RESEARCH ARTICLE

Genome-Wide Linkage Mapping of QTL for Adult-Plant Resistance to Stripe Rust in a Chinese Wheat Population Linmai 2 × Zhong 892

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Abstract

Stripe rust is one of the most devastating diseases of wheat (*Triticum aestivum*) worldwide. Adult-plant resistance (APR) is an efficient approach to provide long-term protection of wheat from the disease. The Chinese winter wheat cultivar Zhong 892 has a moderate level of APR to stripe rust in the field. To determine the inheritance of the APR resistance in this cultivar, 273 F_6 recombinant inbred lines (RILs) were developed from a cross between Linmai 2 and Zhong 892. The RILs were evaluated for maximum disease severity (MDS) in two sites during the 2011–2012, 2012–2013 and 2013–2014 cropping seasons, providing data for five environments. Illumina 90k SNP (single nucleotide polymorphism) chips were used to genotype the RILs and their parents. Composite interval mapping (CIM) detected eight QTL, namely *QYr.caas-2AL*, *QYr.caas-2BL.3*, *QYr.caas-3AS*, *QYr.caas-3BS*, *QYr.caas-5DL*, *QYr.caas-6AL*, *QYr.caas-7AL* and *QYr.caas-7DS.1*, respectively. All except *QYr.caas-2BL.3* resistance alleles were contributed by Zhong 892. *QYr.caas-3AS* and *QYr.caas-3BS* conferred stable resistance to stripe rust in all environments, explaining 6.2–17.4% and 5.0–11.5% of the phenotypic variances, respectively. The genome scan of SNP sequences tightly linked to QTL for APR against annotated proteins in wheat and related cereals genomes identified two candidate genes (autophagy-related gene and disease resistance gene *RGA1*), significantly associated with stripe rust resistance. These QTL and their closely linked SNP markers, in combination with kompetitive allele specific PCR (KASP) technology, are potentially useful for improving stripe rust resistances in wheat breeding.

Introduction

Stripe rust (yellow rust, YR), caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is a very destructive fungal disease of common wheat (*Triticum aestivum*), and it is prevalent in temperate or medium altitude and maritime wheat-growing regions, such as China, India, Pakistan, Australia, USA, Mexico, and northwestern Europe [1,2]. Yield losses caused by YR ranged from 10 to 70% and over 20 significant YR epidemics were documented worldwide during 1954–2010 [3]. During recent years, YR has occurred in about 4.2 million ha and caused heavy yield losses in the southwestern and northwestern China annually [4–6].

Although YR can be controlled by fungicides, this may be limited by management and financial constraints. Resistant cultivars are an economically effective and environmentally friendly approach to control the disease [7]. Resistance to YR can be categorized broadly into all-stage resistance and adult-plant resistance. Generally, all-stage resistance is conferred by major genes that are race-specific in effect and qualitatively inherited [8–10]. However, such resistance is usually not durable and readily overcome by new pathogen races. A singly deployed all-stage resistance gene is effective only for about 3–5 years on average [8]. In contrast, adult-plant resistance (APR) is more likely conferred by minor genes that are typically race non-specific, inherited quantitatively, and has greater potential for durability [11–12]. An APR gene usually contributes partial resistance and combinations of 4–5 APR genes act additively to confer adequate levels of durable resistance [13–14]. For example, *Yr18* and 2–4 additional minor genes have provided effective resistance to YR in China and other countries for over 80 years [15–17]. APR is being increasingly emphasized in breeding for rust resistance worldwide [18–19] mainly because of its potential but not exclusive durability [20].

