Genome-wide Mapping of Quantitative Trait Loci Conferring Resistance to Stripe Rust in Spring Wheat Line PI 660072

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Abstract

Stripe rust, caused by Puccinia striiformis f. sp. tritici (Pst), is one of the most devastating diseases of wheat worldwide. Identifying resistance genes is crucial for developing resistant cultivars to control the disease. Spring wheat PI 660072 (Triticum aestivum) has been identified to possess both adult-plant resistance (APR) and all-stage resistance (ASR) to stripe rust. To elucidate the genetic basis of the resistance in PI 660072, a mapping population consisting of 211 F5 - F7 recombinant inbred lines (RILs) was developed from a cross of PI 660072 with susceptible spring wheat Avocet S. The mapping population was phenotyped for stripe rust responses across five field environments from 2020 to 2022 and genotyped using the 15K SNP (single nucleotide polymorphism) array to map stripe rust resistance loci. The mapping population was also tested at the seedling stage with predominant Chinese Pst races CYR31, CYR32, CYR34 and PST-YX1-3-1 in the greenhouse. Stripe rust resistance genes were identified using the quantitative trait locus (QTL) mapping approach. Two QTL were identified with QYrPI660072.swust-2BL mapped on the long arm of chromosome 2B for ASR and QYrPI660072.swust-4BL on the long arm of chromosome 4B for APR. To facilitate marker-assisted selection breeding, Kompetitive allele specific PCR (KASP) markers, KASP-1269 for QYrPI660072.swust-2BL and KASP-3209 for QYrPI660072.swust-4BL, were developed. These markers could be used to introgress the effective resistance QTL into new wheat cultivars.

Key Message

Two major QTL for resistance to stripe rust were mapped on chromosome 2BL and 4BL in spring wheat PI 660072, and their KASP markers were developed.

Introduction

Wheat stripe rust is a highly contagious disease caused by the fungal pathogen *Puccinia striiformis* f. sp. *tritici* (*Pst*). The pathogen is capable of spreading rapidly over long distances and causing large-scale epidemics (Wellings 2011; Chen 2020). When wheat crops in a large geographic region are affected by stripe rust, the grain yield can be reduced by 5% – 20% (Wellings 2021). In an individual field grown with a highly susceptible wheat cultivar, severe stripe rust can cause a complete loss of grain yield (Chen 2014; Zhou et al. 2022). The most effective approach for controlling stripe rust is to breed resistant cultivars. Unfortunately, many wheat varieties that were once resistant to the disease have become susceptible in China and many other countries (Chen et al. 2009; Kang et al. 2015). It is urgent to identify wheat germplasm and genes for resistance to be used in developing wheat cultivars with effective and durable resistance to stripe rust (Zhou et al. 2015b).

There are two types of stripe rust resistance genes, all-stage resistance (ASR) and adult-plant resistance (APR). ASR refers resistance to the disease throughout all growth stages, whereas plants with only adult-plant resistance (APR) are susceptible at the seedling stage, but become resistant after the seedling stage and the resistance level can increase as plants grow older and often when the weather becomes warmer (Chen 2005, 2013). ASR can be easily detected in the seedling stage and easily transferred into new cultivars. When effective, an ASR gene can provide complete control for the cultivar or cultivars carrying the gene, but the gene can be circumvented by new virulent races of the pathogen. In contrast, APR is usually non-race specific

and therefore durable. However, APR is usually partial, and some APR genes may not provide adequate resistance if stripe rust starts early in the plant growth season and the weather is not warm enough for the full expression of the resistance genes (Chen 2013, 2014). The best approach is to combine both effective ASR and APR genes in wheat cultivars to achieve adequate and durable resistance for control of stripe rust (Chen 2013).

To date, 86 officially named *Yr* genes (Klymiuk et al. 2022; Feng et al. 2023; Zhu et al. 2023) and more than 300 provisionally named genes or quantitative trait loci (QTL) in wheat and its wild relatives have been identified for resistance to stripe rust (Wang and Chen 2017; Pakeerathan et al. 2019; Li et al. 2020). Of the 86 permanently designated genes, about 30 genes confer APR, including *Yr11*, *Yr12*, *Yr13*, *Yr14*, *Yr16*, *Yr18*, *Yr29*, *Yr30*, *Yr34*, *Yr36*, *Yr39*, *Yr46*, *Yr48*, *Yr49*, *Yr52*, *Yr54*, *Yr56*, *Yr58*, *Yr59*, *Yr60*, *Yr62*, *Yr68*, *Yr71*, *Yr75*, *Yr77*, *Yr78*, *Yr79*, *Yr80*, *Yr83* and *Yr86*; and the others confer ASR. Six ASR genes (*Yr5*, *Yr7*, *Yr15*, *YrAS2388*, *YrSP* and *YrU1*) (Klymiuk et al. 2018; Marchal et al. 2018; Wang et al. 2020; Zhang et al. 2020) and three APR genes (*Yr18*, *Yr36* and *Yr46*) (Fu et al. 2009; Krattinger et al. 2009; Moore et al. 2015) have been cloned. Although the number of reported stripe rust resistance genes is quite large, many of the race-specific ASR genes are no longer effective. It is still needed to identify more effective genes and develop molecular markers for more efficiently breeding stripe rust resistant wheat cultivars.

The utilization of marker-assisted selection (MAS) enables breeders to incorporate multiple resistance genes into new cultivars (Zhou et al. 2015a). With the advancing high-throughput sequencing and molecular marker technologies, more effective and user-friendly markers have been developed for various traits, including stripe rust resistance and used in breeding programs through MAS (Barendse et al. 2009; Kump et al. 2011). Simple nucleotide polymorphisms (SNPs) offer an extremely promising approach for exploring genetic variations in crop germplasms and permit the identification of markers closely linked to the target genes or QTL (Wu et al. 2018). By converting SNPs into Kompetitive Allele Specific PCR (KASP) markers, individual markers can be used to screening breeding lines for the specific traits (Rasheed et al. 2016).

