Genome-Wide Linkage Mapping Reveals Stripe Rust Resistance in Common Wheat (*Triticum aestivum*) Xinong1376

Jingmei Mu,¹ Jianhui Wu,¹ Shengjie Liu,¹ Miaofei Dai,¹ Daojie Sun,¹ Shuo Huang,¹ Qilin Wang,¹ Qingdong Zeng,¹ Shizhou Yu,¹ Li Chen,² Zhensheng Kang,^{1,†} and Dejun Han^{1,†}

¹ State Key Laboratory of Crop Stress Biology for Arid Areas, Northwest A&F University, Yangling, 712100, Shaanxi, China

² Extension Center for Agriculture Technology, Agriculture Department of Tibetan Autonomous Region, China

Abstract

Stripe rust, also known as yellow rust, is a significant threat to wheat yield worldwide. Adult plant resistance (APR) is the preferred way to obtain durable protection. Chinese winter wheat cultivar Xinong1376 has maintained acceptable APR to stripe rust in field environments. To characterize APR in this cultivar, 190 F₁₀ recombinant inbred lines (RILs) developed from Xiaoyan81 × Xinong1376 were evaluated for infection type and disease severity in fields either artificially or naturally inoculated. The population along with parents were genotyped using the Illumina 90K single-nucleotide polymorphism arrays. Six quantitative trait loci (QTL) were detected using the inclusive composite interval mapping method. *QYr.nwafu-4AL* and *QYr.nwafu-6BL.3* conferred stable resistance in all environments, and

likely corresponded to a gene-rich region on the long arm of chromosomes 4A and 6B. *QYr.nwafu-5AL*, *QYr.nwafu-5BL*, *QYr.nwafu-3BL.1*, and *QYr.nwafu-3BL.2* were detected only in some environments but enhanced the level of resistance conferred by *QYr.nwafu-4AL* and *QYr.nwafu-6BL.3*. Kompetitive allele-specific PCR (KASP) markers developed for *QYr.nwafu-4AL* and *QYr.nwafu-6BL.3* were confirmed in a subset of RILs and 133 wheat genotypes. The QTL on 4AL and 6BL with their linked KASP markers would be useful for marker-assisted selection to improve stripe rust resistance in breeding programs.

Keywords: cereals and grains, disease management, field crops, fungi

Wheat is grown on 215 million ha each year (CCAFS 2017). As the staple food of humans, the demand for wheat is expected to rise with increases in the population (Ray et al. 2012). Stripe rust, caused by *Puccinia striiformis* f. *tritici*, has become a significant threat to grain yield and quality in most wheat-growing regions, and the crop losses from epidemics range from 5 to 25%, sometimes 100% on highly susceptible wheat cultivars (Chen 2014; Wellings 2011). Although chemical control is currently a major approach to reduce yield losses, the cost and potential environmental effects are a global concern (Chen 2014). Over the long term, stripe rust management should rely on growing resistant cultivars (Chen 2005; McIntosh et al. 1995).

Rust resistance is broadly but not exclusively grouped into seedling stage and adult plant categories. Compared with seedling resistance, adult plant resistance (APR) becomes increasingly effective with age, and more likely to be durable (Chen 2013). To date, more than eighty *Yr* genes have been identified and formally named (McIntosh et al. 2017). Most of them had seedling resistance that has been defeated by predominant virulent races. More than 200 quantitative trait loci (QTL) have also been reported (Bulli et al. 2016; Maccaferri et al. 2015; Zeng et al. 2019a). Because these QTL provided

partial resistance and were easily affected by environmental conditions, cultivars with APR do succumb to yield losses in epidemic situations (Ellis et al. 2014). Therefore, identification of more APR genes to combine with effective all-stage resistance (ASR) genes is important to increase the level of protection and durability of resistance.

Although pyramiding resistance genes for high levels of resistance can be achieved using traditional breeding and phenotypic selection, breeding programs increasingly rely on molecular marker-based selection. Single-nucleotide polymorphism (SNP) markers arrayed in different platforms such as the 9K and 90K chips from Illumina (Cavanagh et al. 2013; Wang et al. 2014) and 660K and 820K chips from Affymetrix (Cui et al. 2017; Winfield et al. 2016), with many advantages over earlier marker types in terms of throughput, cost, and specificity, are now available for crop genetic studies and plant breeding programs (Allen et al. 2011; Colasuonno et al. 2014; Jia and Zhao 2016; Mu et al. 2019; Wu et al. 2017). Genome-wide linkage mapping based on high-throughput SNP arrays provides a rapid approach to find markers linked to specific genes (Wu et al. 2018a; Zeng et al. 2019b).

Wheat cultivar Xinong1376 (pedigree: 84G/Bi16) with 1BL/1RS translocation developed by Northwest A&F University in Shaanxi province has been widely used as the major donor parent in breeding programs due to its good agronomic traits and high grain yield (Wang 1996). Xinong1376 has been included in stripe rust nurseries in Shaanxi and Gansu since 2008 and consistently showed a high level of APR (Han et al. 2012). However, the genetic basis of this resistance remains unknown. Therefore, the objectives of this study were to (i) characterize and dissect the genetic basis of wheat cultivar Xinong1376, (ii) identify QTL for stripe rust APR in an Xiaoyan81 × Xinong1376 RIL population across multiple environments using SNP markers, and (iii) validate the value of linked Kompetitive allele-specific PCR (KASP)-SNP markers with major QTL for marker-assisted breeding.

Materials and Methods

Plant materials. Xinong1376 was crossed as male parent with Xiaoyan81, a major commercial cultivar that is moderately susceptible in the field. In all, 190 F₁₀ recombinant inbred lines (RILs) and the parental lines were assessed for stripe rust response in field

[†]Corresponding authors: Z. Kang; kangzs@nwsuaf.edu.cn; and D. Han; handj@nwsuaf.edu.cn

J. Mu, J. Wu, and S. Liu contributed equally to this work.

Funding: This study was financially supported by the National key “Sci-Tech” project in China (2016ZX08002001), the National Key Research and Development Program of China (number 2016YFD0101802), the earmarked fund for Modern Agro-industry Technology Research System (number CARS-3-1-11), and the key project Science and Technology of Tibetan Autonomous Region, China (number XZ201702NB15).

*The e-Xtra logo stands for “electronic extra” and indicates that two supplementary tables are published online.

The author(s) declare no conflict of interest.

Accepted for publication 10 June 2019.

experiments and genotyped with the 90K SNP array. In total, 133 wheat cultivars and breeding lines from the Yellow and Huai River Valley wheat zone were used to evaluate the polymorphism of markers flanking the major resistance QTL in Xinong1376. Xiaoyan 22 (XY22), Avocet S (AvS), and Mingxian169 (MX169) were planted as susceptible checks throughout this study.

Greenhouse test. Seedling and adult plant tests were conducted under controlled greenhouse conditions to characterize stripe rust resistance in Xinong1376 and Xiaoyan81. CYR34, the most prominent race in China, was used. The avirulence–virulence pattern of this race has previously been reported (Liu et al. 2017; Wu et al. 2016). For the seedling test, approximately 15 seeds were grown in 9-by-9-by-9-cm pots and, for adult plant tests, three plants were grown in large pots (20 cm in diameter by 15 cm in height) filled with soil mixture. Each pot was placed in a growth chamber at a diurnal temperature cycle, gradually changing from 4°C at 2 A.M. to 20°C at 2 P.M. with a 16-h photoperiod, and plants were watered as needed. Seedlings at the two-leaf stage (14 days after sowing) and adult plants at the boot stage were separately inoculated with urediniospores of each race mixed with talc. Briefly, fresh rust spores were evenly mixed with talc powder at an approximate ratio of 1:20 (urediniospores/talc) in a dry 10-ml plastic tube and the spore/talc mixture was spread on the wheat leaves with a cotton swab. Inoculated plants were incubated in a dew chamber at 10°C for 24 h in the dark, and then transferred to a greenhouse at 17 ± 2°C with a photoperiod of 16 h of light (10,000 lux) and 8 h of darkness. Infection type (IT) on the second leaf was recorded when the susceptible check (MX169) showed full sporulation (approximately 15 days after inoculation) using a 0-to-9 scale (Line and Qayoum 1992), where IT 0 = no visible signs or symptom, IT 1 = only tiny necrotic or chlorotic flecks, IT 2 = necrotic or chlorotic blotches (or stripes for adult plants) without any sporulation, IT 3 = necrotic or chlorotic blotches (or stripes) with trace sporulation, IT 4 = necrotic or chlorotic blotches (stripes) with light sporulation, IT 5 = necrotic or chlorotic blotches (stripes) with intermediate sporulation, IT 6 = necrotic or chlorotic blotches (stripes) with moderate sporulation, IT 7 = abundant sporulation with necrotic or chlorotic blotches (stripes), IT 8 = abundant sporulation with chlorosis, and IT 9 = abundant sporulation without necrosis or chlorosis. Plants with ITs 0 to 3 were considered highly resistant, 4 to 6 were moderately resistant, 7 were moderately susceptible, and 8 to 9 was highly susceptible. ITs were recorded once every 3 days and at least two times, and the highest ITs were used for analysis.