To date, 70 YR resistance genes at 67 wheat loci have been formally catalogued [18–21]. Most of these genes are race-specific, and in China, the majorities have been overcome by new races [22]. APR genes at 13 loci have been cataloged, namely, *Yr16* [23], *Lr34/Yr18/Pm38/Sr57* [24–25], *Lr46/Yr29/Pm39/Sr58* [26–27], *Sr2/Yr30* [28], *Yr36* [29–30], *Yr39* [31], *Lr67/Yr46/Pm46/Sr55* [32], *Yr48* [33], *Yr49* [34], *Yr52* [35], *Yr54* [36], *Yr59* [37], and *Yr62* [38]. Some such as *Lr34/Yr18/Pm38/Sr57*, *Lr46/Yr29/Pm39/Sr58*, *Sr2/Yr30* and *Lr67/Yr46/Pm46/Sr55* confer pleiotropic disease resistances. *Lr34/Yr18/Pm38/Sr57* [30], *Yr36* [15], and *Lr67/Yr46/Pm46/Sr55* (Lagudah, pers. comm.) were cloned and appear to have quite different molecular structures to the currently cloned all-stage rust resistance genes.

During the last 15 years more than 160 QTL that reduce YR severity were assigned to 49 chromosomal regions [18,21]. Even allowing for commonality this represents a high level of genetic diversity. Given that combinations of several such QTL (genes) are required to obtain sufficiently high levels of resistance [7,14], the expected reward is durability. Many studies have shown that such resistance can be obtained by visual selection in disease nurseries, but clearly such selection is greatly aided (perhaps even circumvented) by use of molecular markers. Molecular markers can be used in programs that aim to combine both APR and all-stage resistance where it is impossible or extremely difficult to visually assess APR effects in the presence of all-sage resistance genes.

Marker platforms used in the past for linkage map construction and QTL mapping included restriction fragment length polymorphism (RFLP) [7,39–40], amplified fragment length polymorphism (AFLP) [41–42], simple sequence repeats (SSRs) [43–45] and diversity arrays technology (DArT) [22,46]. However, the low level of polymorphism and the large genome size of common wheat ultimately limits mapping resolution. SNP arrays that provide a large number of genome-wide polymorphic, co-dominant markers for high-throughput, cost-effective genotyping are ideal for QTL mapping. The high-density linkage maps constructed with SNP markers can be used for high-resolution QTL analysis and identification of candidate genes

associated with quantitative traits [47–48]. The recently developed wheat 90K SNP array, comprising 81,587 SNPs with a dense coverage of the wheat genome [49], can be used for efficient QTL mapping and construction of high-density maps [47–48,50].

Zhong 892 is a good semi-dwarf winter wheat line, exhibiting a moderate level of resistance to YR and powdery mildew in the field, whereas it is susceptible at the seedling stage, indicating a typical APR. However, little is known about the inheritance of resistance to YR in this cultivar. The objectives of the current study were to identify APR QTL to YR in a Linmai 2 × Zhong 892 RIL population using high-density SNP markers, and to assess the stability of detected QTL across environments.

Materials and Methods

Plant materials

A total of 273 $F_{2:6}$ RILs were developed from the Linmai 2 × Zhong 892 cross. Zhong 892 and Linmai 2 were highly susceptible to currently prevalent *Pst* races CYR29, CYR31, CYR32, and CYR33 at the seedling stage, whereas they showed moderately resistant and moderately susceptible, respectively, at the adult-plant stage in the field. The RILs were generated through single seed descent, where one random spike was harvested in each generation and advanced to the next generation.

Field trials

The $F_{2:6}$ RILs and their parents were evaluated for APR to YR at the Pixian experimental station of Sichuan Academy of Agricultural Sciences (30°05′N, 102°54′E) in Sichuan province by Dr. Ling Wu (a co-author of this manuscript, and a wheat breeder in Sichuan Academy of Agricultural Sciences), and the Qingshui experimental station of Gansu Academy of Agricultural Sciences (34°05′N′, 104°35′E) in Gansu province by Dr. Bin Bai (a co-author of this manuscript, and a wheat breeder in the Qingshui experimental station) during the 2011–2012, 2012–2013 and 2013–2014 cropping seasons, providing data for five environments. Both locations are hotspots for YR in China with ideal conditions for rust infection and spread. Field trials were conducted in randomized complete blocks with three replicates at each location. Each plot consisted of a single row with 1.5 m length and 25 cm between rows. Approximately 50 seeds were sown in each row. Every tenth row was planted with the highly susceptible control cv. Huixianhong. To ensure ample field inoculum, infection rows of cv. Chuanyu 12 and Huixianhong surrounded the experimental areas at Pixian and Qingshui, respectively. Inoculations at both sites each year were performed at the three-leaf stage with a mixture of prevalent Chinese *Pst* races, CYR29, CYR31, CYR32 and CYR33, using spray method (around Jan. 5 at Pixian and April 10 at Qingshui). Maximum disease severities (MDS) [51] of RILs were scored 18–20 d post-flowering, when YR severities on the control Huixianhong reached a maximum level around 8–10 April at Pixian and 7–10 June at Qingshui.

