where σ^2_g , σ^2_{ge} and σ^2_{ϵ} represented the genotypic (RILs), G × E and error variances, respectively, and e and r were the numbers of environments and replicates. In addition, the mean phenotypic values for all five environments, were used to evaluate the genetic effects and find the best confidence region for each QTL (Mu et al. 2019).

SNP calling and clustering

Genomic DNA were extracted from pools of 10-15 plants from each parent and RIL at the jointing stage using the CTAB protocol (Clarke et al. 2009) and DNA quality was assessed using a NanoDrop ND-1000 (Thermo Scientific, Wilmington, DE, USA). The RILs and parents were genotyped by a new wheat 16K SNP array from Mol Breeding (Shijiazhuang in Hebei province; <http://www.molbreeding.com>). The wheat 660K SNP array from CapitalBio Corporation (Beijing; <http://www.capitalbio.com>) was used to genotype the two parents. The distribution of SNPs from the 16K array is shown in Table. S1. The procedure for marker clustering was described in Huang et al. (2021).

Linkage map construction and QTL analysis

A Chi-squared (χ^2) test for goodness of fit to a 1:1 segregation ratio was performed for each SNP before processing by including those <10% missing values and major allele frequencies (MAF) $\leq 5\%$. One marker was selected from each co-segregating marker group using the “BIN” function. The selected markers were used to generate the genetic map using the “MAP” function in IciMapping V4.2 software and drawn in Mapchart V2.3 (Meng et al. 2015; Voorrips 2002). Recombination fractions were converted to cM using the Kosambi function (Kosambi 1943). The phenotypic data including IT, DS, and mean values from all environments were used to identify the QTL. Inclusive composite interval mapping with the additive tool (ICIM-ADD) in IciMapping V4.2 was performed to detect QTL. The phenotypic variances explained (PVE) by individual QTL and additive effects at the LOD peaks were also obtained. Due to low marker density, some QTL mapped in potentially large regions. To further narrow down the flanking intervals of target loci, significant SNPs from 660K SNP array were converted into allele-specific quantitative PCR (AQP) markers by JasonGen Biological Technology Co., Ltd (Beijing; <http://www.jasongen.com>) to genotype the RIL population.

Epistasis

Genotyped SNP markers associated with stripe rust resistance across five environments, were used for pairwise interaction analysis in Network version 2.1 (Yang et al. 2008). QTL effects were evaluated by the mixed linear model (MLM) approach. A “2D genome scan” option was used to map epistatic QTL with or without single-locus effects. additive × additive (A×A) epistatic effects of mapped using the “map epistasis” function. F values were used to control the error rate by permutation tests.

Results

Phenotypic evaluation

The CW357-9 seedlings were resistant (IT 1-2) to PST-Lab.1 and PST-Lab.2, but susceptible (IT 8-9) to PST-V26. CW357-9 was highly resistant (IT 1-2, DS $\leq 5\%$) in field whereas AvS was highly susceptible (IT 8-9) in all experiments. Based on these results, CW357-9 possessed both seedling resistance to two isolates and APR to the mixture of the three isolates used in the field. In the field experiments, both IT and maximum disease severity (MDS) data for RILs showed continuous distributions (Fig. 1), indicating that resistance in CW357-9 was quantitatively inherited. Pearson's correlation coefficients of pairwise comparisons of IT and DS ranged from 0.60-0.85 and 0.58-0.88 ($P < 0.001$) (Table 1), respectively. Broad-sense heritabilities for both IT and DS were 0.92 (Table 2). P values in the ANOVA for IT and DS were highly significant ($P < 0.0001$) for RILs, environments, and line $\times$ environment interactions. Lack of significant variation between the replicates suggested that genetically controlled resistance was the main source of phenotypic variation in the RIL population (Table 2). These results indicated that the QTL conferring resistance was effective in the five environments.