Field test. The 190 F₁₀ RIL population and their parental lines were tested for APR at three experimental sites, including Yangling, Tianshui, and Jiangyou, during the 2016–17 and 2017–18 cropping seasons. All field trials were conducted as a randomized complete block design with two replicates at each location. Each line was planted in a single 1-m row with 25-cm spacing between rows. Each row was sown with approximately 20 seeds of each progeny line or parent. Cultivar XY22 was planted every 20 rows as a susceptible check. The susceptible MX169 and AvS were also grown in the spreader rows around the nursery to increase the inoculum load. Fields in Yangling were artificially inoculated with a mixture of equal quantities of urediniospores of *P. striiformis* f. *tritici* races CYR32, CYR33, and CYR34 at flag leaf emergence. Briefly, a spore suspension was created in a 1:300 ratio of urediniospores/liquid paraffin and sprayed onto MX169 plants using an electric atomizer (3WBS-16 airbrush; Henan Tianweishi Mechanical Co., Ltd.) after sunset when the moisture for infection was favorable. After inoculation, MX169 plants were completely covered with dark plastic bags to keep moisture for 24 h. The fields in Tianshui and Jiangyou were naturally infected because these two locations are considered hot-spots for stripe rust, with ideal conditions for the natural occurrence of this disease. IT and disease severity (DS) were scored for each row when the susceptible checks MX169 and AvS had DS value of 80% or more. IT data were recorded for each line based on the 0-to-9 scale as described above. Disease severities were assessed visually using percentage of diseased leaf area based on the modified Cobb scale

(Peterson et al. 1948). The scales were recorded with 12 degrees (1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100%); those read as 100% represented 37% of the actual rust-covered surface because the development and destructiveness of the underlying mycelium are almost at a maximum when pustules cover 37% of the surface. Thus, the recorded 12 degrees of rustiness corresponded to the actual percentage (0.37, 1.83, 3.7, 7.4, 11.1, 14.8, 18.5, 22.2, 25.9, 29.6, 33.3, and 37.0%, respectively) covered by rust pustules.

Analysis of phenotypic data. Mean IT and DS of F₁₀ RILs were used to examine the variance within individual environments. Analyses of variance (ANOVA) and Pearson's correlation coefficients were performed using the “AOV” tool in the QTL IciMapping V 4.1 software package (Meng et al. 2015; Wang 2009). Broad-sense heritability was calculated as $h^2b = \sigma^2g/(\sigma^2g + \sigma^2ge + \sigma^2e)$, where σ^2g is $(MS_f - MS_{fe})/r$, σ^2ge is $(MS_{fe} - MS_e)/r$, and σ^2e is MS_e ; σ^2g = genetic variance, σ^2ge = genotype–environment interaction variance, σ^2e = error variance, MS_f = mean square of genotypes, MS_{fe} = mean square of genotype–environment interaction, MS_e = mean square of error, r = number of replications, and e = number of environments.

Molecular genotyping. Genomic DNA of parental lines and F₁₀ RILs were extracted from fresh leaves at the jointing stage using the sodium dodecyl sulfonate method (Weining et al. 1994). DNA was dissolved in sterile deionized distilled (dd)H₂O as stock solutions. DNA concentrations were determined using both 1.0% agarose gel electrophoresis and the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). DNA stocks were diluted to 50 ng/μl with ddH₂O for use as templates. All 190 RILs and the parental lines were genotyped using the Illumina 90K SNP chip from CapitalBio Corporation (<http://www.capitalbio.com>). SNP genotype calling and clustering was processed using Genome Studio v1.0 software (Illumina). SNP filtering criteria were followed: monomorphic SNPs and those showing call rates less than 85%, ambiguity in calling, missing rate above 10%, and minor allele frequencies below 5% were excluded from the dataset. Polymorphic SNPs between parents contained homozygous and heterozygous genotypes. Only homozygous genotype differences were assigned to chromosomes according to 90K wheat consensus maps (Wang et al. 2014; Wu et al. 2018a).

Genetic linkage map. SNP markers were tested using a χ^2 test to filter markers with significantly distorted segregation before constructing the genetic linkage maps. Genetic linkage map creation and QTL mapping were performed using IciMapping V4.1 software (<http://www.isbreeding.net>). Redundant markers with the same segregation were merged together using the “BIN” function (Meng et al. 2015). The remaining markers were used to construct the genetic map using the “MAP” function, and the logarithm of odds (LOD) thresholds were set at the range of 6 to 10. The criteria followed marker grouping, ordering, and ripping, with the nnTwoOpt algorithm and SARF criterion, respectively. The genetic map was drawn using Mapchart v2.3 (Voorrips 2002). The chromosome locations of linkage groups were determined according to the 90K wheat consensus map.

QTL mapping. The detection of QTL for IT and DS were based on the BIP function in IciMapping V 4.1, using the inclusive composite interval mapping (ICIM) method. QTL comparisons with previous resistance genes or QTL were made based on two integrated genetic maps. The first consisted of simple-sequence repeat (SSR) markers and the Wheat90K SNP array (Maccaferri et al. 2015), and the second contained known PCR markers (SSR, expressed sequence tag, sequence-tagged site, random amplified polymorphic DNA, and restriction fragment length polymorphism), diversity arrays technology, and SNPs from Wheat90K, Wheat660K, and Wheat820K SNP arrays (F. Cui, personal communication). All genes or QTL were placed in an integrated genetic map based on the locations of flanking markers. The physical locations of flanked markers were determined through a BLAST search (IWGSC RefSeq v1.0). The BLAST hits criteria included an e-value threshold of 10^{−5} and minimum similarity cutoff greater than 95% between query and database sequences.

Conversion of SNP to KASP markers. SNP markers flanking stable QTL were converted to KASP markers for use in breeding programs. KASP markers were developed using a similar approach described in Ramirez-Gonzalez et al. (2015) and Wu et al. (2017, 2018c). Briefly, the sequences of the flanking markers were uploaded to PolyMarker (<http://www.polymarker.info/>), and only chromosome-specific KASP markers were synthesized. KASP assays were conducted in a 384-well plate format following the protocol of LGC Genomics. Reaction mixtures consisted of final volumes of 5 μ l containing 2.5 μ l of genomic DNA (50 to 100 ng), 2.5 μ l of 2 $\times$ KASP master mix (V4.0; LGC Genomics), and 0.014 μ l of primer mix (12 mM each allele-specific primer and 30 mM common primer). A Veriti 384-well thermal cycler (Applied Biosystems) was used with cycling conditions of denaturation at 95°C for 15 min, nine cycles of 95°C for 20 s, touchdown starting at 65°C for 60 s (decreasing 0.8°C per cycle), finally followed by 30 to 40 cycles of amplification (95°C for 20 s and 57°C for 60 s). End-point fluorescence data were

visualized with a microplate reader (FLUOstar Omega; BMG LAB-TECH) and analyzed using Klustering Caller software (LGC).

Results

Greenhouse test. Xiaoyan81 along with the susceptible checks MX169 and AvS were susceptible (IT 7 to 9) to CYR34 either seedlings or adults. While Xinong1376 was susceptible (IT 8 to 9) to CYR34 in seedling test, but highly resistant (IT 1 to 2) to CYR34 in adult-plant stage test.