Genotyping

Genomic DNA was extracted from five bulked leaves each line using a modified CTAB procedure [52]. All 273 lines and their parents were genotyped with the Illumina 90K iSelect assay [49] by Capital Bio Corporation (Beijing, China; http://www.capitalbio.com). Genotypic clusters for each SNP were determined using the manual option of Genome Studio version 1.9.4 with the polyploid clustering version 1.0.0 (Illumina; http://www.illumina.com), based on data from all genotypes. The default clustering algorithm implemented in Genome Studio was initially used to classify each SNP call into three distinct clusters corresponding to the AA, BB and

AB genotypes expected for bi-allelic SNPs. These SNP markers were described by Wang et al. [49] and only co-dominant SNP markers were used for genetic mapping. The chromosome location of each SNP was based on wheat SNP consensus map [49].

Statistical analysis

The MDS were evaluated in five environments during three cropping seasons, and data from each environment and the arithmetic means for each line were used for analysis of variance (ANOVA) and subsequent QTL mapping. ANOVA and computation of correlation coefficients were performed by the SAS V9.0 (SAS Institute Inc., Cary, NC). The contributions of lines (RILs) and environments were evaluated by PROC MIXED, where environments were treated as fixed effects, and lines, line × environment interaction and replicates nested in environments were all treated as random. The information in the ANOVA table was used to calculate broad sense heritability (h_b^2) for YR: $h_b^2 = \sigma_g^2/(\sigma_g^2 + \sigma_{ge}^2/r + \sigma_\varepsilon^2/re)$, where σ_g^2, σ_{ge}^2 and σ_ε^2 were estimates of genotypic, genotype (line) × environment interaction and residual error variances, respectively, and e and r were the numbers of environments and replicates per environment.

Genetic linkage map construction and QTL analysis

The genotypic data for SNP markers were used to construct genetic linkage maps with the software Joinmap V4.0 (http://www.kyazma.com) [53] and maps were made by MapChart V2.2 (http://www.earthatlas.mapchart.com) [54]. Map distances (in centimorgans, cM) were calculated based on the Kosambi mapping function [55].

Composite interval mapping (CIM) was performed using the software QTL Cartographer V2.5 (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm) [56]. The walking speed chosen for all QTL was 2.0 cM, with $P = 0.001$ in stepwise regression. Based on 2,000 permutations at a probability of 0.01, the LOD score to declare significant QTL for MDS was 2.0–2.5 in all five environments and the averaged data, thus the LOD score 2.5 was set as the threshold for declaring significant QTL. The proportion of phenotypic variance (R^2) explained by a single QTL was determined by the square of the partial correlation coefficient, and the total R^2 in a simultaneous fit was calculated through multiple linear regressions using the SAS REG procedure (SAS Institute Inc., Cary, NC). Individual environment QTL overlapping within a 20 cM interval were considered common. In this study, the genotype of Zhong 892 was defined as 2, and the genotype of Linmai 2 was defined as 0. Thus, the allele from Zhong 892 reduced YR MDS when the additive effect was negative. QTL detected in at least two environments were included in the results.

Search for candidate genes for stripe rust resistance

In order to identify candidate genes involved in QTL for stripe rust resistance detected in the Linmai 2/Zhong 892 population, the EST sequences (about 50 bp upstream and 50 bp downstream) corresponding to the SNP markers [49] located in the regions underlying the QTL were used to BLAST against the NCBI nucleotide database (http://www.ncbi.nlm.nih.gov/) and European Nucleotide Archive (http://www.ebi.ac.uk/ena). BLAST hits were filtered to an e-value threshold of 10^{-5} with an identity higher than 75%.