Genetic linkage map

Of the 20,995 SNPs, 5,104 (24.4%) showed polymorphism between the parents. By using the "BIN" function in QTL IciMapping 4.2, redundant polymorphic SNPs were removed showing $>10\%$ missing data and distorted segregation. Finally, 841 SNPs were chosen to construct the genetic linkage map; they were distributed in 22 linkage groups spanning 3,533.11 cM. The A, B, and D genomes included 290 (34.48%), 374 (44.47%), and 177 (21.05%) markers covering lengths of 1,268.96, 1,356.53, and 1,012.27 cM with average marker intervals of 4.38, 3.63, and 5.72 cM, respectively. Only chromosome 2D had two linkage groups; the other chromosomes were each represented by a single linkage group (Table S1).

Additive QTL for stripe rust resistance

Two QTL for seedling resistance to race PST-Lab.1 were detected on chromosomes arms 2BL and 4DL, however did not confer resistance in field indicating that isolate PST-V26 was prevalent in field experiments. Both IT and DS data from the field environments were used to detect QTL at the adult plant stage. Four consistent QTL on chromosome arms 1BL, 2AL, 3DL, and 6BS, designated as *QYrCW357-1BL*, *QYrCW357-2AL*, *QYrCW357-3DL*, and *QYrCW357-6BS*, respectively, were identified in all five environments using the ICIM method in QTL IciMapping 4.2. All detected QTL were derived from the resistant parent CW357-9 (Table 3; Fig. 2A and B). *QYrCW357-1BL* with the largest effect was closely linked to markers AX-110020417 and 16K-16852 and explained 19.8-28.8% and 23.9-29.1% of variation in IT and DS, respectively (Table 3; Fig. 2A). *QYrCW357-2AL* located in a 1.3 cM interval spanned by markers 16K-4252 and 16K-4207, explained 4.0-10.0% and 5.8-12.9% of the phenotypic variation in IT and DS, respectively, across environments (Table 3; Fig. 2B). *QYrCW357-6BS*, linked to 16K-15955 and AX-109914318, explained 2.8-8.1% (IT) and 2.6-8.3% (DS) of the phenotypic variances, respectively. QTL on 3DL, flanked by 16K-9333 and 16K-9526, explained 2.8-8.1% and 9.1-16.4% of the variation in IT and DS, respectively. All QTL had additive effects (Table 3).

Epistatic interaction detected by QTLNetwork version 2.1

Significant epistatic interactions were detected across all field traits using QTL Network version 2.1. Two different intervals on 1BL and 2AL corresponding to the markers *AX-110020417-16K-16852* (*QYrCW357-1BL* or *Yr29*) and *16K-4252-AX-110974948* (*QYrCW357-2AL*), showed an estimated additive by additive interaction (A*A) effects of 0.24-0.34 and 3.24-4.50 in IT and DS, respectively (Table 4). Although the QTL on 1BL and 2AL showed significant interaction for reduced stripe rust severity, the presence of the 3DL and 6BS QTL and some additional environmentally sensitive QTL were necessary to reach the consistently low response of CW357-9 (Table 3).

QTL combinations and interaction

In order to investigate the effects of QTL combinations, RILs were classified into five genotypic groups based on all field tests (Table S2). RILs with all four QTL *QYrCW357-1BL*, *QYrCW357-2AL*, *QYrCW357-3DL*, and *QYrCW357-6BS* were more resistant (lower IT and DS) than all others, displaying almost similar resistance levels to CW357-9 (Fig. 3A, B; Table S2). Among these genes the combination of *QYrCW357-1BL* and *QYrCW357-2AL* showed the most significant effect in reducing stripe rust severity (Fig. 3C, D). RILs with none of the four stable QTLs had mean IT and DS values of 8.2 and 88.6%, respectively; RILs with only one QTL (1BL or 2AL) had mean values of 6.9 and 71.0% for the 1BL locus (similar to Avocet-*Yr29* in Table S3) and 7.2 and 76.1% for 2AL, respectively (Fig. 3C, D). The group combining *QYrCW357-1BL* and *QYrCW357-2AL*, with mean IT and DS of 4.6 and 36.0%, respectively, showed significant effect in reducing IT and DS (Fig. 3C, D, E and F).