Field test. The response of 190 RILs and parental lines at the adult plant stage displayed significant variation in all environments. Xinong1376 exhibited a high level of resistance (IT 1, DS $\leq$ 10%), and that of Xiaoyan81 was susceptible (IT 7, DS $\geq$ 70%). Both IT and DS data for the RILs showed a normal distribution in all field tests, suggesting that the APR was quantitatively inherited (Fig. 1). ANOVA showed significant phenotypic variation for both IT and DS between different lines, environments, and line–environment

Fig. 1. Frequency distributions of Xiaoyan81 $\times$ Xinong1376 recombinant inbred lines for stripe rust **A**, infection type (IT) and **B**, disease severity (DS) in field trials at Jiangyou in 2016–2017 (2017JY) and 2017–18 (2018JY), Yangling in 2016–17 (2017YL) and 2017–18 (2018YL), and Tianshui in 2016–17 (2017TS) and 2017–18 (2018TS). Values for the parents Xiaoyan81 and Xinong1376 are indicated by arrows.

Table 1. Analysis of variance and estimate of broad-sense heritability of stripe rust infection type (IT) and disease severity (DS) among Xiaoyan81 $\times$ Xinong1376 recombinant inbred lines tested at Yangling, Tianshui, and Jiangyou in 2017 and 2018

Source of variation	IT				DS			
	df	Mean square	F value	P value	df	Mean square	F value	P value
Lines	189	19.96	15.33	<0.0001	189	2,352.87	13.65	<0.0001
Replicates/environment	6	6.14	4.71	...	6	2,309.33	13.39	...
Environments	5	209.38	160.76	<0.0001	5	19,322.70	112.05	<0.0001
Line–Environments	944	3.42	2.63	<0.0001	944	386.25	2.24	<0.0001
Error	1,101	1.30	...	...	1,101	172.44	...	...
$h^2 b$	0.85	...	...	...	0.85	...	...	...

Table 2. Correlation coefficients (r) of infection type (IT) and disease severity (DS) of Xiaoyan81 $\times$ Xinong1376 recombinant inbred lines (RILs) across six environments^a

Environment	r value based on DS (IT) ^b					
	2017JY	2017YL	2017TS	2018JY	2018YL	2018TS
2017JY	1	...	...	...	...	...
2017YL	0.84 (0.76)	1	...	...	...	...
2017TS	0.75 (0.65)	0.76 (0.68)	1	...	...	...
2018JY	0.85 (0.75)	0.80 (0.78)	0.65 (0.76)	1	...	...
2018YL	0.65 (0.70)	0.75 (0.76)	0.76 (0.83)	0.86 (0.76)	1	...
2018TS	0.70 (0.80)	0.72 (0.74)	0.74 (0.71)	0.81 (0.75)	0.73 (0.71)	1

^a Year and location: 2017 and 2018 represent the RIL populations grown during the 2016–17 and 2017–18 cropping seasons, respectively; and JY, YL, and TS denote Jiangyou, Yangling, and Tianshui, respectively.

^b r Values calculated with IT are shown in parentheses. All r values are significant at $P < 0.001$.

Table 3. Summary of quantitative trait loci (QTL) for stripe rust resistance detected in the Xiaoyan81 × Xinong1376 recombinant inbred line populations across six environments

QTL, environments ^a	Left marker	Right marker	Genetic position ^b	Physical interval ^c	LOD ^d	PVE ^e	ADD ^f
<i>QYr.nwafu-4AL</i>							
2017JY_IT	IWB6683	IWB49885	137	617–622	12.5	25.9	–12.5
2017YL_IT	IWB7070	IWB6683	131	617	12.7	13.2	–9.1
2017TS_IT	IWB6683	IWB49885	137	617–622	13.2	27.5	–13.8
2017JY_DS	IWB6683	IWB49885	137	617–622	10.4	18.7	–8.5
2017YL_DS	IWB7070	IWB6683	131	617	15.2	20.2	–10.2
2017TS_DS	IWB6683	IWB49885	137	617–622	11.3	23.5	–10.8
2017_mean	IWB6683	IWB49885	137	617–622	13.5	24.7	–11.1
2018JY_IT	IWB6683	IWB49885	137	617–622	18.7	30.5	–17.5
2018YL_IT	IWB7070	IWB6683	131	617	8.4	25.3	–9.1
2018TS_IT	IWB7070	IWB6683	131	617	15.8	28.7	–16.2
2018JY_DS	IWB6683	IWB49885	137	617–622	14.2	32.8	–19.8
2018YL_DS	IWB6683	IWB49885	137	617–622	10.2	20.9	–9.5
2018TS_DS	IWB6683	IWB49885	137	617–622	9.8	18.2	–8.2
2018_mean	IWB6683	IWB49885	137	617–622	12.8	25.5	–11.2
<i>QYr.nwafu-6BL.3</i>							
2017JY_IT	IWB52926	IWB10268	12	605–617	7.7	20.2	–9.8
2017YL_IT	IWB10268	IWB10041	14	605–696	6.9	26.7	–11.9
2017TS_IT	IWB2097	IWB10268	12	605–613	12.8	23.6	–10.5
2017JY_DS	IWB2097	IWB10268	12	605–613	8.3	17.4	–12.3
2017YL_DS	IWB52926	IWB10268	12	605–617	10.1	25.2	–15.2
2017TS_DS	IWB2097	IWB10268	12	605–613	8.9	15.8	–9.3
2017_mean	IWB2097	IWB10268	12	605–613	11.5	25.5	–8.5
2018JY_IT	IWB52926	IWB10268	12	605–617	9.8	23.4	–9.2
2018YL_IT	IWB10268	IWB10041	14	605–696	11.2	25.6	–12.9
2018TS_IT	IWB10268	IWB10041	14	605–696	10.6	18.8	–11.1
2018JY_DS	IWB10268	IWB10041	14	605–696	7.5	15.6	–8.2
2018YL_DS	IWB52926	IWB10268	12	605–617	8.3	20.1	–11.4
2018TS_DS	IWB52926	IWB10268	12	605–617	7.9	16.4	–6.9
2018_mean	IWB2097	IWB10268	12	605–613	13.8	23.2	–10.1
<i>QYr.nwafu-5AL</i>							
2017JY_IT	IWB5776	IWB63558	102	463–598	5.4	8.2	–5.9
2017YL_IT	IWB5776	IWB63558	102	463–598	6.3	7.1	–5.1
2017JY_DS	IWB5776	IWB63558	102	463–598	4.7	9.3	–8.4
2017YL_DS	IWB5776	IWB63558	102	463–598	5.8	8.5	–6.1
2017_mean	IWB5776	IWB63558	102	463–598	8.1	8.9	–7.2
2018JY_IT	IWB5776	IWB63558	102	463–598	5.1	9.5	–10.2
2018YL_IT	IWB5776	IWB63558	102	463–598	4.5	8.9	–8.1
2018JY_DS	IWB5776	IWB63558	102	463–598	5.4	9.2	–9.8
2018YL_DS	IWB5776	IWB63558	102	463–598	6.4	7.5	–5.5
2018_mean	IWB5776	IWB63558	102	463–598	6.5	9.6	–9.3
<i>QYr.nwafu-3B.1</i>							
2017TS_IT	IWA4841	IWB12466	65	669–674	4.6	9.3	–4.2
2017TS_DS	IWA4841	IWB12466	65	669–674	5.8	10.8	–5.3
2018TS_IT	IWA4841	IWB12466	65	669–674	6.5	8.3	–3.1
2018TS_DS	IWA4841	IWB12466	65	669–674	5.4	9.7	–5.4
2017TS_IT	IWA4841	IWB12466	65	669–674	5.6	9.5	–3.2
2017TS_DS	IWA4841	IWB12466	65	669–674	4.2	8.1	–5.1
2018TS_IT	IWA4841	IWB12466	65	669–674	5.4	8.5	–3.9
2018TS_DS	IWA4841	IWB12466	65	669–674	4.6	9.6	–5.8
<i>QYr.nwafu-3B.2</i>							
2017JY_IT	IWB6460	IWB32266	121	590–606	4.5	10.6	6.6
2017YL_IT	IWB6460	IWB32266	121	590–606	3.7	8.9	5.4
2017TS_IT	IWB6460	IWB32266	121	590–606	4.1	9.1	3.2
2017JY_DS	IWB6460	IWB32266	121	590–606	3.5	7.2	4.8
<i>QYr.nwafu-5BL</i>							
2017YL_DS	IWB7873	IWB13284	320	670–672	3.8	8.4	5.5
2017TS_DS	IWB7873	IWB13284	320	670–672	4.3	9.5	4.8
2017_mean	IWB7873	IWB13284	320	670–672	3.9	9.1	6.3
2018JY_IT	IWB7873	IWB13284	320	670–672	4.4	8.2	3.6
2018YL_IT	IWB7873	IWB13284	320	670–672	5.4	9.7	4.2
2018TS_IT	IWB7873	IWB13284	320	670–672	4.8	8.2	4.9
2018JY_DS	IWB7873	IWB13284	320	670–672	3.6	9.1	3.9
2018YL_DS	IWB7873	IWB13284	320	670–672	4.3	10.1	4.1
2018TS_DS	IWB7873	IWB13284	320	670–672	5.3	8.7	3.4
2018_mean	IWB7873	IWB13284	320	670–672	5.8	9.8	4.3