Results

Phenotypic evaluation

The mean MDS of the susceptible control Huixianhong were over 80% across all environments. The averaged MDS for the 273 RILs were 48.3%, 44.5%, 56.6%, 35.2% and 42.8%, ranging

between 5.0–95.1%, 2.0–90.0%, 3.1–96.2%, 3.1–74.5% and 2.6–84.2% in Pixian 2012, Pixian 2013, Pixian 2014, Qingshui 2013 and Qingshui 2014, respectively (S1 Fig), indicating polygenic variation. Zhong 892 was rated with a mean MDS of 23.3%, 34.6%, 40.7%, 25.0% and 20.0% in Pixian 2012, Pixian 2013, Pixian 2014, Qingshui 2013 and Qingshui 2014, respectively, whereas Linmai 2 had mean MDS of 53.2%, 46.8%, 60.2%, 38.3% and 42.7% in the five environments, respectively (S1 Fig). The MDS were significantly correlated (0.47–0.61, $P < 0.01$) across environments, and the broad-sense heritability of YR MDS was 0.85. ANOVA of MDS revealed significant differences ($P < 0.01$) among RILs, environments, and line × environment interactions (Table 1).

QTL for APR to YR

Eight QTL were identified on different chromosomes, namely *QYr.caas-2AL*, *QYr.caas-2BL.3*, *QYr.caas-3AS*, *QYr.caas-3BS*, *QYr.caas-5DL*, *QYr.caas-6AL*, *QYr.caas-7AL* and *QYr.caas-7D*S.1 (Table 2; Fig 1). The resistance alleles of the QTL on 2AL, 3AS, 3BS, 5DL, 6AL, 7AL and 7DS were contributed by Zhong 892, whereas *QYr.caas-2BL.3* was from Linmai 2.

A major and consistent QTL for YR resistance, *QYr.caas-3AS*, was flanked by *Kukri_rep_c102131_891* and *Kukri_c96747_274* with genetic distances of 2.2 and 1.5 cM, respectively, and explained 9.2%, 6.2%, 13.0%, 17.4%, 6.5% and 15.8% of the phenotypic variances in Pixian 2012, Pixian 2013, Pixian 2014, Qingshui 2013 Qingshui 2014 and the averaged MDS, respectively. The second consistently detected QTL with a relatively large effect, *QYr.caas-3BS*, between *IAAV5662* and *BS00056257_51* with genetic distances of 1.1 and 1.2 cM, respectively, explained 5.0 to 11.5% of the phenotypic variances in five environments and the averaged MDS. The third QTL, *QYr.caas-7AL*, between *Kukri_c41603_111* and *Excalibur_c25335_306* with genetic distances of 2.0 and 1.2 cM, respectively, accounted for 12.0%, 7.7%, 5.0% and 6.6% of the phenotypic variances in Pixian 2013, Qingshui 2013, Qingshui 2014, and the averaged MDS, respectively. The fourth QTL, *QYr.caas-7DS.1*, between *tplb0024a09_2369* and *RAC875_c29314_291* with genetic distances of 10.0 and 2.3 cM, respectively, explained 7.8%, 6.7%, 5.7% and 9.1% of the phenotypic variances in Pixian 2013, Pixian 2014, Qingshui 2013, and the averaged MDS, respectively (Table 2; Fig 1).

Three QTL, *QYr.caas-2AL*, *QYr.caas-2BL.3* and *QYr.caas-6AL*, were found in Pixian 2014, Qingshui 2013 and the averaged MDS, and explained 4.4–7.2%, 5.6–7.6% and 4.3–7.8% of the phenotypic variances, respectively (Table 2; Fig 1). *QYr.caas-2AL* was flanked by *wsnp_Ex_c16627_25162391* and *BS00092550_51* with genetic distances of 6.5 and 1.6 cM, respectively; *QYr.caas-2BL.3* was located in the interval of *Ra_c21099_1781* and *IACX8602* with genetic distances of 2.0 and 0.9 cM, respectively; *QYr.caas-6AL* was mapped on chromosome 6AL between *Ku_c45494_267* and *BS00040166_51* with genetic distances of 0.8 and 7.3 cM, respectively (Fig 1).