Marker-assisted selection (MAS) based on AHQ markers

Genotyping of the 238-accession panel for polymorphic AQP markers *AX-110020417*, *AX-110974948*, *AX-109466386* and *AX-109995005* represented for *QYrCW357-1BL*, *QYrCW357-2AL*, *QYrCW357-3DL*, and *QYrCW357-6BS*, respectively, suggested these markers can be used in MAS (Table S4). Wheat lines with both *QYrCW357-1BL* (or *Yr29*) and *QYrCW357-2AL* were on the average more resistant than lines without them, but some accessions containing the QTL were very susceptible, indicating that the effects of the two QTL alone were influenced by genetic background and environment. However, wheat lines combining all four loci had the lowest average DS in Yangling and Tianshui (Figure 5). Sequences for the AQP markers *AX-110020417*, *AX-110974948*, *AX-109466386*, and *AX-109914318* are provided in Table S4.

Discussion

There is now overwhelming evidence that pyramiding multiple partially effective resistance genes with additive or positive interaction in a single wheat cultivar can lead to more durable resistance than a single highly effective all-stage resistance gene. The data also suggest that the level of resistance required to protect yield potential and to prevent significant disease spread will require about four genes (Huang et al. 2019; Zeng et al. 2019; Huang et al. 2021). In addition, the number of epistatic interactions are frequently larger than the number of additive QTL, and the importance and number of epistatic

interactions in terms of both the number of loci involved and effects may be greater than the additive QTL (Malmberg 2005). CW357-9 is such a wheat genotype, combining four partial APR QTL with both epistatic and additive effects.

Four stable QTL for APR in CW357-9

QYrCW357-1BL with largest effect on APR, spanned by the markers *AX-110020417* and *16K-16852*, was mapped on chromosome arm 1BL (Table 3; Fig. 2A). *Yr29*, the only designated *Yr* gene on 1BL, closely linked to, and commonly identified by, marker *csLV46G22* has been mapped in many studies (William et al. 2003, Cobo et al. 2018; Kolmer et al. 2012; Lan et al. 2014; Lan et al. 2015; Ponce-Molina et al. 2018; Rosewarne et al. 2012). This multi-pathogen resistance allele (*Lr46/Yr29/Pm39/Sr58/Ltn2*) was widely used in CIMMYT germplasm (Singh et al. 2013; Lan et al. 2015; Rosewarne et al. 2006). Genotyping of CW357-9 and *Yr29* carriers (Pavon 76, Attila, AVS *Yr29NIL*) with the PCR markers *AX-110020417* and *csLV46G22*, showed that CW357-9 and *Yr29* shared an allele that differed from AvS. CW357-9 also showed leaf tip necrosis in our field. Thus, *QYrCW357-1BL* in CW357-9 should be *Yr29*.

The second QTL *QYrCW357-2AL* conferring APR explained 4.0-12.9% of the phenotypic variation in IT and DS was flanked by markers *16K-4252* and *AX-110974948* (Table 3; Fig. 2B). Many genes for APR were previously mapped on chromosome arm 2AL, including *QYr.caas-2AL* in Zhong 892 (Liu et al. 2015), *QYrqin.nwafu-2AL* in QN142 (Zeng et al. 2019), and *Yrxy2* in Xiaoyan54 (Zhou et al. 2011) closely linked to the markers *IWB11764*, *AX-94895021*, and *Xgwm794*, respectively. Two additional QTL closely linked to markers *IWA7339* and *IWA544*, respectively, were detected by genome-wide association analysis (GWAS) (Fig. 4, Table S3). Based on the integrated genetic (Zeng et al. 2019) and physical maps (Fig. 4, Table S5), *YrCW357-2AL* appeared to be a new stripe rust resistance gene.

QYrCW357-3DL with minor but stable effects was detected on 3DL (Table 3). In a previous study, *QYrsn.nwafu-3DL* in Shaannong 33 flanked by *AX-109466386* (180,398,197) and *AX-110284733* (414,838,337) was mapped in a similar physical region (Huang et al. 2021). Genotyping of *QYrsn.nwafu-3DL*-linked AQP marker *AX-109466386* showed that CW357-9 and Shaannong 33 had the same allele that differed from AvS. In addition, similar level of explanation was found for PVE. Based on the integrated map (Huang et al. 2021), these results indicated that *QYrCW357-3DL* and *QYrsn.nwafu-3DL* should be same, and different from other QTL/genes in the region.