^a 2017 and 2018 represent the field experiments during the 2016–2017 and 2017–2018 cropping seasons, respectively; JY, YL, and TS denote Jiangyou, Yangling, and Tianshui, respectively; IT and DS denote infection type and disease severity, respectively; and mean denotes the average value at different environments each year.

^b Peak position in centimorgans (cM) from the first linked marker of the relevant linkage group.

^c Physical location in mega base (Mb) from linked markers in wheat genome.

^d LOD = logarithm of odds score.

^e PVE = percentages of the phenotypic variance explained by individual QTL.

^f ADD = additive effect of the resistance allele. Negative signs indicate the resistance allele from Xinong1376.

Genetic linkage map. In total, 9,728 SNP markers out of the 90K SNP array showed polymorphism between the parental lines. Among these, 119 SNPs with more than 10% missing datapoints were excluded. The remaining 9,609 SNPs fell into 859 bins. After removing redundant markers, a final 1,948 markers were used to create the

A

101.8 IWA1691
104.1 IWB35214
106.4 IWB36596
107.8 IWA1529
110.3 IWB54608
112.2 IWA6733
113.4 IWA4243
114.2 IWB8103
115.1 IWB39801

124.3 IWB36641
128.0 IWB23723
129.7 IWB12421
130.8 IWB7070
132.0 IWB6683

141.3 IWB49885
141.6 IWB42362
143.5 IWB37469
143.8 IWB43191

149.7 IWB12737
151.3 IWB64647
152.7 IWB60934
155.2 IWB58552

B

38.8 IWA3981
55.0 IWA3756
60.5 IWA3774
68.3 IWB7070
70.5 Yr51
71.0 IWA8475
73.2 IWB49885
74.2 XwPt-9901
74.4 Xgwm219
76.1 Xbarc78
78.8 XwPt-2151
80.1 XwPt-6440
85.7 Yr60
92.3 XwPt-1007

C

0.0 IWB36050
0.3 IWB5901
1.2 IWB5832
3.7 IWB21359
4.5 IWB26364
6.0 IWB34441
7.2 IWA3947
8.4 IWB54401
9.2 IWB72540
10.1 IWB52926
10.6 IWB2097
13.7 IWB10268
14.0 IWB10041
16.4 IWB35852
17.5 IWB48362
17.8 IWB19988
18.7 IWB4109
19.8 IWA8853
21.2 IWB41570
21.4 IWB10379
22.0 IWA349
23.7 IWB63870
24.5 IWB6367

D

33.9 IWA7257
34.1 gwm132
34.4 gdm113
35.3 IWB10041
36.8 IWB10268
37.8 IWA4169
38.4 gwm361
39.7 barc136
42.3 gwm58
43.5 gwm626
44.0 IWA4501
46.8 AX-111486170
50.2 XwPt-6329
56.8 XwPt-5176

E

91.5 IWA2926
92.3 IWA6126
92.6 IWB2190
93.5 IWB21834
93.7 IWA5380
94.9 IWB46680
95.7 IWB71393
96.6 IWB20039
97.4 IWB11967
98.5 IWB218
99.4 IWB73945
100.0 IWB5776
103.1 IWB63558
103.7 IWB55464
105.9 IWB49048
109.7 IWB71951
109.9 IWB36264
111.1 IWB87358
111.3 IWB55590
111.6 IWB36245
113.6 IWB53912
114.7 IWB71451
115.8 IWA6949
116.5 IWA4434
117.8 IWB35869
118.6 IWB7544
118.9 IWB43972
119.5 IWB22035
120.6 IWB65371

F

55.1 barc151
64.0 cfd12
66.8 gwm126
69.2 wmc410
71.5 barc261
78.9 IWB5776
81.9 IWB63558
92.0 wmc727
92.5 gwm291
92.9 IWA2646
94.1 IWA2558
96.0 Yr34/Yr48

G

291.9 IWB6771
292.2 IWB38713
293.3 IWB70324
294.7 IWB8583
300.3 IWB7732
300.9 IWA6416
302.2 IWB10952
302.5 IWA3972
305.6 IWA8031
306.1 IWB71624
306.9 IWB6532
310.0 IWB34550
311.4 IWB62049
311.9 IWB62048
315.1 IWB6886
317.3 IWB64466
319.0 IWB7873
320.7 IWB13284
321.2 IWB44440
321.7 IWB29005
322.0 IWB11392
323.7 IWB6924
329.3 IWB53204
329.6 IWB2982
329.8 IWB25892
330.4 IWB34210
330.9 IWB47574
332.1 IWB35477
334.3 IWB50382
334.6 IWA3185
336.0 IWB56872
339.6 IWB56115

H

102.8 IWA6867
105.3 IWB6383
111.2 wmc537
113.0 wmc415
119.5 IWA5488
123.4 XwPt6135
161.2 XwPt1733
166.7 gwm604
167.2 wmc75
170.2 XwPt2707
173.6 barc142
175.3 barc275
200.1 IWB7873
201.1 IWB13284

I

56.5 IWB8457
56.8 IWA1548
56.9 IWA4941
56.9 IWB12466
56.9 IWB9688
67.4 IWB58033
68.2 IWB63601
68.7 IWB26919
69.3 IWB63113
70.7 IWB9872
71.2 IWB53113
71.8 IWB49250
72.0 IWB32558
72.0 IWB71475
74.2 IWB65338
77.0 IWB21829
78.0 IWB42559
80.0 IWB61164
101.8 IWB12510
104.0 IWB46704
104.3 IWB8208
104.8 IWB28234
105.2 IWB53363
106.7 IWA2550
107.3 IWB50759
107.8 IWB5630
108.1 IWB27335
109.3 IWB4894
110.3 IWB1189
111.2 IWB7068
113.5 IWB8954
115.1 IWB6460
115.1 IWB2266
126.8 IWB54838
134.4 IWB9884
134.9 IWB9884

2746 Plant Disease / Vol. 103 No. 11

translocation line contributed by Xinong1376 that was confirmed in our previous study (Supplementary Table S1). Only linkage groups with significant stripe rust resistance QTL are presented.

QTL mapping. Six QTL were identified on chromosomes 4AL, 6BL, 5AL, and 3BL using the ICIM method. Five were from Xinong1376 and *QYr.nwafu-5BL* was from Xiaoyan81. *QYr.nwafu-4AL*, *QYr.nwafu-6BL.3*, *QYr.nwafu-5AL*, and *QYr.nwafu-5BL* were detected in almost all six environments; *QYr.nwafu-3BL.1* and *QYr.nwafu-3BL.2* were each detected in one environment. *QYr.nwafu-4AL*, with a major effect in reducing stripe rust, was

flanked by SNP markers *IWB49885* and *IWB7070*, explaining 25.3 to 30.5% of the phenotypic variation in IT and 18.2 to 32.8% in DS. *QYr.nwafu-6BL.3*, with the closest markers *IWB2097* and *IWB10268* spanning 3.1 cM, explained 18.8 to 26.7% of the variation in IT and 15.6 to 25.2% in DS. *QYr.nwafu-5AL* flanked by markers *IWB5776* and *IWB63558*, *QYr.nwafu-3BL.1* close to *IWA4841* and *IWB12466*, *QYr.nwafu-3BL.2* linked with *IWB6460* and *IWB32666*, and *QYr.nwafu-5BL* in the interval of *IWB7873* and *IWB13284* explained the phenotype variation below 10%, respectively. The physical locations for these flanked SNP markers were also obtained based on a BLAST search (IWGSC RefSeq v.1.0) (Table 3; Fig. 2).