Spring wheat PI 660072 was developed by the Wheat Health, Genetics and Quality Research Unit of the US Department of Agriculture, Agricultural Research Service (USDA-ARS) and Washington State University and deposited in the USDA-ARS National Small Grains Collection (Wang et al. 2012). The line was selected from the progeny from a cross of stripe rust susceptible spring wheat Avocet S (AvS) and resistant spring wheat line PI 180957 originally from India. In the previous study, PI 660072 was resistant to US *Pst* races PST-114 and PST-127 and moderately resistant to PST-43 and PST-100 in the seedling tests and highly resistant in the fields under natural *Pst* infection in Washington State before its registration, and it was thus concluded to have both ASR and high-temperature adult-plant resistance (Wang et al. 2012). PI 660072 has continually shown high resistance to stripe rust in the United States (Wang MN and Chen XM, unpublished data). In China, PI 660072 was also highly resistant to the predominant *Pst* races in both greenhouse seedling and field adult-plant tests (Zhou et al. 2015b). However, the genetic basis of the stripe rust resistance in PI 660072 was not clear. The objectives of this study were to: 1) genetically characterize the stripe rust resistance in PI 660072 and map its resistance genes using a whole-genome QTL mapping approach, 2) assess the stability of the resistance genes across different environments, 3) determine if the genes confer ASR and APR and 4) to develop KASP markers to be used in MAS.

Materials and methods

Plant materials

Used as the male parent, PI 660072 was crossed to AvS for developing a mapping population. AvS, an Australian spring wheat selection, is susceptible to most *Pst* races in Australia, China, the United States and many other countries, and was used as the recurrent parents in developing near-isogenic lines and mapping populations to identifying stripe rust resistance in many wheat genotypes (Wellings et al. 2004; Lin et al. 2007; Sui et al. 2009; Cheng et al. 2010; Li et al. 2011; Ren et al. 2012; Wang et al. 2012; Sharma-Poudyal et al. 2013; Xu et al. 2013; Cheng et al. 2014; Lu et al. 2014; Zhou et al. 2014; Zhou et al. 2015b; Xiang et al. 2016; Feng et al. 2023). From the cross AvS/PI 660072, a mapping population consisting of 211 recombinant inbred lines (RIL) was developed using the single-seed descent method, and its F_5 - F_7 generations were used in the phenotypic tests for stripe rust responses.

Field tests

The 211 RILs, together with PI 660072 and AvS, were evaluated for stripe rust response at Mianyang (MY) (31°33'N, 104°55'E, 485 m above sea level) in Sichuan province in 2020–2022 using the F_5 - F_7 generations, respectively and at Yangling (YL) (34°17'N, 108°04'E, 530 m above sea level) in Shaanxi province in 2021 and 2022 using the F_6 and F_7 generations, respectively. MY is an area where *Pst* is able to overwinter and occurs naturally with no need of artificial inoculation. The predominant *Pst* races in Shannxi and Sichuan provinces were CYR32, CYR33, and CYR34 in recent years (Ma et al. 2016; Wang 2017; Li et al. 2018; Zhao et al. 2023). In the YL nurseries, the plants were inoculated with a mixture of the prevalent races CYR32 and CYR34. The field tests were conducted in a completely randomized block design with three replications. In each replication, approximately 30 seeds for each line were sown in a row of 100 cm with 25 cm between rows. The parents and susceptible check were planted every 20 rows throughout the field. ITs were recorded based on the 0–9 scale, with 0–3 classified as resistant, 4–6 moderately resistant and 7–9 susceptible (Line et al. 1992; Wan et al. 2004), and disease severity (DS) was recorded as percentage of leaf areas infected. Both IT and disease severity (DS) of each row were recorded three times starting when DS on MX169 reached 80% and plants were at the adult stage with the second and third notes taken 7 and 14 days after the first note, respectively.

Seedling tests

Seedling tests were conducted in a greenhouse. Chinese predominate *Pst* races CYR31, CYR32 and CYR34 and a new local isolate (PST-YX1-3-1) were used for evaluating the stripe rust responses of PI 660072, AvS and selected F_7 RILs. CYR32, CYR34 and PST-YX1-3-1 were used in phenotyping the mapping populations. In the seedling phenotyping tests, the two parents and their F_8 RILs were seeded in a 9 × 9 × 9 cm pot with approximately 10 seeds and grown in the greenhouse with the temperature and light conditions optimal for wheat seedling growth. The seedlings were inoculated at the two-leaf stage with urediniospores of a single race. Inoculated plants were kept in a dew chamber in the dark at 8 ℃ for approximately 24 h. The seedlings were then moved to a growth chamber at 16 ℃ with a daily 16 h light for stripe rust development.

Approximately 15 days later, the infection type (IT) data were recorded based on a 0–9 scale (Line and Qayoum 1992). Chinese wheat cultivar Mingxian 169 (MX169) was used as a susceptible check.

Genotyping the parental lines and RILs

Genomic DNAs of the parents and the 211 F_6 RILs were extracted from fresh seedling leaves using the cetyltrimethylammonium bromide (CTAB) method (Anderson et al. 1993). The stock DNA solutions were determined for their quality and concentration using a NanoDrop ND-1000 (Thermo Scientific, Wilmington, DE, USA) and were diluted to 50 ng/µl using deionized distilled water (ddH_2O) to be used for genotyping. The parents and RILs were genotyped using the 15K Illumina® iSelect wheat SNP array by Zhongyujin Marker Biotechnology Co., Ltd. (Beijing, China).

Statistical analyses and QTL mapping

The IT data from greenhouse, and IT and DS data from each environment were used for the analysis of variance (ANOVA) and subsequent QTL mapping. ANOVA and Pearson's correlation coefficients were calculated using QTL IciMapping V4.1 (Meng et al. 2015). The broad-sense heritability (h^2) of stripe rust resistance was estimated using the following formula: $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_{ge}^2/e + \sigma_\epsilon^2/re)$, where σ_g^2, σ_{ge}^2 and σ_ϵ^2 are estimates of the genotype, genotype × environment interaction and error variances, respectively, and *e* and *r* are the number of environments and number of replicates for each environment, respectively (Yang et al. 2005). For the plotting purpose, the step was set to 1 cM and the PIN (probability of SNP being included in the model) was set to 0.001. For QTL mapping, the threshold value of the logarithm of odds (LOD) score for each chromosome analysis was set to 2.5 to declare a significant QTL. To determine the additive effects of QTL, boxplots were used to demonstrate the effects of QTL combinations on the mean IT and DS of RILs that shared the same number of beneficial alleles.

Developing KASP markers

To find more close markers for stripe rust resistance QTL, the parents were genotyped using the 660K SNP chip. SNP markers near or at the peaks of the identified resistance QTL based on the results obtained from both 15K and 660K SNP chips were selected for developing KASP markers by Zhongyujin Marker Biotechnology Co., Ltd. (Beijing, China). The developed KASP markers were validated using the parents and RILs.