Dong et al. (2017) mapped *QYr.ucw-6B* (*Yr78*) close to the marker *IWA7257*. Several studies confirmed that *QYrsn.nwafu-6BS* in Shaannong 33, *QYr.wgp-6B.1* in Stephens, *QYr.sun-6BS* in Janz, and *QYrMa.wgp-6BS* in Madsen were *Yr78* (Huang et al. 2021; Dong et al. 2017; Liu et al. 2018). In our study, *QYrCW357-6BS*, flanked by the markers *16K-15955* and *AX-109914318*, explained 6.6-11.7% of the phenotypic variation in IT and DS (Table 4). Genotyping of CW357-9 and AvS showed different allele with the marker *IWA7257*, indicating that *YrCW357-6BS* is likely *Yr78*.

***Yr29* and *Yr78* are frequently combined in Chinese wheat germplasm** Combinations of *QYrCW357-1BL* (*Yr29*) on chromosome arm 1BL and *QYrCW357-6BS* (*Yr78*) on 6BS, were detected in several Shaanxi

wheat cultivars, including Qinnong 142 (Zeng et al. 2019), Shaanong 33 (Huang et al. 2021), and Xinong3517 (Huang unpublished data). These cultivars were highly resistant in the field at Shaanxi, Gansu, and Sichuan provinces, which are hotspot regions for over-season survival of *Pst* and have frequent occurrence of stripe rust. In this study, most carrier varieties with *Yr29* and *Yr78* were from Sichuan (12, 21.4%), Henan (9, 16.1%), and Shandong (8, 14.3%). Similar results reported in Huang et al. (2021) indicate varieties carrying *Yr29* and *Yr78* are common in these provinces.

Gene–gene interaction contributing to stripe rust resistance for MAS

A method of MAS based on QTLs with epistatic effects was proposed (Liu et al. 2003). Changwu 357-9 with desirable agronomic traits and high-level durable resistance to stripe rust can be used as a parent for marker-assisted breeding for favorable epistatic interactions. Based on the epistatic analysis of field IT and DS, *QYrCW357-1BL* (or *Yr29*) and *QYrCW357-2AL* showed significant interaction (Table 4, Figure 2). *Yr29* is present in many wheat cultivars around the world and has remained effective for more than 60 years (Cobo et al. 2017). The novel locus on chromosome arm 2AL interacted with *Yr29* and other genes to confer an acceptable level of resistance to stripe rust in Chinese wheat Changwu 357-9 (Figure C, D, E, and F; Table S2). Based on genotyping of the flanking AQP markers, these results suggested that these markers can be used for developing new cultivars with high-level of durable resistance to stripe rust (Table S4). In addition, further exploration may provide insight for understanding the interactions observed between *YrCW357-1BL* or *Yr29* and *YrCW357-2AL* in this study as well as functional mechanisms that contribute to this resistance gene network.

Abbreviations

ANOVA, analysis of variance

APR, adult plant resistance

AQP, allele-specific quantitative PCR

CIMMYT, International Maize and Wheat Improvement Center

cM, centiMorgan

DS, disease severity

GWAS, genome-wide association analysis

ICIM, inclusive composite interval mapping

IT, infection type

IWGSC, International Wheat Genome Sequencing Consortium

KASP, kompetitive allele-specific polymerase chain reaction

LOD, likelihood-of-odds

MAS, marker-assisted selection

PCR, polymerase chain reaction

Pst, *Puccinia striiformis* f. sp. *tritici*

QTL, quantitative trait locus

RIL, recombinant inbred line

SNP, single nucleotide polymorphism

SSR, simple sequence repeat

Conclusion

CW357-9 with durable resistance to stripe rust for more than a decade carries a 4-gene combination of APR genes, including *Yr29*, *Yr78*, *QYrCW357-2AL* and *QYrCW357-3DL* with additive and epistatic effects. The QTL on chromosome arms 2AL and 3DL were novel. The key points from this work were: 1) *QYrCW357-2AL* and *QYrCW357-3DL* can be selected to enrich the overall stripe rust resistance gene pool for breeding; 2) the combination of *Yr78* and *Yr29* is frequent among wheat cultivars and breeding lines in China; and 3) the discovery of favorable epistatic interaction between *Yr29* and *QYrCW357-2AL*. Finally, *CW357-9* not only represents a useful breeding parent but the markers developed here can be used in MAS to develop new cultivars with potentially durable resistance. Field trials in disease nurseries will still be required to determine that lines with the selected resistance gene combination confer an acceptable level of protection from stripe rust.