To show the effects of individual QTL on stripe rust reaction, the 190 lines that carried different allelic combination at the three QTL on 4A, 6B, and 5A were grouped based on the presence of the closest markers. RILs with only one QTL had a level of resistance similar to that of the susceptible parent Xiaoyan81. RILs with combinations 4AL+5AL, 6BL+5AL, and 4AL+6BL showed comparatively lower response than those with only one QTL. Those with three QTL combinations were even more resistant, whereas RILs with no QTL were most susceptible. The mean IT and DS scores of the different combinations are presented in Figure 3. These results clearly showed that the degree of resistance in Xinong1376 depended upon the additive effect of several QTL with variable individual effects.

Conversion of SNP to KASP markers. *QYr.nwafu-4AL* and *QYr.nwafu-6BL.3*, with large effects detected across all test environments, can be useful for marker-assisted selection. SNP markers within the vicinity of the QTL were used for genotyping a panel of 133 Chinese wheat varieties. Among these flanking SNP markers and flanking sequences submitted to PolyMarker, six chromosome-specific SNPs were selected for conversion to KASP markers and to genotype the parents and panel to confirm polymorphisms. KASP markers from SNP markers *IWB6683*, *IWB7070*, *IWB42362*, and *IWB12421* were for *QYr.nwafu-4AL* and KASP markers from *IWB52926* and *IWB10041* were for *QYr.nwafu-6BL.3*. Genotyping results are presented in Supplementary Table S2, and the primer sequences are provided in Table 4. Xinong936 and Xinong182 shared at least three marker alleles with *QYr.nwafu-4AL*. These two accessions showed APR in fields, indicating that they probably had the same QTL. No other genotypes in the panel carried both markers flanking *QYr.nwafu-6BL.3*, suggesting that this QTL was only unique to Xinong1376.

Discussion

Xinong1376 has shown durable APR in field nurseries since its release in 1992. Five QTL were identified in Xinong1376 on

Fig. 3. Effects of quantitative trait loci (QTL) combinations on stripe rust scores in the Xiaoyan81 × Xinong1376 recombinant inbred line population in Yangling (YL), Tianshui (TS), and Jiangyou (JY) and the mean value (Average) across all environments. Box plots (minima and maxima are black dots, medians are crosses, and the first and third quartiles are boxes) for **A**, infection type (IT) and **B**, maximum disease severity (MDS) associated with the identified QTL and their combinations. X-axis refers to number of QTL combinations and Y-axis refers to the value of IT and DS.

Table 4. Primer sequences of Kompetitive allele-specific PCR (KASP) markers developed from single-nucleotide polymorphism markers closely linked to stripe rust resistance quantitative trait loci (QTL) with major effect

KASP names ^a	QTL name	Primer sequence (5'–3')
IWB6683-4A-A	<i>QYr.nwafu-4AL</i>	GAAGGTGACCAAGTTCATGCTggctcgggtgatggaGcC
IWB6683-4A-B	<i>QYr.nwafu-4AL</i>	GAAGGTGCGGAGTCAACGGATTggcctcgggtgatggaGcA
IWB6683-4A-C	<i>QYr.nwafu-4AL</i>	acctGAGTtggaagcgcgG
IWB7070-4A-A	<i>QYr.nwafu-4AL</i>	GAAGGTGACCAAGTTCATGCTacAtTcaGggcttcgacaaG
IWB7070-4A-B	<i>QYr.nwafu-4AL</i>	GAAGGTGCGGAGTCAACGGATTacAtTcaGggcttcgacaaT
IWB7070-4A-C	<i>QYr.nwafu-4AL</i>	cgtatcgcttgaaactctca
IWB42362-4A-A	<i>QYr.nwafu-4AL</i>	GAAGGTGACCAAGTTCATGCTaaagcactgcaggagcgT
IWB42362-4A-B	<i>QYr.nwafu-4AL</i>	GAAGGTGCGGAGTCAACGGATTaaagcactgcaggagcgC
IWB42362-4A-C	<i>QYr.nwafu-4AL</i>	tgatcaggttgctagctttattttG
IWB12421-4A-A	<i>QYr.nwafu-4AL</i>	GAAGGTGACCAAGTTCATGCTcgccacaaactactctatctgT
IWB12421-4A-B	<i>QYr.nwafu-4AL</i>	GAAGGTGCGGAGTCAACGGATTcgccacaaactactctatctgC
IWB12421-4A-C	<i>QYr.nwafu-4AL</i>	gcgatcttcattgccggaT
IWB52926-6B-A	<i>QYr.nwafu-6BL.3</i>	GAAGGTGACCAAGTTCATGCTtgccgaaatttgctccaaaG
IWB52926-6B-B	<i>QYr.nwafu-6BL.3</i>	GAAGGTGCGGAGTCAACGGATTtgccgaaatttgctccaaaA
IWB52926-6B-C	<i>QYr.nwafu-6BL.3</i>	tgatctgctagaacagtttgcT
IWB10041-6B-A	<i>QYr.nwafu-6BL.3</i>	GAAGGTGACCAAGTTCATGCTgccagctgctgaccaaC
IWB10041-6B-B	<i>QYr.nwafu-6BL.3</i>	GAAGGTGCGGAGTCAACGGATTgccagctgctgaccaaT
IWB10041-6B-C	<i>QYr.nwafu-6BL.3</i>	ctcagggtgtcatcgagtG

^a KASP names: A = primers with the added FAM adapter, B = primers with the added HEX adapter, and C = common primers.

chromosomes 4AL, 6BL, 5AL, and 3BL and named *QYr.nwafu-4AL*, *QYr.nwafu-6BL.3*, *QYr.nwafu-5AL*, *QYr.nwafu-3BL.1*, and *QYr.nwafu-3BL.2*, respectively. *QYr.nwafu-4AL* and *QYr.nwafu-6BL.3* showed significant effects in almost all environments, whereas the other QTL showed minor effects and expressed only in some environments. An additional QTL from Xiaoyan81, *QYr.nwafu-5BL*, had a major effect in reducing stripe rust response.

QYr.nwafu-4AL was mapped near the distal end of chromosome arm 4AL (Fig. 2A). Thus far, five APR QTL for stripe rust have been reported on the telomere region of 4AL. Most of the QTL were assigned on the bin 4AL4-0.80-1.00 based on an integrated bin map (Qi et al. 2004; Sourdille et al. 2004). These QTL contained *QYrst.orr-4AL* in Stephens (Vazquez et al. 2012), *QYrid.ui-4A* in IDO444 (Chen et al. 2012), *QYr-4A* in Sachem (Singh et al. 2013), *QYr.sgi-4A* in Kariaga (Agenbag et al. 2014), and *QYrms.orz-4AL* in NSA-980995 (Vazquez et al. 2015), and two QTL from a genome-wide association study, including *QYr.ucw-4A* (Maccaferri et al. 2015) and *QYr.wsu-4A* (Bulli et al. 2016). *QYr.nwafu-4AL* flanked by marker *IWB49885* and *IWB6683* also fell into the same chromosome bin based on marker bin location (https://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi) (Sourdille et al. 2004). Thus, it indicated that *QYr.nwafu-4AL* shared a common genomic region with these QTL. Two formally named genes, *Yr51* and *Yr60*, were also in the proximal region. *Yr51* was close to *QYr.sgi-4A*, with a 4.3-cM distance (Randhawa et al. 2013), and *Yr60* was approximately 10.1 cM distant from *Yr51* (Herrera-Foessel et al. 2015). However, both genes conferred ASR. *Yr51* was from Pakistan wheat landrace AUS27858 and *Yr60* was from Mexican common wheat Almop, whereas Xinong1376 was sourced from Chinese landraces (Wang 1996). The different resistance sources and resistance types indicated that *QYr.nwafu-4AL* was different from *Yr51* and *Yr60*. Because *QYr.nwafu-4AL* explained 25.5% of the mean variation against stripe rust, conferring a moderate level of resistance to stripe rust, combining this QTL with additional genes or QTL will achieve high levels of resistance in the field.