Results

Phenotypic evaluation

The infection types of AvS and PI 660072 tested with the *Pst* races or isolates were shown in Table 1. AvS was susceptible (IT 8–9), whereas PI 660072 was resistant to CYR31 (IT 2), CYR32 (IT 3), CYR34 (IT 2) and PST-YX1-3-1(IT 2). In the field tests, AvS had IT 9 across the three years and two locations, and its final DS values ranged from 90–100%. In contrast, PI 660072 was highly resistant (IT 2) in all field tests with DS 10–20%. The mean values of IT and DS of the RILs ranged from 0 to 9 and 0 to 100%, respectively, suggesting that the stripe rust resistance in PI 660072 was quantitatively inherited (Fig. 1a, b). The broad-sense heritability values of IT and DS were 0.86 and 0.87, respectively. The ANOVA analysis revealed significant

differences ($P < 0.001$) among RILs, environments, and line × environment interactions, indicating that the resistance genes in the RIL population are the primary source of phenotypic variation (Table 2). The correlation coefficients for IT and DS ranged from 0.73–0.95 and 0.68–0.95 ($P < 0.001$) based on the YL and MY data, respectively (Table 3).

Table 1
Infection types of AvS, PI 660072, and 15 selected F_7 recombinant inbred lines tested with *Puccinia striiformis* f. sp. *tritici* (*Pst*) races or isolates CYR32, CYR34 and PST-YX1-3-1 in the seedling stage under controlled greenhouse conditions

		Infection types produced by *Pst* race isolate			
Wheat line	QTL	CYR31	CYR32	CYR34	PST-YX1-3-1[a]
AvS	None	9	9	9	9
PI 660072	2BL, 4BL	2	3	2	2
RIL-5	2BL	2	2	3	2
RIL-19	2BL	1	2	4	2
RIL-32	2BL	1	2	5	4
RIL-53	2BL	1	2	2	2
RIL-94	2BL	1	2	5	1
RIL-166	2BL	2	2	5	3
RIL-179	2BL	2	2	5	2
RIL-182	2BL	1	2	5	1
RIL-209	2BL	1	2	3	3
RIL-18	4BL	7	7	7	7
RIL-40	4BL	7	7	7	7
RIL-44	4BL	7	7	7	7
RIL-76	4BL	7	7	7	7
RIL-128	4BL	7	7	7	7
RIL-131	4BL	7	7	7	7

[a] PST-YX1-3-1 is an isolate obtained from stripe rust collection in Zhongxing Town, Youxian District, Mianyang, Sichuan in 2023, and its race based on the Chinese differentials has not been determined.

Table 2

Analysis of variance and the estimates of broad-sense heritability (h^2) of the infection type (IT) and disease severity (DS) in the recombinant line (RIL) population of AvS × PI 660072 tested at Mianyang (MY) and Yangling (YL) in 2020–2022

Source	IT			DS		
	df[a]	MS[b]	*F* value	*df*	MS	*F* value
Line	210	25.38	12.59***[c]	210	3322.62	12.65***
Environments	3	268.91	133.40***	3	14068.55	53.57***
Line/environment	630	38.86	19.28***	630	9110.39	34.69***
Error	840	2.02		840	262.63	
h^2	0.86			0.87		

[a] *df*, degree of freedom

[b] MS, Mean square

[c] "***" denotes the significant level of $P < 0.001$

Table 3
Correlation coefficients (*r*) of infection type (IT) and disease severity (DS) of the recombinant inbred lines of AvS × PI 660072 tested in different environments

Trait	Environment[a]	20MY	21MY	21YL	22MY	22YL
IT	20MY	1.00***[b]				
	21MY	0.73***	1.00***			
	21YL	0.75***	0.75***	1.00***		
	22MY	0.75***	0.68***	0.71***	1.00***	
	22YL	0.95***	0.74***	0.79***	0.78***	1.00***
DS	20MY	1.00***				
	21MY	0.75***	1.00***			
	21YL	0.78***	0.92***	1.00***		
	22MY	0.73***	0.68***	0.73***	1.00***	
	22YL	0.95***	0.76***	0.79***	0.77***	1.00***

[a] MY and YL denote Mianyang and Yangling, respectively. 20, 21 and 22 denote 2020, 2021 and 2022, respectively

[b] The *r* values based on the DS data are given in parentheses. "***" denotes the *r* value is significant at $P < 0.001$

Inheritance of stripe rust resistance

To determine the number of genes conferring stripe rust resistance in PI 660072, genetic analysis was performed. In the F_5 generation tested in Mianyang in 2020, 170 lines were resistant and 41 lines susceptible, fitting a 3:1 ratio ($\chi^2 = 3.20$; $P = 0.06$). In the F_6 generation at Mianyang in 2021, 174 lines were resistant and 37 lines susceptible, marginally fitting the 3:1 ratio ($\chi^2 = 5.88$; $P = 0.01$). In the F_6 generation tested at Yangling, 172 lines were resistant and 39 lines susceptible, also marginally fitting the 3:1 ratio ($\chi^2 = 4.44$; $P = 0.03$). In the F_7 generation tested at Mianyang in 2022, 167 lines were resistant and 44 lines susceptible, fitting the 3:1 segregation ratio ($\chi^2 = 1.72$; $P = 0.16$). In the F_7 generation tested at Yangling, 170 lines were resistant and 41 lines susceptible, fitting the 3:1 segregation ratio ($\chi^2 = 3.20$; $P = 0.06$). When the F_8 generation tested at the seedling stage in the greenhouse with CYR32, 167 lines were resistant and 44 lines susceptible, fitting the 3:1 segregation ratio ($\chi^2 = 1.72$; $P = 0.16$); with CYR34, 167 lines were resistant and 44 lines susceptible, fitting the 3:1 segregation ratio ($\chi^2 = 1.72$; $P = 0.16$); and with PST-YX1-3-1, 174 lines were resistant and 37 lines susceptible, fitting the 3:1 segregation ratio ($\chi^2 = 5.88$; $P = 0.01$) (Table 4). The genetic analyses indicated two genes for stripe rust resistance.

