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Genome-wide Mapping for Stripe Rust Resistance Loci in Common Wheat Cultivar Qinnong 142

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Abstract

Stripe rust caused by *Puccinia striiformis* f. sp. *tritici* threatens worldwide wheat production. Growing resistant cultivars is the best way to control this disease. Chinese wheat cultivar Qinnong 142 (QN142) has a high level of adult-plant resistance to stripe rust. To identify quantitative trait loci (QTLs) related to stripe rust resistance, we developed a recombinant inbred line (RIL) population from a cross between QN142 and susceptible cultivar Avocet S. The parents and 165 F_6 RILs were evaluated in terms of their stripe rust infection type and disease severity in replicated field tests with six site-year environments. The parents and RILs were genotyped with single-nucleotide polymorphism (SNP) markers. Four stable QTLs were identified in QN142 and mapped to chromosome arms 1BL, 2AL, 2BL, and 6BS. The 1BL QTL was probably the known resistance gene *Yr29*, the 2BL QTL was in a resistance gene-rich region, and the 2AL and 6BS QTLs might be new. Kompetitive allele specific polymerase chain reaction markers developed from the SNP markers flanking these QTLs were highly polymorphic in a panel of 150 wheat cultivars and breeding lines. These markers could be used in marker-assisted selection for incorporating the stripe rust resistance QTL into new wheat cultivars.

Wheat (*Triticum aestivum* L.) is a major crop throughout the world. Stripe rust (yellow rust) caused by *Puccinia striiformis* Westend. f. sp. *tritici* Erikss. occurs in almost all wheat-producing regions. This disease causes significant economic losses in terms of reduced grain production and the expenses incurred for disease management (Beddow et al. 2015; Hovmøller et al. 2010; McIntosh et al. 1995). In general, stripe rust causes yield losses of 5 to 25% in susceptible wheat cultivars, but it may cause complete yield losses in severe epidemics (Chen 2005; Wellings 2011). As the largest stripe rust epidemic region throughout the world, China frequently suffers severe yield losses (Chen 2014; Li and Zeng 2002; Wan et al. 2007). Growing resistant cultivars is an economical, effective, and environmentally friendly way to control this disease (Li and Zeng 2002; McIntosh et al. 1995; Wiesner-Hanks and Nelson 2016).

Both all-stage resistance (ASR) and adult-plant resistance (APR) have been used for breeding wheat cultivars with resistance to stripe rust (Chen 2013). ASR often confers complete resistance during all growth stages and is easy to select during the breeding process, but it is mostly race-specific and not durable (Johnson 1981; McDonald and Linde 2002). By contrast, APR usually confers partial resistance during the adult-plant stage, and it is mostly race-nonspecific and more durable. However, APR can be relatively difficult to incorporate into new cultivars compared with ASR (Chen 2013; Park and McIntosh 1994). Many wheat cultivars that exhibit durable resistance possess APR (Boyd 2005; Chen 2013; McIntosh 1992; Navabi et al. 2005; Singh et al. 2005). Over 300 genes or quantitative trait loci (QTLs) for either ASR or APR to *P. striiformis* f. sp. *tritici* in various wheat genotypes have been mapped to all 21 chromosomes (Maccaferri et al. 2015; McIntosh et al. 2016, 2017; Rosewarne et al. 2013; Wang and Chen 2017). However, many of the ASR genes are no longer effective against the current *P. striiformis* f. sp. *tritici* populations, and many of the APR QTLs have only small effects. Moreover, not all of these genes or QTLs have molecular markers that can be used for marker-assisted selection (MAS) in breeding programs. Therefore, more resistance genes or QTLs and suitable user-friendly markers should be identified.