QYr.caas-5DL flanked by *wsnp_Ex_c508_1008029* and *wsnp_Ex_c22984_32207214* with genetic distances of 1.7 and 4.1 cM, respectively, explained 5.8%, 6.5% and 4.7% of the phenotypic variances in Pixian 2013, Qingshui 2013, and the averaged MDS, respectively (Table 2; Fig 1).

To identify the combined effects of these QTL, the flanking markers were used to select RILs possessing the corresponding QTL. Among 42 combinations (genotypes) of eight resistance QTL, a significant additive effect for stripe rust was found in the RILs possessing 5–7 QTL (S1 Table). Results indicated that the more resistance genes a line possessed, the lower the disease severity was (Fig 2). When 5–7 genes were combined in a line, the MDS was less than 30% on average (Fig 2).

Table 1. Analysis of variance of MDS for stripe rust response in the Linmai 2 × Zhong 892 RIL population.

Source of variance	Df	Mean square	F value
Replicate (environment)	2	1571	8.8**
Environment	4	38028	212.0**
Line	272	3125	17.4**
Line × Environment	1088	311	1.7**
Error	1654	179	

** Significant at $P < 0.0001$

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Discussion

Comparisons of QTL with previous reports

QYr.caas-3AS. The QYr.caas-3AS was derived from Zhong 892, and it was detected consistently across all environments. A number of QTL were previously found on chromosome 3A [57–59]. As this is a first report of a QTL for YR resistance on chromosome 3AS, it is likely to be a new gene.

QYr.caas-3BS. Several QTL for YR resistance on chromosome 3BS were reported previously [24,27,33,40,42,60–61]. Most of these QTL are probably in the same region [57]. For example, loci *Xfba190* [40], and *Xgwm493* [60] are close to *Xgwm389* and *Xgwm533* [24]. *Yr30* [40], *Sr2* [62], pseudo-black chaff [63] and a Fusarium head blight resistance gene *Fhb1* [64] were all closely linked to *Xgwm533*. QYr.caas-3BS derived from Zhong 892 showed consistent resistance to YR across all environments. The SNP markers closely linked to QYr.caas-3BS were *IAAV5662* and *BS00056257_51*. SSR marker *Xgwm533* and SNP marker *BS00056257_51* belong to the same bin (3BS9-0.57–0.78). The molecular marker for *csSr2* [65] was not present in Zhong 892 indicating absence of *Sr2/Yr30*. Nevertheless, QYr.caas-3BS was likely to be the same as some of the QTL described above. Further study is needed to test the allelism between QYr.caas-3BS and other previously reported QTL.

QYr.caas-5DL. Suenaga et al. [24] identified *QYr.jirc-5DL* on chromosome 5DL closely linked to SSR locus *Xwmc215*; this explained 3.9% of the phenotypic variance. Imtiaz et al. [66] found *QYr.nsw-5DL* on chromosome 5DL, closely linked to *Xgwm583*, explaining 6.1% of the phenotypic variance. The SSR markers *Xwmc215* and *Xgwm583*, and the SNP marker *wsnp_Ex_c22984_32207214* belong to the same bin (5DL5-0.76–1.00). Thus, QYr.caas-5DL was likely to be the same as the QTL described previously.

QYr.caas-7AL. Dedryver et al. [42] identified an APR QTL *QYr.inra-7A* on chromosome 7A in wheat cultivar Recital; it was located between AFLP markers *Xbcd129b* and *Xfba127c*. Zwart et al. [67] identified *QYr.sun-7A* on chromosome 7A in wheat cultivar CPI133872; this was mapped between AFLP markers *Wpt-7214* and *Wpt-4877*. Due to different kinds of markers used in those and current studies, it is difficult to determine whether they are the same or not.