Table 4
The number of genes for resistance to stripe rust in the AvS × PI 660072 determined by genetic analysis based on the infection type data of adult plants in Mianyang (MY) and Yangling (YL) in 2020 (20), 2021 (21), 2022 (22) and seedling stage with CYR32, CYR34 and PST-YX1-3-1 in 2023

Test	R[a]	S[b]	Total	No. of genes	χ^2	*P*-values
20MY(F_5)	170	41	211	2	3.20	0.06
21MY(F_6)	174	37	211	2	5.88	0.01
22MY(F_7)	167	44	211	2	1.72	0.16
21YL(F_6)	172	39	211	2	4.44	0.03
22YL(F_7)	170	41	211	2	3.20	0.06
CYR32	167	44	211	2	1.72	0.16
CYR34	167	44	211	2	1.72	0.16
PST-YX1-3-1	174	37	211	2	5.88	0.01

[a] R, RILs showing IT < 7

[b] S, RILs showing IT ≥ 7

Genetic linkage maps

A total of 13,946 SNPs were found between the two parents using the 15K SNP array, of which 13,198 SNPs had known chromosomal locations. After filtering the redundant markers based on “BIN”, 3,869 SNPs were used to construct genetic linkage maps. These markers were distributed across 21 linkage groups corresponding to the 21 wheat chromosomes, spanning a total length of 5,884.82 cM (Fig. 2). The number of markers per chromosome in the genetic linkage map ranged from 7 for chromosome 1D to 248 for chromosome 3A, with an average of 94 SNP markers (Table 5). The map lengths of individual chromosomes ranged from 35.10 cM (chromosome 6A) to 994.95 cM (chromosome 7D). The mean distance between adjacent SNP markers in the genetic linkage map varied across chromosomes, ranging from 0.37 cM for chromosome 6A to 29.93 cM for chromosome 1D. The genetic maps were subsequently used to locate the stripe rust resistance QTL.

Table 5
Summary of chromosome assignment, number of SNPs, map length and marker density of the genetic maps of the AvS × PI 660072 recombinant inbred line population

Chromosome	No. of SNPs	Map length (cM)	Mean SNP distance (cM)
1A	98	382.44	3.90
1B	12	236.82	19.73
1D	7	209.51	29.93
2A	46	354.94	7.72
2B	87	351.74	4.04
2D	105	214.90	2.05
3A	248	278.34	1.12
3B	33	307.41	9.32
3D	43	218.87	5.09
4A	74	297.17	4.02
4B	66	135.32	2.05
4D	66	247.24	3.75
5A	55	231.87	4.22
5B	244	237.06	0.97
5D	33	137.75	4.17
6A	95	35.10	0.37
6B	49	271.85	5.55
6D	100	298.95	2.99
7A	58	307.08	5.29
7B	220	135.51	0.62
7D	235	994.95	4.23

QTL mapping

To identify the resistance genes for stripe rust in PI 660072, QTL analysis was conducted using the genotypic data and the stripe rust phenotype data from the five field environments and the seedling tests with three races in the greenhouse. Two major QTL for resistance to stripe rust were identified in the mapping population and were located on chromosomes 2BL and 4BL, named *QYrPI660072.swust-2BL* and *QYrPI660072.swust-4BL*, respectively (Fig. 3a, b). Both QTL were from PI 660072. *QYrPI660072.swust-2BL* was located between SNP markers *AX-109547533* and *AX-111640532* at the 376,495,312 bp and 682,801,798 bp positions, respectively, about 21.0 cM apart. This QTL explained about 10.04–24.77%

phenotypic variation explained (PVE), with the LOD value of 3.78 – 11.63. *QYrPI660072.swust-4BL* was located between *AX-109412222* and *AX-108847266*, at 362,135,412 bp and 429,339,058 bp positions, respectively, about 1.5 cM apart. This QTL explained 20.50 – 28.18% PVE, and its LOD value ranged from 13.59 to 18.10 (Table 6).

Table 6
Summary of two quantitative trait loci (QTL) for stripe rust resistance identified based on mean disease severity (DS) and infection type (IT) of the 211 recombinant inbred lines from cross AvS × PI 660072 tested in Mianyang (MY) and Yangling (YL) in 2020 (20), 2021 (21) and 2022 (22) and seedling stage with CYR32, CYR34 and *Pst*-YX1-3-1 in 2023

QTL	Environment	Marker interval	IT			DS		
			LOD	PVE (%)	Add	LOD[a]	PVE (%)[b]	Add[c]
QYrPI660072.swust-2BL	20MY	*AX-109547533* *AX-111640532*	9.92	17.32	-0.98	7.97	14.53	-9.83
	21MY		10.54	18.44	-0.87	11.22	19.62	-12.12
	21YL		7.82	12.89	-0.74	11.63	19.43	-14.54
	22MY		6.05	10.04	-0.69	7.81	14.27	-9.11
	22YL		8.74	15.24	-1.03	6.61	12.42	-10.18
QYrPI660072.swust-4BL	CYR32	*AX-108847266* *AX-109412222*	10.16	24.34	-0.87	10.33	24.77	-9.57
	CYR34		5.16	12.91	-0.60	5.31	13.14	-6.40
	Pst-YX1-3-1		3.78	11.10	-0.52	3.83	11.15	-5.19
	20MY		17.19	25.74	-1.20	15.58	24.61	-12.85
	21MY		15.53	22.83	-0.97	14.10	20.50	-12.42
	21YL		15.26	24.74	-1.04	16.48	22.99	-15.87
	22MY		13.59	23.33	-1.06	14.66	22.50	-11.51
	22YL		17.96	27.40	-1.39	18.10	28.18	-15.40
	CYR32		-	-	-	-	-	-
	CYR34		-	-	-	-	-	-
	Pst-YX1-3-1		-	-	-	-	-	-

[a] LOD, logarithm of odds score

[b] PVE, percentage of the phenotypic variance explained by individual QTL

[c] Add, the additive effect of resistance allele. A negative value indicates that the resistance allele is from PI 660072

Development and validation of KASP markers

The scanning of the 660K SNP chip for the two parents resulted in 65 different SNPs on chromosome 2B and 37 polymorphic SNPs on 4B between the parents. From the 65 SNPs on 2B, 24 were initially selected for converting to KASP markers, and 3 KASP markers were finally developed. From the 37 SNPs on chromosome 4B, 14 were initially selected for developing KASPs, and 4 KASP markers were eventually developed. The 3 KASP markers on chromosome 2BL were *KASP-072-1269*, *KASP-072-7058* and *KASP-072-4948*, and the 4 on chromosome 4BL were *KASP-072-3209*, *KASP-072-9918*, *KASP-072-2222* and *KASP-072-1793.* The corresponding SNPs of the three 2BL KASP markers were *AX-108941269* (at the 682,743,322 bp position, *AX-108757058* (640,086,397 bp) and *AX-11114948* (655,225,005 bp); and those on 4BL were *AX-109493209* (385,557,144 bp), *AX-110399918* (376,431,384 bp), *AX-109412222* (362,135,412 bp) and *AX-111501793* (404,838,495 bp), respectively. KASP markers *KASP-072-1269* on 2BL and *KASP-072-3209* on 4BL were tightly linked to the target QTL (Fig. 3). The genotypes of seven recombinant RILs with their phenotypes in the genetic interval between markers *AX-109412222* and *AX-108847266* of *QYrPI660072.swust-4BL* are shown in Table 7, indicating that the QTL region has a high recombination frequency.