Declarations

Authors' contribution statement

S Huang designed and conducted the experiments, analyzed the data, and wrote the manuscript. YB Zhang, H Ren, X Li, X Zhang, CL Zhang, QD Zeng, and QL Wang participated in creation of the genetic populations and assisted in analysis of the SNP array data. YB Zhang, H Ren, ZY Zhang, XT Wang and SJ Liu participated in greenhouse and field experiments and contributed to genotyping. RP Singh, S Bhavani, and ZS Kang participated in revision of the manuscript. JH Wu, DJ Han and ZS Kang conceived and directed the project and revised the manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest.

Data availability

All data, models, or code generated or used during the study are available from the corresponding author by request.

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Tables

Table 1. Correlation coefficients (*r*) for stripe rust infection type (IT) and disease severity (DS) in the AvS × CW357-9 RIL population tested in five field environments

Environment ^a	<i>r</i> value based on IT (DS) ^b			
	18YL	2018JY	2019JY	2019YL
2018-JY	0.70(0.72)	-	-	-
2019-JY	0.85(0.88)	0.73(0.80)	-	-
2019-YL	0.71(0.70)	0.67(0.63)	0.69(0.67)	-
2019-TS	0.61(0.61)	0.76(0.75)	0.60(0.58)	0.67(0.63)

^a YL, TS, and JY, Yangling, Tianshui, and Jiangyou, respectively.

^b All *r* values were significant at $P = 0.001$

Table 2. Analysis of variance (ANOVA) for stripe rust infection type (IT) and disease severity (DS) data for the AvS × CW357-9 RIL population evaluated at Yangling and Jiangyou in 2017 and 2018 and Tianshui in 2018

Source of variation	IT				DS			
	<i>df</i>	Mean square	<i>F</i> value	<i>P</i> -value	<i>df</i>	Mean square	<i>F</i> value	<i>P</i> -value
RILs	166	38.8	39.3	<0.0001	166	6850.3	54.3	< 0.0001
Replicates	1	6.8	6.9		1	1872.6	14.8	
Environments	4	217.8	220.9	< 0.0001	4	37363.6	296.2	< 0.0001
Line × environment	660	3.1	3.1	< 0.0001	660	561.9	4.5	< 0.0001
Error	662	0.99			662	126.1		
h ² b	0.92				0.92			

Table 3. Summary of stripe rust APRQTL detected in the AvS × CW357-9 RIL population using IciMapping 4.1

QTL	Environment ^a	Marker interval		Genetic Position	LOD ^b	PVE ^c	Add ^d
<i>QYrCW357-1BL</i>	2018YL-IT	<i>AX-110020417</i>	<i>16K-16852</i>	152	10.5	25.7	-0.9
	2018YL-DS	<i>AX-110020417</i>	<i>16K-16852</i>	153	10.6	25.8	-11.8
	2018JY-IT	<i>AX-110020417</i>	<i>16K-16852</i>	153	12.1	28.8	-1.1
	2018JY-DS	<i>AX-110020417</i>	<i>16K-16852</i>	153	10.2	24.9	-13.3
	2019YL-IT	<i>AX-110020417</i>	<i>16K-16852</i>	153	9.8	24.3	-1.0
	2019YL-DS	<i>AX-110020417</i>	<i>16K-16852</i>	153	12.4	29.1	-15.3
	2019JY-IT	<i>AX-110020417</i>	<i>16K-16852</i>	153	8.0	19.8	-1.0
	2019JY-DS	<i>AX-110020417</i>	<i>16K-16852</i>	153	9.8	23.9	-13.7
	2019TS-IT	<i>AX-110020417</i>	<i>16K-16852</i>	153	9.8	24.5	-1.0
	2019TS-DS	<i>AX-110020417</i>	<i>16K-16852</i>	153	10.9	26.4	-14.5
	IT_mean	<i>AX-110020417</i>	<i>16K-16852</i>	153	11.4	27.5	-1.0
	DS_mean	<i>AX-110020417</i>	<i>16K-16852</i>	153	11.8	27.9	-13.7
<i>QYrCW357-2AL</i>	2018YL-IT	<i>16K-4252</i>	<i>AX-110974948</i>	54	3.5	4.0	-0.4
	2018YL-DS	<i>16K-4252</i>	<i>AX-110974948</i>	54	4.1	6.3	-5.9
	2018JY-IT	<i>16K-4252</i>	<i>AX-110974948</i>	54	5.4	5.9	-0.6
	2018JY-DS	<i>16K-4252</i>	<i>AX-110974948</i>	54	3.9	4.6	-6.5
	2019YL-IT	<i>16K-4252</i>	<i>AX-110974948</i>	54	4.1	4.5	-0.5
	2019YL-DS	<i>16K-4252</i>	<i>AX-110974948</i>	54	4.2	4.6	-6.7
	2019JY-IT	<i>16K-4252</i>	<i>AX-</i>	54	3.7	3.8	-0.5