QYr.caas-7DS.1. The pleiotropic APR gene *Lr34/Yr18/Pm38* located on the short arm of chromosome 7D [68–70], and closely linked to *Xgwm295*. Many other studies also detected QTL in this region, explaining 20–40% of the phenotypic variances [16,24,40,67,71–76]. The closest marker *BS00022203_51* linked to *QYr.caas-7DS.1* in bin 7DS5-0.36–0.61 is near *Xgwm295* in bin 7DS4-0.61–1.00. The available information indicates that *QYr.caas-7DS.1* is different from *Yr18*. Firstly, tests with the STS marker *csLv34* [25] were negative and secondly, *Lr34/Yr18/Pm38* is linked with the phenotypic marker LTN (leaf tip necrosis), which was not observed in the present materials under conditions where LTN was clearly expressed in other

Table 2. QTL for stripe rust response in the Linmai 2 × Zhong 892 RIL population across five environments.

QTL[a]	Closest marker	Distance (cM)	Pixian 2012			Pixian 2013			Pixian 2014			Qingshui 2013			Qingshui 2014			Average		
			LOD[b]	R^{2c}	Add[d]	LOD	R^2	Add	LOD	R^2	Add	LOD	R^2	Add	LOD	R^2	Add	LOD	R^2	Add
QYr. caas-2AL	BS00092550_51	1.6							2.6	4.4	-3.7	4.4	7.2	-7.9				3.0	5.1	-5.0
QYr. caas-2BL.3	IACX8602	0.9							3.0	5.6	3.9	5.3	7.6	9.7				3.4	5.9	4.9
QYr. caas-3AS	Kukri_c96747_274	1.5	5.4	9.2	-8.1	3.7	6.2	-5.9	8.1	13.0	-5.2	10.8	17.4	-11.8	3.7	6.5	-3.1	10.0	15.8	-6.0
QYr. caas-3BS	IAAV5662	1.1	3.5	5.9	-6.9	2.6	5.0	-3.1	7.4	11.5	-5.1	6.9	10.5	-10.6	5.7	9.4	-3.8	6.8	10.5	-5.5
QYr. caas-5DL	wsnp_Ex_c508_1008029	1.7				3.4	5.8	-6.4				3.8	6.5	-10.7				2.9	4.7	-4.5
QYr. caas-6AL	Ku_c45494_267	0.8							2.6	4.3	-4.0	4.6	7.8	-9.7				3.0	4.9	-4.2
QYr. caas-7AL	Excalibur_c25335_306	1.2				7.2	12.0	-8.3				4.1	7.7	-8.0	2.9	5.0	-3.0	3.1	6.6	-4.6
QYr. caas-7DS.1	RAC875_c29314_291	2.3				4.2	7.8	-5.8	3.5	6.7	-4.9	2.7	5.7	-3.7				4.8	9.1	-4.6
Total R^{2e}				10.2			33.4			36.9			42.8			18.3			48.7	