Table 7 SNP genotypes of seven recombinant inbred lines in the genetic interval *AX-109412222* and *AX-108847266* of *QYrPI660072.swust-4BL* (A, the resistant genotype from PI 660072; B, the susceptible genotype from AvS)

Marker[a]	SNP genotype								
	PI 660072	RIL-146	RIL-33	RIL-31	RIL-81	RIL-117	RIL-181	RIL-80	AvS
AX-111022564	A	A	A	A	B	B	B	B	B
AX-110359713	A	A	A	A	B	B	B	B	B
AX-109412222	A	A	A	A	A	B	B	B	B
KASP-072-3209	A	A	A	A	A	A	B	B	B
AX-109015565	A	A	A	A	B	B	B	B	B
AX-108847266	A	A	A	B	B	B	B	B	B
AX-109368728	A	A	B	B	B	B	A	B	B
AX-109296842	A	B	B	B	B	B	A	A	B
Phenotype	R	R	R	R	R	S	S	S	S

[a] The eight markers were from the 15K SNP and KASP marker development

Effects of the individual QTL and their combination

To estimate the individual and combined effects of the two QTL on IT and DS, the 211 RILs were grouped into different genotypic groups based on the presence of markers highly associated with the two QTL. These genotypes were further grouped into 4 groups based on the number of QTL. RILs without any of the two QTL had a mean infection type (MIT) 6 and mean DS (MDS) 54.9%. RILs carrying only *QYrPI660072.swust-2BL* had MIT 4 and MDS 30%. RILs carrying only *QYrPI660072.swust-4BL* had MIT 3 and MDS 23%. RILs carrying the two QTL had MIT 2 and MDS 11%. As expected, these results demonstrated that RILs carrying the two QTL had the highest level of resistance. RILs carrying only *QYrPI660072.swust-4BL* had lower IT and DS than those carrying only *QYrPI660072.swust-2BL* (Fig. 4a, b). The results showed that combining different QTL could increase the level of resistance through additive interactions.

Determination of resistance types of the QTL

Based on the SNP marker data, 9 lines with *QYrPI660072.swust-2BL* and 6 lines with *QYrPI660072.swust-4BL* were identified from the 211 RILs. These 15 lines were either highly or moderately resistant at the adult-plant stage in all field tests. These lines were further tested with CYR31, CYR32, CYR34 and PST-YX1-3-1 at the seedling stage in the greenhouse, and their infection types produced by the four *Pst* races or isolates are presented in Table 1. The nine lines with *QYrPI660072.swust-2BL* were resistant to the four races (IT 0–4) similar to their field resistance (IT 1–4). The six lines with *QYrPI660072.swust-4BL* were intermediate to susceptible to the three races (IT 5–8), which were different to their high or moderate resistance (IT 2–5) in the field tests. Based on these results, we concluded that *QYrPI660072.swust-2BL* confers ASR, and *QYrPI660072.swust-4BL* confers APR. The results confirmed the conclusion of both ASR and HTAP resistance in PI 660072 in the previous study (Wang et al. 2012).

Discussion

To achieve sustainable control of stripe rust, it is critical to identify and use effective genes to develop new cultivars with high-level and durable resistance (Chen 2013, 2020). In the present study, we identified two QTL, *QYrPI660072.swust-2BL* and *QYrPI660072.swust-4BL*, conferring resistance to stripe rust in wheat line PI 660072. The two major QTL were identified through QTL mapping using the stripe rust phenotypic data of five field environments. Two KASP markers, *KASP-072-1269* for the 2BL QTL and *KASP-072-3209* for the 4BL QTL, were developed and could be used in MAS for incorporating the QTL into wheat cultivars to improve resistance to stripe rust.

QYrPI660072.swust-2BL, mapped on chromosome 2BL, was effective against the tested predominant Chinese *Pst* races in the greenhouse seedling tests and consistently detected in all five field environments. This ASR QTL was mapped between SNP markers *AX-109547533* and *AX-111640532*, corresponding to the 376,495,312 bp and 682,801,798 bp positions, respectively on 2BL of the Chinese Spring reference genome (IWGSC RefSeq v1.0 2BL). Many stripe rust resistance genes or QTL have been reported on 2BL (Wang and Chen 2017; Liu et al. 2019; Nicolas et al. 2019). Five permanently named stripe rust resistance genes, *Yr5* (Yan et al. 2003), *Yr7* (Yao et al. 2006), *Yr43* (Sui et al. 2009), *Yr44* (Cheng et al. 2010) and *Yr53* (Xu et al. 2013) have been mapped to 2BL and conferred ASR. *Yr5*, *Yr7* and *YrSP* have been cloned, and their physical position was 685.27 Mb on 2BL in the Chinese Spring reference genome (IWGSC RefSeq v1.0) (Marchal et al. 2018). *Yr43* in a hard white spring wheat cultivar 'IDO377s' (PI 591045) is flanked between *Xwgp103* and *Xwgp110*, with a genetic distance of 4.4 and 5.5 cM, respectively (Cheng et al. 2010). *Yr43* was susceptible to the *Pst* CYR32, CYR33 and CYR34 at the seedling stage (Liu et al. 2017). *Yr44* is originally from a soft white spring wheat cultivar 'Zak' (PI 607839). This gene is 3.9 and 9.4 cM to its frank markers *Xwgp100* and *XpWB5/N1R1*, respectively (Sui et al. 2009). *Xwgp100* is on the same side as *Xgwm501* that is around 672,082,697 bp on 2BL of the Chinese Spring genome (IWGSC RefSeq v1.0 2BL). *Yr53* in durum wheat PI 480148 originally from Ethiopia was mapped between *XLRRrev/NLRRrev*$_{350}$ and *Xwmc441*, about 5.6 cM to the latter marker (Xu et al. 2013). *Xwmc441* is around 598,064,318 bp on 2BL in the Chinese Spring genome (IWGSC RefSeq v1.0 2BL). Because *QYrPI660072.swust-2BL* had no linked markers between the flanking markers, this interval also includes *Yr44* and *Yr53*. However, according to a previous study, *Yr44* was susceptible to Chinese *Pst* races CYR32, CYR33 and CYR34 at the seedling stage (Wang et al. 2019).

Therefore, *QYrPI660072.swust-2BL* should be different from *Yr44*. Further research is needed to narrow the target interval and determine whether *Yr53* and *QYrPI660072.swust-2BL* are the same gene.