110974948							
	2019JY-DS	16K-4252	AX-110974948	54	4.2	3.2	-6.4
	2019TS-IT	16K-4252	AX-110974948	54	4.9	5.4	-0.5
	2019TS-DS	16K-4252	AX-110974948	54	5.1	4.6	-7.3
	IT_mean	16K-4252	AX-110974948	54	5.1	4.3	-0.5
	DS_mean	16K-4252	AX-110974948	54	4.6	4.5	-6.5
QYrCW357-3DL	2018YL-IT	16K-9514	16K-9526	22	2.5	3.3	-0.4
	2018YL-DS	16K-9334	16K-9468	4	3.6	5.4	-5.4
	2018JY-IT	16K-9334	16K-9468	4	4.0	4.2	-0.5
	2018JY-DS	16K-9333	16K-9415	1	3.7	4.6	-6.5
	2019YL-IT	16K-9514	16K-9526	20	6.0	8.1	-0.6
	2019YL-DS	16K-9514	16K-9526	20	6.1	8.3	-9.1
	2019JY-IT	16K-9334	16K-9468	4	3.1	3.0	-0.5
	2019JY-DS	16K-9334	16K-9468	4	3.7	2.8	-5.9
	2019TS-IT	16K-9334	16K-9468	4	2.5	2.6	-0.4
	2019TS-DS	16K-9514	16K-9526	19	2.9	2.8	-5.7
	IT_mean	16K-9334	16K-9468	4	4.7	3.8	-0.4
	DS_mean	16K-9334	16K-9468	4	4.1	3.9	-6.0
QYrCW357-6BS	2018YL-IT	16K-15955	AX-109914318	52	5.3	6.6	-0.6
	2018YL-DS	16K-15955	AX-109914318	52	7.3	12.4	-8.2
	2018JY-IT	16K-15955	AX-109914318	52	7.4	8.9	-0.7
	2018JY-DS	16K-15955	AX-109914318	52	6.7	8.8	-9.1
	2019YL-IT	16K-15955	AX-109914318	51	9.2	11.6	-0.7
	2019YL-DS	16K-15955	AX-109914318	51	8.9	11.0	-10.4

2019JY-IT	16K-15955	AX-109914318	53	7.0	7.9	-0.7
2019JY-DS	16K-15955	AX-109914318	50	4.4	3.4	-6.6
2019TS-IT	16K-15955	AX-109914318	53	7.7	9.2	-0.7
2019TS-DS	16K-15955	AX-109914318	51	10.2	10.4	-10.9
IT_mean	16K-15955	AX-109914318	51	10.0	9.3	-0.7
DS_mean	16K-15955	AX-109914318	52	10.6	11.7	-10.4

^a YL, TS, and JY, r Yangling, Tianshui, and Jiangyou, respectively; Mean, average data from five environments.

^b LOD, logarithm of odds score.

^c PVE, percentage of phenotypic variance explained by individual QTL.

^d Add, additive effect of resistance allele. A negative value indicates that the resistance allele is from CW357-9.