It is important to map QTLs and to identify closely linked markers to combine with resistance QTLs in wheat cultivars. During the last 20 years, different types of molecular markers have been developed, such as simple sequence repeat (SSR) (Röder et al. 1998) and resistance gene analog polymorphism (Chen et al. 1998), and used for mapping wheat genes (Ren et al. 2012a, b; Rosewarne et al. 2013; Zhou et al. 2014). These polymerase chain reaction (PCR) markers have been applied successfully in MAS, but the densities of these types of markers are about 10–20 cM per marker (Semagn et al. 2010; Yan et al. 2006). Thus, more cost-effective markers and high-throughput genotyping platforms are still required for MAS (St. Clair 2010; Yang et al. 2015). More recently, single nucleotide polymorphism (SNP) markers have been developed, which are abundant, mostly codominant, and distributed throughout the genome, and applied in studies of wheat genetics (Allen et al. 2013; Rasheed et al. 2016; Wu et al. 2017, 2018). SNP markers have many advantages for high-throughput genotyping, including their low cost, allele specificity, and high efficiency (Colasuonno et al. 2014; Gupta et al. 2008; Long et al. 2017; Semagn et al. 2014). SNP chips containing different numbers of markers, including 9K, 15K, 35K, 50K, 55K, 90K, 660K, and 820K, are commercially available, and some of these chips have been used widely to identify genes for various traits in wheat (Allen et al. 2017; Cavanagh et al. 2013; Jia and Zhao 2016; Muqaddasi et al. 2017; Wang et al. 2014; Winfield et al. 2016).

Wheat cultivar Qinnong 142 (QN142) developed from the cross of Zhengmai 8329/87135-2-1-2-9 by the Baoji Academy of Agricultural Sciences, Shaanxi, China, is a high-yielding cultivar with resistance to stripe rust. This cultivar has been grown in the Yellow and Huai River Valley wheat zone of China since 2005, and it has been widely used as a parent in breeding programs. We have tested QN142

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for resistance to stripe rust in fields located in Shaanxi and Gansu provinces since 2008 and found that it is consistently resistant at the adult-plant stages (Zeng et al. 2014). However, the genetic basis of its stripe rust resistance is unknown. Thus, the objectives of this study were (i) to characterize and dissect the genetic structure of stripe rust resistance in an Avocet S (AvS) × QN142 recombinant inbred line (RIL) population, (ii) to identify QTLs for APR to stripe rust across multiple environments using molecular markers, (iii) to assess the additive effects of the QTLs on reducing stripe rust severity, and (iv) to develop kompetitive allele-specific PCR (KASP) markers for the QTLs for use in MAS.

Materials and Methods

Wheat materials. In total, 165 F_6 and F_7 RILs developed from a single F_1 plant of AvS × QN142 through single-seed descent were used for genetic mapping. The resistant parent QN142, originally from the China Agriculture Research System, and the susceptible parent AvS, originally from Australia, were used as references in all phenotypic and genotypic experiments. Wheat cultivars Mingxian 169 (MX169) and Xiaoyan 22 (XY22) were used as susceptible checks in the field experiments. A panel comprising 150 wheat cultivars and breeding lines from the Yellow and Huai River Valley wheat zone was used to validate the KASP markers flanking the identified QTL or to determine the marker polymorphisms.

Seedling tests. Seedling tests were conducted under controlled greenhouse conditions to characterize the stripe rust ASR in QN142. Chinese *P. striiformis* f. sp. *tritici* race CYR23 was used in the tests, and its avirulence/virulence pattern was reported by Wu et al. (2016). For the seedling tests, 10 to 15 plants of AvS, QN142, and their RILs were grown in 9 × 9 × 9 cm pots. Details of the inoculation procedure were described previously by Wu et al. (2018). The infection types were recorded at 18 to 21 days after inoculation using a scale that ranged from 0 to 9 (Line and Qayoum 1992; Wan and Chen 2014). Plants with infection type (IT) ranging from 0 to 6 were considered resistant, and plants with IT from 7 to 9 were considered susceptible. All tests were conducted three times in order to confirm and clarify the IT.