QYrPI660072.swust-4BL, mapped on chromosome 4BL, was consistently associated with stripe rust APR in all five field environments. This QTL was mapped between markers *AX-109412222* and *AX-108847266* corresponding to the 362,135,412 bp and 429,339,058 bp positions, respectively on 4BL in the Chinese Spring genome (IWGSC RefSeq v1.0 4BL). So far, more than ten genes or QTL have been reported on chromosome 4BL. *QYrus.vt-4BL* for APR in wheat line USG 3555 was mapped between SSR markers *gwm165* and *gwm149* corresponding to the 412,716,441 bp and 544,649,745 bp positions respectively on 4BL in the Chinese Spring genome (IWGSC RefSeq v1.0) (Christopher et al. 2013). *QYrhm.nwafu-4B* in wheat Humai 15 was mapped to an interval of 3.4 cM on chromosome 4BL, flanked by SNP markers *AX-111150955* and *Xgwm251* corresponding to the interval between 523,448,600 and 568,556,138 bp, respectively on 4BL in the Chinese Spring genome (IWGSC RefSeq v1.0) (Yuan et al. 2018). Permanently named stripe rust resistance genes on 4BL include *Yr50* (Liu et al. 2013) and *Yr62* (Lu et al. 2014). *Yr50* is an ASR gene mapped between SSR markers *Xbarc1096* and *Xwmc47*, about 8.0 cM and 7.2 cM apart to these markers, respectively (Liu et al. 2013). The two markers correspond to the positions 105,145,886 and 644,865,926 bp, respectively on 4BL of the Chinese Spring genome (IWGSC RefSeq v1.0). The HTAP resistance gene *Yr62* in PI 192252 was mapped between SSR markers *Xgwm251* and *Xgwm192* (Lu et al. 2014). The *Xgwm251* is around the 568,556,138 bp position on 4BL in the Chinese Spring genome (IWGSC RefSeq v1.0). Based on the physical positions, some of the above-mentioned genes are different from *QYrPI660072.swust-4BL*, but *QYrus.vt-4BL* is close to *QYrPI660072.swust-4BL*. The relationship between *QYrPI660072.swust-4BL* and *QYrus.vt-4BL* needs a further study.

The present study showed that the combination of *QYrPI660072.swust-2BL* and *QYrPI660072.swust-4BL* increased the level of resistance through additive interactions. The selected RILs carrying the two genes, as well as PI 660072, are valuable sources to be used in wheat breeding programs for developing cultivars resistant to stripe rust. The two QTL can be used in combination and also with other genes for stripe rust resistance. The KASP markers for the two QTL should be useful for MAS in pyramiding them with other resistance genes.

Declarations

Author contribution statementXZ made the cross, participated in field experiments and contributed to the genotyping experiment, analyzed the data, detected the QTL and prepared the first draft of the manuscript. XZ, YW and JS contributed to the collection of samples and phenotypic data. YW, YL and HC tested the resistant parental and single-gene lines. JS, YW, GJ, LZ and HC assisted in analyzing the data. JS, XL, SY, GL, KH and YR participated in field experiments. MW provided seed of the resistant parent and assistance to the study. XC and XZ conceived and directed the project and revised the manuscript. All authors revised and approved the manuscript for submission.

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Conflict of interest The authors declare that they have no conflict of interest.

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Figures

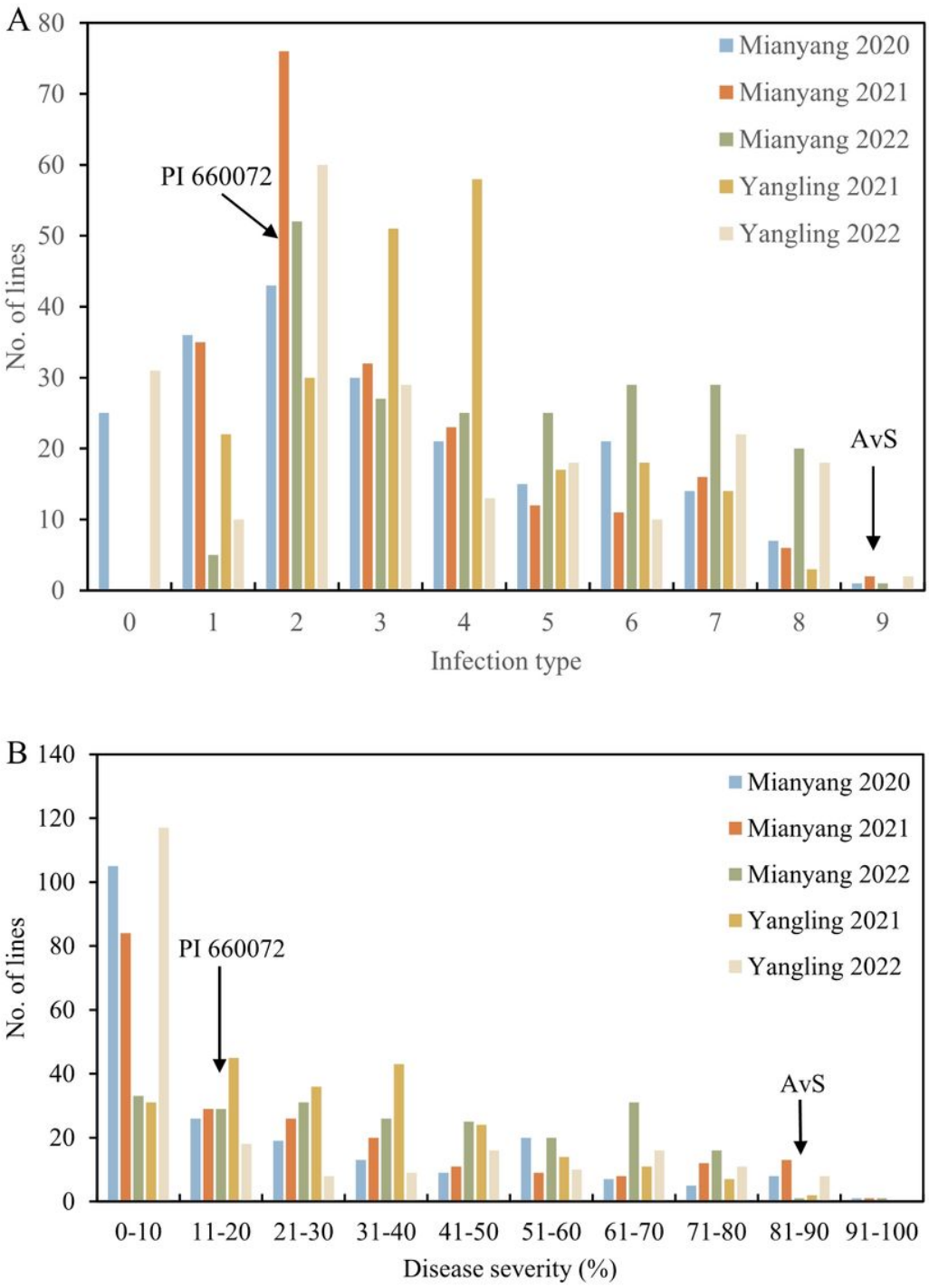

Figure 1

Frequency distributions of mean infection types (IT) and disease severity (DS) values for 211 recombinant inbred lines from cross AvS × PI 660072 tested in Mianyang (MY) and Yangling (YL) in 2020-2022. Arrows indicate the values of the parent lines