Table 4. Summary of stripe rust resistance QTL detected in the AvS × CW357-9 RIL population in the adult-pant stages using IciMapping 4.1

Traits	QTL1 ^a	Flanking interval	QTL2 ^b	Flanking interval	A1*A2 ^c effect
2018YL-IT	<i>QYrCW357-1BL(Yr29)</i>	<i>AX-110020417-16K-16852</i>	<i>QYrCW357-2AL</i>	<i>16K-4252-AX-110974948</i>	0.26**
2018YL-DS		<i>AX-110020417-16K-16852</i>		<i>16K-4252-AX-110974948</i>	3.55**
2018JY-IT		<i>AX-110020417-16K-16852</i>		<i>16K-4252-AX-110974948</i>	0.24*
2018JY-DS		<i>AX-110020417-16K-16852</i>		<i>16K-4252-AX-110974948</i>	3.24*
2019YL-IT		<i>AX-110020417-16K-16852</i>		<i>16K-4252-AX-110974948</i>	0.34**
2019YL-DS		<i>AX-110020417-16K-16852</i>		<i>16K-4252-AX-110974948</i>	3.98**
2019JY-IT		<i>AX-110020417-16K-16852</i>		<i>16K-4252-AX-110974948</i>	0.34*
2019JY-DS		<i>AX-110020417-16K-16852</i>		<i>16K-4252-AX-110974948</i>	4.50**
2019TS-IT		-		-	-
2019TS-DS		<i>AX-110020417-16K-16852</i>		<i>16K-4252-AX-110974948</i>	3.64*
IT_mean		<i>AX-110020417-16K-16852</i>		<i>16K-4252-AX-110974948</i>	0.25*
DS_mean		<i>AX-110020417-16K-16852</i>		<i>16K-4252-AX-110974948</i>	4.08**

^aFirst QTL and interval of a pair of interacting QTL

^b Second QTL and interval of a pair of interacting QTL

^c A1*A2 is the additive × additive interaction or epistatic effect across different environments

* P < 0.005; ** P < 0.001

Figures

Fig. 1

Figure 1

Frequency distributions of stripe rust infection type (IT) and disease severity (DS) recorded in five environments for the AvS × CW357-9RIL population during the 2017-2018 and 2018-2019 cropping seasons.

Fig. 2

Figure 2

Graphical display of stripe rust QTL mapping regions on chromosome arms 1BL (A) and 2AL (B) in five environments, corresponding to their genetic and physical maps locations (red bars), respectively.

Fig. 3

Figure 3

Effects of QTL combinations on IT and DS for the AvS x CW357-9 RIL population tested at Yangling (YL), Tianshui (TS), and Jiangyou (JY). (A, B) Effects of different QTL combinations in reducing stripe rust severity. (C, D) Validation of the combined effect of YrCW357-1BL (Yr29) and QYrCW357-2AL in RILs. (E, F) Interaction of QYrCW357-1BL (Yr29) and QYrCW357-2AL.

Fig. 4

Figure 4

Integrated physical map of QYrCW357-2AL compared with previously reported QTL for APRon chromosome arm 2AL (10-Mb scale map). Blue bars indicate interval size between flanking markers, and a dot indicates a single linked marker for a QTL. QYrCW357-2AL was shown in red. Numbers in parentheses indicate references: (1) this study; (2) Bansal et al. (2009); (3) Eriksen et al. (2004); (4) Zhou et al. (2011); (5) Liu et al. (2015); (6) Boukhatem et al. (2017); (7) Jighly et al. (2015); (8) Naruoka et al. (2015); (9) Naruoka et al. (2015); (10) Zegeye et al. (2014); (11) Chen et al. (2016); and (12) Zeng et al. (2019).

Fig. 5

Figure 5

Effects of QTL combinations for both QYrCW357-1BL and QYrCW357-2AL in a panel of 241 wheat lines based on the mean disease severities.

Supplementary Files

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- [SupplementaryTable1.xlsx](#)
- [SupplementaryTable2.xlsx](#)
- [SupplementaryTable3.xlsx](#)
- [SupplementaryTable4.xlsx](#)
- [SupplementaryTable5.xlsx](#)