Several stripe rust APR QTL on 6BL have been reported (Bulli et al. 2016; Zeng et al. 2019c). To estimate the relationship of *QYr.nwafu-6BL.3* from other resistance QTL on 6BL, all genes or QTL were placed in an integrated map based on the genetic locations of flanking markers (Bulli et al. 2016; Maccaferri et al. 2015) and physical location based on the Chinese Spring sequence (IWGSC RefSeq v.1.0). *QYr-6B* from Pavon76 (William et al. 2006), *QYr.inra-6B* from Renan (Dedryver et al. 2009), *QYrdr.wgp-6BL.2* in Druchamp (Hou et al. 2015), and *QYr.nwafu-6BL.1* in Friedrichswerther (Wu et al. 2018b) were placed at 42.3 to 43.5 cM, a physical region of about 417 to 521 Mb. *QYr.nwafu-6BL.2* (Zeng et al. 2019c) was in the region of 50.2 to 56.8 cM. Three QTL were concentrated in 33.9 to 39.7 cM of genetic distance, including *QYr.wsu-6B.1* (Bulli et al. 2016) and *QYr.ucw-6B* (Maccaferri et al. 2015) in the interval of 33.9 to 35.3 cM (genomic region of about 631 to 720 Mb) and *QYr.wgp-6B.1* from Stephens (Santra et al. 2008) in 34.5 to 35.0 cM (region of 690 Mb) whereas, in our study, *QYr.nwafu-6BL.3* was in an interval of 35.3 to 36.8 cM (617 to 622 Mb) according to the flanked markers location (Fig. 2B). *QYr.nwafu-6BL.3* seems to be in a proximate region with those three QTL. However, they were accurately distinct in the physical location. Thus, the concise location relationships with the three QTL needs to be further confirmed.

QYr.nwafu-5AL was mapped with flanking markers *IWB5776* and *IWB63558* (Fig. 2C), and the QTL was identified with high LOD scores in all tested environments, except in Tianshui. The QTL on 5AL identified in previous studies was mainly distributed in two genomic regions. The first region included *Yr34* in wheat line WAWHT2046 (AUS22857/Kulin/Blade), *Yr48* in a synthetic derivative spring wheat PI610750 (Croc/Aegilops tauschii (synthetic 205)/Kauz) (Qureshi et al. 2018), *QYrdr.wgp-5AL* in Druchamp (Vilmorin27/N.R./Yeoman), and *QYrms.orz-5AL* in NSA-98-0995 (Hou et al. 2015; Vazquez et al. 2015). *QYrtb.pau-5A* as high-temperature adult-plant (HTAP) resistance in *Triticum boeoticum* accession Pau5088 was in the second location (Chhuneja et al. 2008). *QYr-5A* in a hard red spring wheat Opata 85 (Boukhatef et al. 2002)

and *QYr.caas-5AL* in Pingyuan 50 (Lan et al. 2010) were proximal. However, *QYr.nwafu-5AL* was located in the distinct region according to the integrated map (Maccaferri et al. 2015; Somers et al. 2004). The pedigree also verified their differences, although Pingyuan 50 was an old Chinese wheat landrace, with an origin similar to Xinong1376. However, Pingyuan 50 displayed slow rusting response (DS 30 to 70%) in the same field (Wu et al. 2018b). Therefore, *QYr.nwafu-5AL* seems to be a novel QTL. Additional haplotype analysis is needed for verification.

QYr.nwafu-5BL was mapped with flanking markers *IWB7873* and *IWB13284*. Several QTL were previously located on chromosome 5BL. *QYr.jirc-5BL* from Oligoculm, *QYr.caas-5BL.1* from Libellula, and *QYrco.wgp-5B* from Coda (Case et al. 2014; Lu et al. 2009; Suenaga et al. 2003) should be in the same region (105 to 119 cM, 43.33 to 50.37%). *QYr.caas-5BL.2* from Libellula and *QYr.sun-5B* from Janz seem to be located in the same interval (166.7 to 173.6 cM, 70.2 to 73.1%) (Bariana et al. 2010; Lu et al. 2009) (Fig. 2D). The previously identified QTL were at least 34 cM away from *QYr.nwafu-5BL* (200.1 cM, 84.31%) (Maccaferri et al. 2015). Because *QYr.nwafu-5BL* accounted for only 7.2 to 10.6% of the overall percentages of the phenotypic variance, it would be useful when combined with other genes for resistance.

QYr.nwafu-3BL.1 and *QYr.nwafu-3BL.2* were identified in well-separated regions with more than 20 cM of genetic distance (Fig. 2E). Because both QTL were unstable across environments, they are potentially less useful than other QTL detected in this study. Several QTL have been reported on 3BL. *QYrms.orz-3BL* was detected in hard red winter wheat NSA 980995 (Vazquez et al. 2015). *QYrex.wgp-3BL* was detected in Express and *QYrid.ui-3B.2* in IDO444 (Chen et al. 2012; Lin and Chen 2009) as HTAP resistance. Their resistance sources and resistance type seem different from Xinong1376; thus, additional studies are required for useful comparisons with the current results.

KASP markers tightly linked with major QTL *QYr.nwafu-4AL* and *QYr.nwafu-6BL.3* can be used to pyramid these genes with other genes in breeding programs. *QYr.nwafu-6BL.3* was mapped at less than 5 cM genetic interval by markers *IWB2097* and *IWB10268*; therefore, they can be used for marker-based selection. We also determined that the flanking markers of the two stable QTL were highly polymorphic among the wheat cultivars and breeding lines, indicating the uniqueness of these markers for the incorporation of these genes into other backgrounds to develop new cultivars through marker-assisted selection.

Acknowledgments

We thank R. A. McIntosh, Plant Breeding Institute, University of Sydney, for critical review of this manuscript.

Literature Cited

- Agenbag, G. M., Pretorius, Z. A., Boyd, L. A., Bender, C. M., MacCormack, R., and Prins, R. 2014. High-resolution mapping and new marker development for adult plant stripe rust resistance QTL in the wheat cultivar Kariaga. *Mol. Breed.* 34:2005-2020.
- Allen, A. M., Barker, G. L., Berry, S. T., Coghill, J. A., Gwilliam, R., Kirby, S., Robinson, P., Brenchley, R. C., D'Amore, R., McKenzie, N., Waite, D., Hall, A., Bevan, M., Hall, N., and Edwards, K. J. 2011. Transcript-specific, single-nucleotide polymorphism discovery and linkage analysis in hexaploid bread wheat (*Triticum aestivum* L.). *Plant Biotechnol. J.* 9:1086-1099.
- Bariana, H. S., Bansal, U. K., Schmidt, A., Lehmensiek, A., Kaur, J., Miah, H., Howes, N., and McIntyre, C. L. 2010. Molecular mapping of adult plant stripe rust resistance in wheat and identification of pyramided QTL genotypes. *Euphytica* 176:251-260.
- Boukhatef, N., Baret, P. V., Mingeot, D., and Jacquemin, J. M. 2002. Quantitative trait loci for resistance against yellow rust in two wheat-derived recombinant inbred line populations. *Theor. Appl. Genet.* 104:111-118.
- Bulli, P., Zhang, J., Chao, S., Chen, X. M., and Pumphrey, M. 2016. Genetic architecture of resistance to stripe rust in a global winter wheat germplasm collection. *G3 (Bethesda)* 6:2237-2253.
- Case, A. J., Naruoka, Y., Chen, X. M., Garland-Campbell, K. A., Zemetra, R. S., and Carter, A. H. 2014. Mapping stripe rust resistance in a Brundage × Coda winter wheat recombinant inbred line population. *PLoS One* 9:e91758.
- Cavanagh, C. R., Chao, S., Wang, S., Huang, B. E., Stephen, S., Kiani, S., Forrest, K., Saintenac, C., Brown-Guedira, G. L., Akhunova, A., See, D., Bai, G., Pumphrey, M., Tomar, L., Wong, D., Kong, S., Reynolds, M., Da Silva,