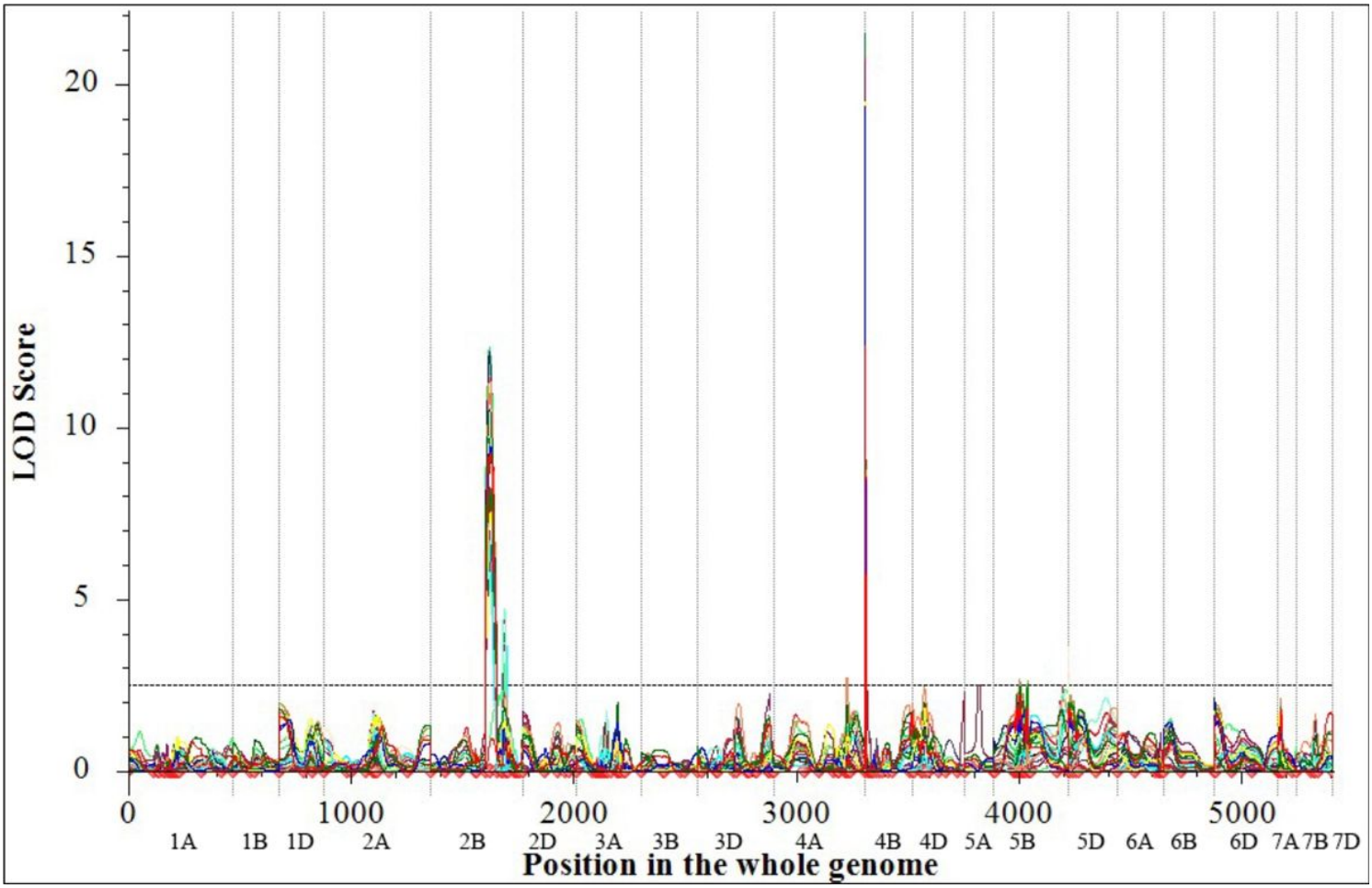

Figure 2

Stripe rust resistance quantitative trait loci detected by the biparental population analysis in the AvS × PI 660072 recombination inbred line populations across five field environments in 21 chromosome based on mean infection type (IT) and mean disease severity (DS)