a QTL were detected with a LOD threshold 2.5 for declaring QTL based on 2,000 permutations at $P = 0.01$.
b Logarithm of odds score.
c Percentage of phenotypic variance explained by the QTL.
d Additive effect of resistance allele.
e The total R^2 in a simultaneous fit was calculated through multiple linear regressions.
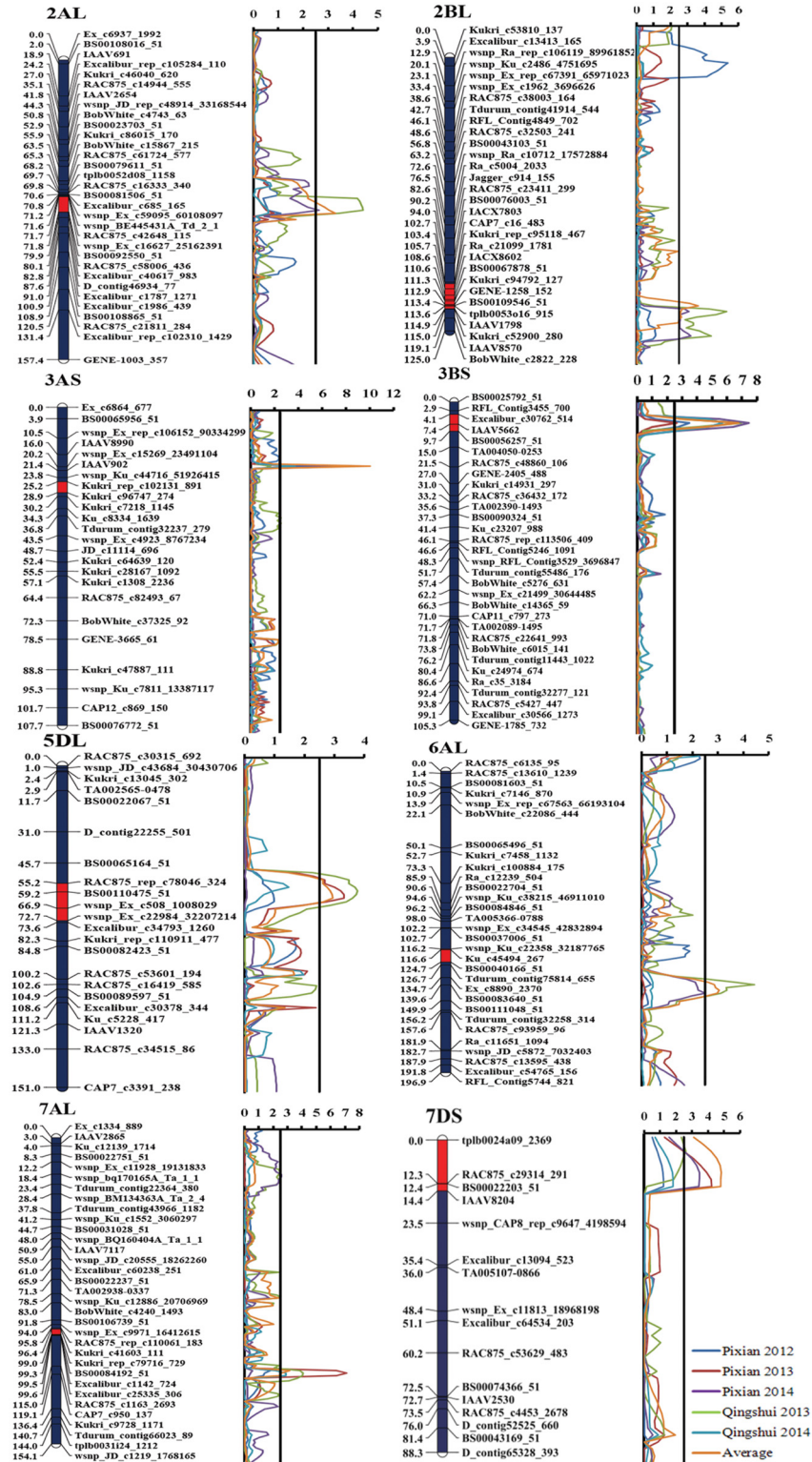
doi:10.1371/journal.pone.0145462.t002

Fig 1. LOD contours obtained by composite interval mapping of QTL for stripe rust response in the Linmai 2 × Zhong 892 RIL population. Pixian 2012, Pixian 2013, Pixian 2014, Qingshui 2013, Qingshui 2014, and averaged MDS are indicated with deep blue, red, purple, green, light blue and orange colours. LOD thresholds of 2.5 are indicated by solid vertical lines.
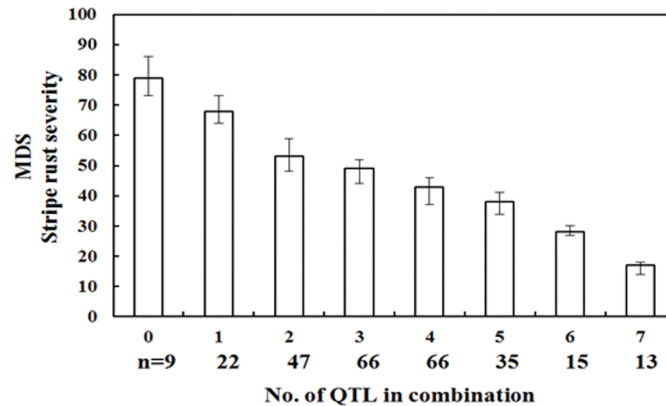
doi:10.1371/journal.pone.0145462.g001

Fig 2. The effects of QTL combinations on stripe rust severity among different classes. The number in abscissa indicates the number of QTL combined in each subset of RILs. The error bars indicate the standard error of sample means.

materials. In addition, the effect of *QYr.caas-7DS.1* on YR response was much less than normally observed on materials with *Yr18*.