- M. L., Bockelman, H., Talbert, L., Anderson, J. A., Dreisigacker, S., Baenziger, S., Carter, A., Korzun, V., Morrell, P. L., Dubcovsky, J., Morell, M. K., Sorrells, M. E., Hayden, M. J., and Akhunov, E. 2013. Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. *Proc. Nat. Acad. Sci. U.S.A.* 110: 8057-8062.
- CCAFS. 2017. 2016 Annual Report to CGIAR Consortium. CGIAR Research Program on Climate Change, Agriculture and Food Security (CCAFS), Copenhagen, Denmark.
- Chen, J. L., Chu, C., Souza, E. J., Guttieri, M. J., Chen, X. M., Xu, S., Hole, D., and Zemetra, R. 2012. Genome-wide identification of QTL conferring high-temperature adult-plant (HTAP) resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*) in wheat. *Mol. Breed.* 29:791-800.
- Chen, X. M. 2005. Epidemiology and control of stripe rust (*Puccinia striiformis* f. sp. *tritici*) on wheat. *Can. J. Plant Pathol.* 27:314-337.
- Chen, X. M. 2013. High-temperature adult-plant resistance, key for sustainable control of stripe rust. *Am. J. Plant Sci.* 4:608-627.
- Chen, X. M. 2014. Integration of cultivar resistance and fungicide application for control of wheat stripe rust. *Can. J. Plant Pathol.* 36:311-326.
- Chhuneja, P., Kaur, S., Garg, T., Ghai, M., Kaur, S., Prashar, M., Bains, N. S., Goel, R. K., Keller, B., Dhaliwal, H. S., and Singh, K. 2008. Mapping of adult plant stripe rust resistance genes in diploid A genome wheat species and their transfer to bread wheat. *Theor. Appl. Genet.* 116:313-324.
- Colasunanno, P., Gadaleta, A., Giancespro, A., Nigro, D., Giove, S., Incerti, O., Mangini, G., Signorile, A., Simeone, R., and Blanco, A. 2014. Development of a high-density SNP-based linkage map and detection of yellow pigment content QTL in durum wheat. *Mol. Breed.* 34:1563-1578.
- Cui, F., Zhang, N., Fan, X., Zhang, W., Zhao, C., Yang, L., Pan, R., Chen, M., Han, J., Zhao, X., Ji, J., Tong, Y., Zhang, H., Jia, J., Zhao, G., and Li, J. 2017. Utilization of a Wheat660 K SNP array-derived high-density genetic map for high-resolution mapping of a major QTL for kernel number. *Sci. Rep.* 7: Article 3788.
- Dedryver, F., Paillard, S., Mallard, S., Robert, O., Trottet, M., Nègre, S., Verplancke, G., and Jahier, J. 2009. Characterization of genetic components involved in durable resistance to stripe rust in the bread wheat "Renan". *Phytopathology* 99:968-973.
- Ellis, J. G., Lagudah, E. S., Spielmeier, W., and Dodds, P. N. 2014. The past, present and future of breeding rust resistant wheat. *Front. Plant Sci.* 5:641.
- Han, D. J., Zhang, P. Y., Wang, Q. L., Zeng, Q. D., and Wu, J. H. 2012. Identification and evaluation of resistance to stripe rust in 1980 wheat landraces and abroad germplasm. *Sci. Agric. Sin.* 45:5013-5023 [In Chinese with English summary].
- Herrera-Foessel, S. A., Singh, R. P., Lan, C. X., Huerta-Espino, J., Calvo-Salazar, V., Bansal, U. K., Bariana, H. S., and Lagudah, E. S. 2015. *Yr60*, a gene conferring moderate resistance to stripe rust in wheat. *Plant Dis.* 99:508-511.
- Hou, L., Chen, X. M., Wang, M. N., See, D. R., Chao, S. M., Bulli, P., and Jing, J. X. 2015. Mapping a large number of QTL for durable resistance to stripe rust in winter wheat Druchamp using SSR and SNP markers. *PLoS One* 10: e0126794.
- Jia, J., and Zhao, G. 2016. Wheat660 SNP array developed by CAAS.
- Lan, C. X., Liang, S. S., Zhou, X. C., Zhou, G., Lu, Q. L., Xia, X. C., and He, Z. H. 2010. Identification of genomic regions controlling adult-plant stripe rust resistance in Chinese landrace Pingyuan 50 through bulked segregant analysis. *Phytopathology* 100:313-318.
- Lin, F., and Chen, X. M. 2009. Quantitative trait loci for non-race-specific, high-temperature adult-plant resistance to stripe rust in wheat cultivar Express. *Theor. Appl. Genet.* 118:631-642.
- Line, R. F., and Qayoum, A. 1992. Virulence, aggressiveness, evolution, and distribution of races of *Puccinia striiformis* (the cause of stripe rust of wheat) in North America 1968-87. *Tech. Bull. No. 1788. USDA-ARS, Washington, DC, U.S.A.*
- Liu, B., Liu, T., Zhang, Z., Jia, Q., Wang, B., Gao, L., Peng, Y., Jin, S., and Chen, W. 2017. Discovery and pathogenicity of CYR34, a new race of *Puccinia striiformis* f. sp. *tritici* in China. *Acta Phytopathol. Sin.* 47:681-687 [In Chinese with English summary].
- Lu, Y. M., Lan, C. X., Liang, S. S., Zhou, X. C., Liu, D., Zhou, G., Lu, Q. L., Jing, J. X., Wang, M. N., Xia, X. C., and He, Z. H. 2009. QTL mapping for adult-plant resistance to stripe rust in Italian common wheat cultivars Libellula and Strampelli. *Theor. Appl. Genet.* 119:1349-1359.
- Maccaferri, M., Zhang, J., Bulli, P., Abate, Z., Chao, S., Cantu, D., Bossolini, E., Chen, X. M., Pumphrey, M., and Dubcovsky, J. 2015. A genome-wide association study of resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*) in a worldwide collection of hexaploidy spring wheat (*Triticum aestivum* L.). *G3 (Bethesda)* 5:449-465.
- McIntosh, R. A., Dubcovsky, J., Rogers, W. J., Morris, C., and Xia, X. C. 2017. Catalogue of Gene Symbols for Wheat: 2017 Supplement. <https://shigen.nig.ac.jp/wheat/komugi/genes/macgene/supplement2017.pdf>
- McIntosh, R. A., Wellings, R. A., and Park, R. F. 1995. Pages 20-26 in: *Wheat Rusts: An Atlas of Resistance Genes*. CSIRO Publications, East Melbourne, VIC, Australia.
- Meng, L., Li, H., Zhang, L., and Wang, J. 2015. QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. *Crop J.* 3:269-283.
- Mu, J., Huang, S., Liu, S., Zeng, Q., Dai, M., Wang, Q., Wu, J., Yu, S., Kang, Z., and Han, D. 2019. Genetic architecture of wheat stripe rust resistance revealed by combining QTL mapping using SNP-based genetic maps and bulked segregant analysis. *Theor. Appl. Genet.* 132:443-455.
- Peterson, R. F., Campbell, A. B., and Hannah, A. E. 1948. A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. *Can. J. Res.* 26c: 496-500.
- Qi, L. L., Echalié, B., Chao, S., Lazo, G. R., Butler, G. E., Anderson, O. D., Akhunov, E. D., Dvořák, J., Linkiewicz, A. M., Ratnasiri, A., Dubcovsky, J., Bermudez-Kandianis, C. E., Greene, R. A., Kantety, R., La Rota, C. M., Munkvold, J. D., Sorrells, S. F., Sorrells, M. E., Dilbirligi, M., Sidhu, D., Erayman, M., Randhawa, H. S., Sandhu, D., Bondareva, S. N., Gill, K. S., Mahmoud, A. A., Ma, X. F., Gustafson, J. P., Conley, E. J., Nduati, V., Gonzalez-Hernandez, J. L., Anderson, J. A., Peng, J. H., Lapitan, N. L., Hossain, K. G., Kalavacharla, V., Kianian, S. F., Pathan, M. S., Zhang, D. S., Nguyen, H. T., Choi, D. W., Fenton, R. D., Close, T. J., McGuire, P. E., Qualset, C. O., and Gill, B. S. 2004. A chromosome bin map of 16,000 expressed sequence tag loci and distribution of genes among the three genomes of polyploid wheat. *Genetics* 168:701-712.
- Qureshi, N., Bariana, H. S., Zhang, P., McIntosh, R. A., Bansal, U. K., Wong, D., Hayden, M. J., Dubcovsky, J., and Shankar, M. 2018. Genetic relationship of stripe rust resistance genes *Yr34* and *Yr48* in wheat and identification of linked KASP markers. *Plant Dis.* 102:413-420.
- Ramirez-Gonzalez, R. H., Uauy, C., and Caccamo, M. 2015. PolyMarker: A fast polyploid primer design pipeline. *Bioinformatics* 31:2038-2039.
- Randhawa, M., Bansal, U., Valarik, M., Klocova, B., Dolezel, J., and Bariana, H. 2013. Molecular mapping of stripe rust resistance gene *Yr51* in chromosome 4AL of wheat. *Theor. Appl. Genet.* 127:317-324.
- Ray, D. K., Ramankutty, N., Mueller, N. D., West, P. C., and Foley, J. A. 2012. Recent patterns of crop yield growth and stagnation. *Nat. Commun.* 3: Article 1293.
- Santra, D. K., Chen, X. M., Santra, M., Campbell, K. G., and Kidwell, K. K. 2008. Identification and mapping QTL for high-temperature adult-plant resistance to stripe rust in winter wheat (*Triticum aestivum* L.) cultivar "Stephens". *Theor. Appl. Genet.* 117:793-802.
- Singh, A., Pandey, M. P., Singh, A. K., Knox, R. E., Ammar, K., Clarke, J. M., Clarke, F. R., Singh, R. P., Pozniak, C. J., DePauw, R. M., McCallum, B. D., Cuthbert, R. D., Randhawa, H. S., and Fetch, T. G. 2013. Identification and mapping of leaf, stem and stripe rust resistance quantitative trait loci and their interactions in durum wheat. *Mol. Breed.* 31:405-418.
- Somers, D. J., Isaac, P., and Edwards, K. 2004. A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 109:1105-1114.
- Sourdille, P., Singh, S., Cadalen, T., Brown-Guedira, G. L., Gay, G., Qi, L., Gill, B. S., Dufour, P., Murigneux, A., and Bernard, M. 2004. Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). *Funct. Integr. Genomics* 4:12-25.
- Suenaga, K., Singh, R. P., Huerta-Espino, J., and William, H. M. 2003. Microsatellite markers for genes *Lr34/Yr18* and other quantitative trait loci for leaf rust and stripe rust resistance in bread wheat. *Phytopathology* 93: 881-890.
- Vazquez, M. D., Peterson, C. J., Riera-Lizarazu, O., Chen, X., Heesacker, A., Ammar, K., Crossa, J., and Mundt, C. C. 2012. Genetic analysis of adult plant, quantitative resistance to stripe rust in wheat cultivar "Stephens" in multi-environment trials. *Theor. Appl. Genet.* 124:1-11.
- Vazquez, M. D., Zemetra, R., Peterson, C. J., Chen, X. M., Heesacker, A., and Mundt, C. C. 2015. Multi-location wheat stripe rust QTL analysis: Genetic background and epistatic interactions. *Theor. Appl. Genet.* 128:1307-1318.
- Voorrips, R. E. 2002. MapChart: Software for the graphical presentation of linkage maps and QTL. *J. Hered.* 93:77-78.
- Wang, H. 1996. Accumulation and distribution of dry matter on wheat variety Xinong1376. *J. Northwest A&F Univ.* 5:31-34.
- Wang, J. 2009. Inclusive composite interval mapping of quantitative trait genes. *Acta Agron. Sin.* 35:239-245.
- Wang, S., Wong, D., Forrest, K., Allen, A., Chao, S., Huang, B. E., Maccaferri, M., Salvi, S., Milner, S. G., Cattivelli, L., Mastrangelo, A. M., Whan, A., Stephen, S., Barker, G., Wieseke, R., Plieske, J., Lilleno, M., Mather, D., Appels, R., Dolferus, R., Brown-Guedira, G., Korol, A., Akhunova, A. R., Feuillet, C., Salse, J., Morgante, M., Pozniak, C., Luo, M. C., Dvorak, J., Morell, M., Dubcovsky, J., Ganai, M., Tuberosa, R., Lawley, C., Mikoulitch, I., Cavanagh, C., Edwards, K. J., Hayden, M., and Akhunov, E. 2014. Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. *Plant Biotechnol. J.* 12:787-796.
- Weining, S., Ko, L., and Henry, R. J. 1994. Polymorphisms in the α -amyl 1 gene of wild and cultivated barley revealed by the polymerase chain reaction. *Theor. Appl. Genet.* 89:509-513.
- Wellings, C. R. 2011. Global status of stripe rust: A review of historical and current threats. *Euphytica* 179:129-141.
- William, H. M., Singh, R. P., Huerta-Espino, J., Palacios, G., and Suenaga, K. 2006. Characterization of genetic loci conferring adult plant resistance to leaf rust and stripe rust in spring wheat. *Genome* 49:977-990.
- Winfield, M. O., Allen, A. M., Burridge, A. J., Barker, G. L., Benbow, H. R., Wilkinson, P. A., Coghill, J., Waterfall, C., Davassi, A., Scopes, G., Pirani,