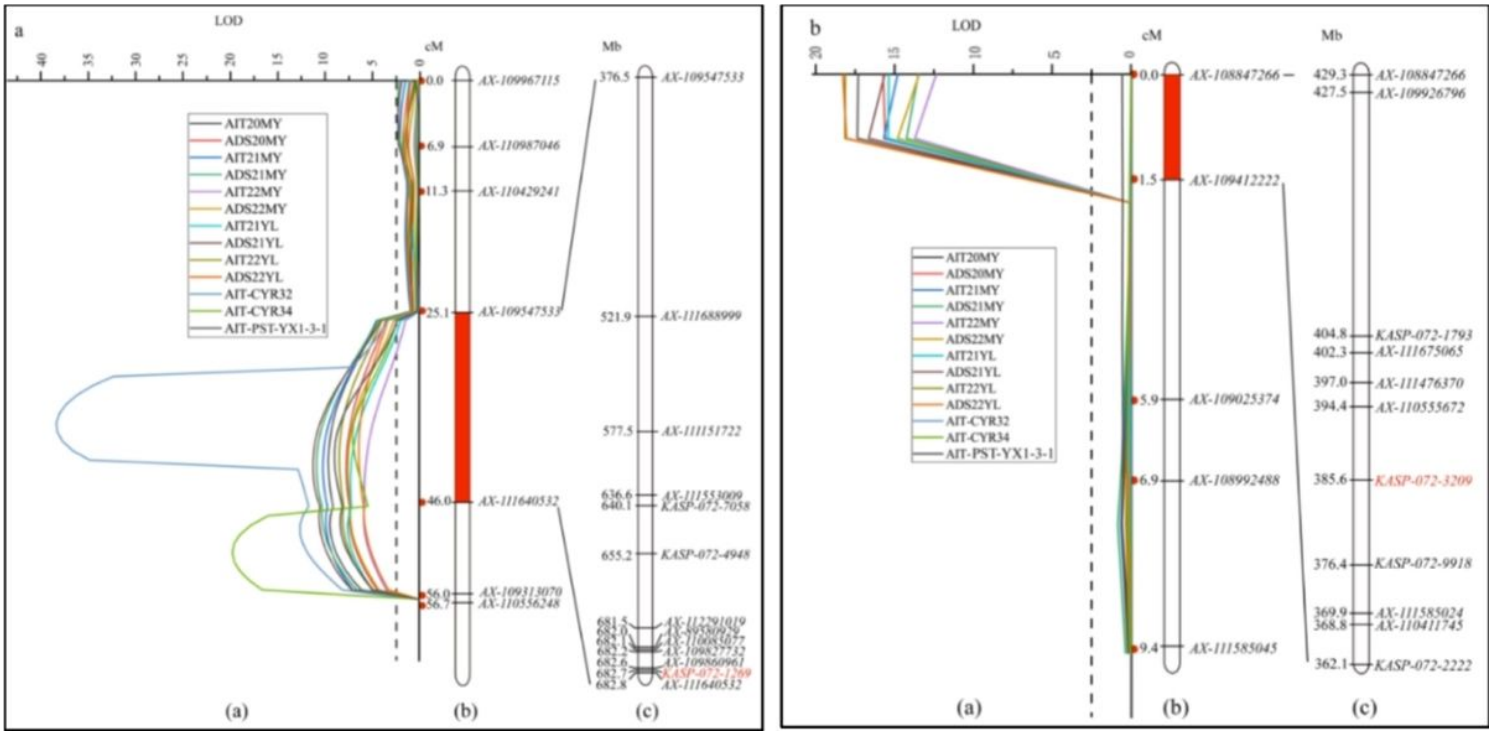

Figure 3

Stripe rust resistance quantitative trait loci (QTL) on the genetic maps of chromosomes 2BL and 4BL. (**a**) The plot of LOD values calculated from 15K SNP genotyping and stripe rust phenotypic data. The red rectangle on the genetic map indicates the corresponding QTL region. (**b**) The genetic distance (cM) between markers calculated by QTL IciMapping V4.1. (**c**) The physical position of a portion of SNP markers with differences in the parents between flanking markers

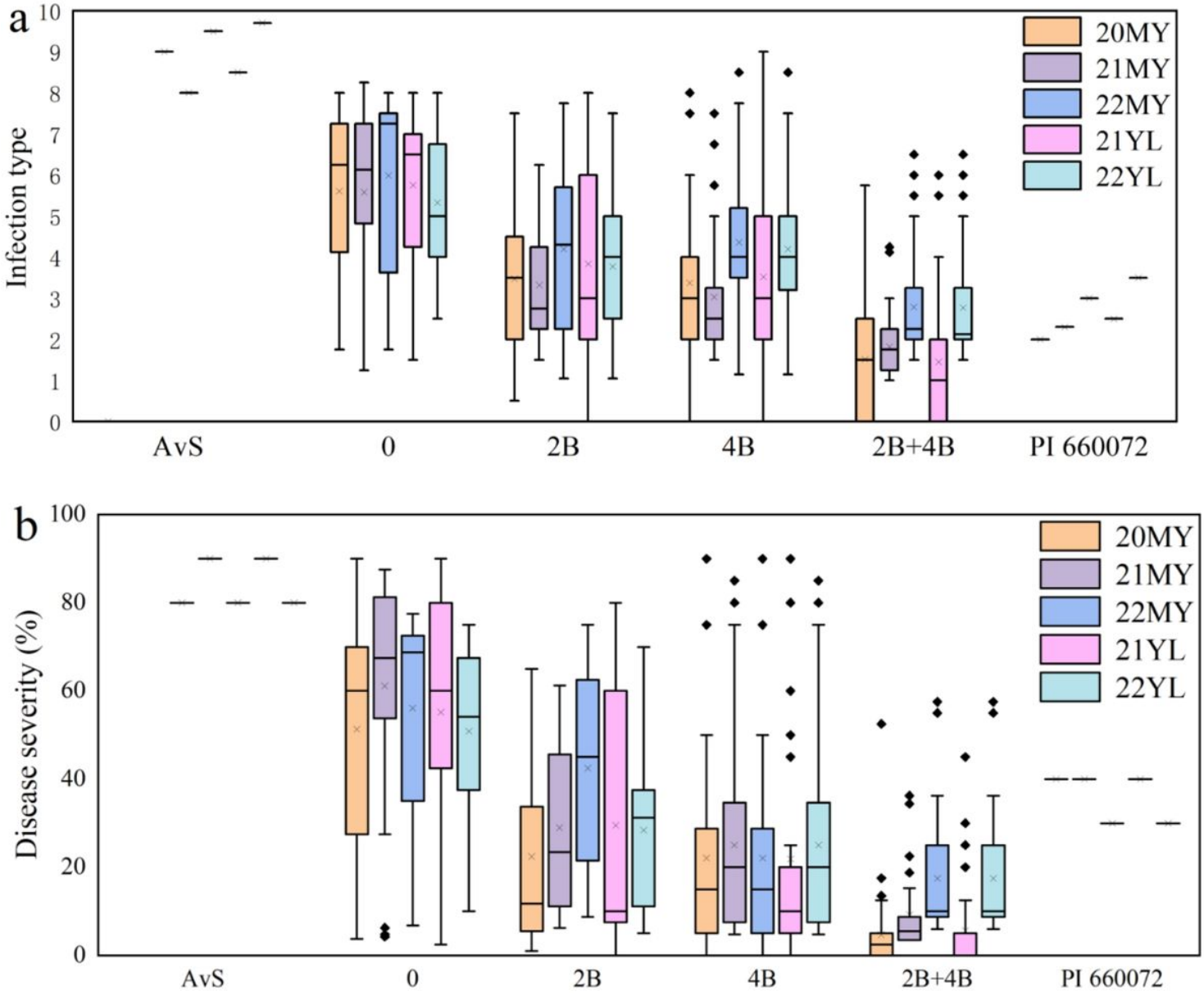

Figure 4

Effects of combined quantitative trait loci (QTL) on stripe rust using infection type **(a)**and disease severity **(b)** data for the AvS × PI 660072 recombinant inbred line population data from Mianyang (MY) and Yangling (YL). Y-axes, 'QTL combination'