Candidate genes related to stripe rust resistance

With the rapid development of gene chip technologies in wheat, the SNP markers play more and more important role in the development of high-density genetic linkage maps [77] and genetic diversity studies [78]. The wheat 90K SNP arrays were mainly developed from expressed genes, and the availability of EST sequence data corresponding to SNP markers makes it possible to identify candidate genes by BLAST against the database of common wheat, *Brachypodium* and other cereals genome sequences.

The bioinformatics analysis of SNP markers tightly linked to stripe rust resistance QTL indicated that the closest marker IACX8602 for *QYr.caas-2BL.3* corresponded to the autophagy-related gene [79]. The autophagy-related proteins ATG4 and ATG8 are crucial for autophagy biogenesis and play important role in resistance response to fungus infection, such as *Blumeria graminis* f. sp. *tritici* [79]. Another SNP marker on chromosome 7DS (*RAC875_c29314_291*) corresponded to a putative disease resistance gene *RGA1* [80], at a distance of 2.3 cM from the LOD contour peak of *QYr.caas-7DS*. However, since the resistance response to fungus is a very complicated biological process, a more detailed experimental analysis should be carried out to confirm the role of these genes on stripe rust resistance.

Potential application of QTL for MAS in wheat breeding

The present study indicated that any combination of 4–5 APR genes with minor or intermediate effects in a line may provide a higher level of resistance to YR, which is consistent with previous reports [7,22,45,81]. In all QTL combinations, *QYr.caas-3AS* and *QYr.caas-3BS* showed high and stable resistance than the others, and their additive effects played more important role than interaction effects in this study (S1 Table).

QTL identified across multiple environments should be useful for marker-assisted selection (MAS) [82]. In the present study, *QYr.caas-3AS*, *QYr.caas-3BS*, *QYr.caas-7AL* and *QYr.caas-7DS* showed consistent effects across multiple environments. *QYr.caas-3AS* and *QYr.caas-3BS* were tightly linked to *Kukri_c96747_274* and *IAAV5662*, with genetic distances of 1.5 and 1.1 cM, respectively; they should provide accurate selection in wheat breeding. KASP is a uniplex

SNP genotyping platform that offers cost-effective and scalable flexibility in applications that require small to moderate numbers of markers, such as marker-assisted selection, and QTL fine mapping [83]. The QTL reported in the present study, *QYr.caas-3AS*, *QYr.caas-3BS*, *QYr. caas-7AL* and *QYr.caas-7DS*, and their closely linked SNP markers *Kukri_c96747_274*, *IAAV5662*, *Excalibur_c25335_306* and *RAC875_c29314_291*, could be potentially used for MAS and pyramiding of stripe rust APR genes in wheat breeding using KASP technology.

Supporting Information

S1 Fig. Frequency distributions of MDS for stripe rust responses in the Linmai 2 × Zhong 892 RIL population. A, Pixian 2012; B, Pixian 2013; C, Pixian 2014; D, Qingshui 2013; E, Qingshui 2014; F, averaged MDS. Mean MDS for the parents, Linmai 2 and Zhong 892, are indicated by arrows.
(TIF)

S1 Table. Mean stripe rust severity of lines with different QTL combinations from the Linmai 2/Zhong 892 RIL population.
(DOCX)

Acknowledgments

Author Contributions

Conceived and designed the experiments: ZHH CJX XCX. Performed the experiments: JDL. Analyzed the data: JDL. Contributed reagents/materials/analysis tools: LW BB WEW. Wrote the paper: JDL ZHH CJX XCX. Participated in field trials: LW BB WEW.

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