- A., Webster, T., Brew, F., Bloor, C., King, J., West, C., Griffiths, S., King, I., Bentley, A. R., and Edwards, K. J. 2016. High-density SNP genotyping array for hexaploid wheat and its secondary and tertiary gene pool. *Plant Biotechnol. J.* 14:1195-1206.
- Wu, J., Huang, S., Zeng, Q., Liu, S., Wang, Q., Mu, J., Yu, S., Han, D., and Kang, Z. 2018a. Comparative genome-wide mapping versus extreme pool-genotyping and development of diagnostic SNP markers linked to QTL for adult plant resistance to stripe rust in common wheat. *Theor. Appl. Genet.* 131:1777-1792.
- Wu, J., Liu, S., Wang, Q., Zeng, Q., Mu, J., Huang, S., Yu, S., Han, D., and Kang, Z. 2018b. Rapid identification of an adult plant stripe rust resistance gene in hexaploid wheat by high-throughput SNP array genotyping of pooled extremes. *Theor. Appl. Genet.* 131:43-58.
- Wu, J., Wang, Q., Chen, X., Wang, M., Mu, J., Lv, X., Huang, L., Han, D., and Kang, Z. 2016. Stripe rust resistance in wheat breeding lines developed for central Shaanxi, an overwintering region for *Puccinia striiformis* f. sp. *tritici* in China. *Can. J. Plant Pathol.* 38:317-324.
- Wu, J., Wang, Q., Kang, Z., Liu, S., Li, H., Mu, J., Dai, M., Han, D., Zeng, Q., and Chen, X. 2017. Development and validation of KASP-SNP markers for QTL underlying resistance to stripe rust in common wheat cultivar P10057. *Plant Dis.* 101:2079-2087.
- Wu, J., Zeng, Q., Wang, Q., Liu, S., Yu, S., Mu, J., Huang, S., Sela, H., Distelfeld, A., Huang, L., Han, D., and Kang, Z. 2018c. SNP-based pool genotyping and haplotype analysis accelerate fine-mapping of the wheat genomic region containing stripe rust resistance gene *Yr26*. *Theor. Appl. Genet.* 131:1481-1496.
- Zeng, Q., Wu, J., Huang, S., Yuan, F., Liu, S., Wang, Q., Mu, J., Yu, S., Chen, L., Han, D., and Kang, Z. 2019a. SNP-based linkage mapping for validation of adult plant stripe rust resistance QTL in common wheat cultivar Chakwal 86. *Crop J.* 7:176-186.
- Zeng, Q., Wu, J., Liu, S., Chen, X., Yuan, F., Su, P., Wang, Q., Huang, S., Mu, J., Han, D., Kang, Z., and Chen, X. M. 2019b. Genome-wide mapping for stripe rust resistance loci in common wheat cultivar Qinnong 142. *Plant Dis.* 103:439-447.
- Zeng, Q., Wu, J., Liu, S., Huang, S., Wang, Q., Mu, J., Yu, S., Han, D., and Kang, Z. 2019c. A major QTL co-localized on chromosome 6BL and its epistatic interaction for enhanced wheat stripe rust resistance. *Theor. Appl. Genet.* 132:1409-1424.