Field tests. The parents and F_6 RIL population, as well as MX169 and XY22, were grown in fields at Yangling, Shaanxi province (34°17′N, 108°04′E, altitude 519 m), Tianshui, Gansu province (34°27′N, 105°56′E, altitude 1,697 m), and Jiangyou, Sichuan province (31°53′N, 104°47′E, altitude 571 m) during the 2015–2016 and 2016–2017 cropping seasons. The fields at Yangling were inoculated at the jointing stage by spraying a mixture of urediniospores from the prevalent races CYR32 and CYR33 suspended in liquid paraffin (1:300) onto the susceptible checks planted as spreader rows. The experiments in Tianshui and Jiangyou were conducted under natural infection with *P. striiformis* f. sp. *tritici* because these locations were in the "hotspot" stripe rust regions. All of the experiments were arranged according to a randomized complete block design with two replicates in the 2015–2016 growing season and three replicates in the 2016–2017 growing season. Thirty seeds from each line were planted in a 120-cm row in each replicate with a 30-cm space between rows. IT and disease severity (DS) were scored for each row at 18–20 days postflowering when the stripe rust severity levels in MX169, AvS, and XY22 reached 90 to 100% around 15–20 May at Yangling, 10–15 June at Tianshui, and 10–15 April at Jiangyou. The IT data were recorded based on the scale from 0 to 9 mentioned above, and the DS data were scored with the modified Cobb scale (Peterson et al. 1948). For each line, the score was recorded at least twice or more, and the IT data and the last set of DS data treated as the maximum DS (MDS) were used for phenotypic and QTL analyses.

Phenotypic analysis. Analyses of variance (ANOVAs) were conducted using the IT and MDS data to determine significant differences among RILs and environments. ANOVA and Pearson's correlation coefficient analyses were conducted with the AOV function in the QTL IciMapping (version 4.1) software with the default parameters. The broad sense heritability (h_b^2) of stripe rust resistance was estimated with the following formula:

$$h_b^2 = \frac{\sigma_g^2}{\sigma_g^2 + \left(\sigma_{ge}^2/e\right) + \left(\sigma_\varepsilon^2/re\right)} \quad (1)$$

where σ_g^2 represents genetic variance, σ_{ge}^2 represents the genotype (line) × environment interaction variance, σ_ε^2 represents the residual error, and e and r are the numbers of environments and replicates, respectively. The minimum number of QTLs was also estimated using Wright's method (Wright 1968): $n = (GR)^2/4.27(\sigma_g^2)$, where GR comprises the phenotype ranges of the RILs multiplied by the narrow sense heritability, σ_g^2 is the genetic variance of the RIL population, and 4.27 was used as an inbreeding index for the F_6 generation, as described by Ren et al. (2017).

DNA extraction and marker genotyping. DNA was extracted from fresh leaves of the parents and F_6 RILs sampled at the jointing stage from the field in Yangling according to the method described by Song et al. (1994). The RILs and parents were genotyped at the CapitalBio Corporation (Beijing, China) using 35K and 660K iSelect SNP arrays. Low-quality SNPs with more than 10% missing values and major allele frequencies above 95% were removed. In addition, 1,375 SSR markers (Somers et al. 2004) selected to cover the genome were screened for polymorphic markers between the resistant and susceptible parents. The sequences of the SSR primers were acquired from the GrainGenes website (https://wheat.pw.usda.gov/GG3/), and the primers were synthesized by Invitrogen Trading Co. (Shanghai, China). Polymorphic SSR markers were used to genotype the RIL population according to the method described by Wu et al. (2017). The sequences of the 660K SNP markers were provided by Jizeng Jia, Chinese Academy of Agricultural Sciences.

Linkage map construction and QTL mapping. The SNP and SSR markers were tested for segregation using the χ^2 test. Markers that differed significantly from a one-locus segregation ratio were excluded. Redundant markers with identical segregation were first binned using the BIN function in QTL IciMapping version 4.1 (Meng et al. 2015; Wang 2009). One marker was chosen to represent each bin based on the least amount of missing data or selected randomly if the markers had the same number of missing values. The filtered markers were then used to construct linkage maps with the MAP function in QTL IciMapping version 4.1, and the linkage groups were drawn using Mapchart version 2.3 (Voorrips 2002). To finalize the linkage map, marker ordering and ripping were performed using the RECORD and COUNT algorithms, respectively. The positions of the linkage groups on wheat chromosomes were determined based on both previously published maps (Sourdille et al. 2004) and 35K integrated maps (Allen et al. 2017).

The inclusive composite interval mapping (ICIM) of additive functionality in IciMapping was used to detect QTLs based on the mean IT and MDS scores in each environment. The threshold logarithm of odds (LOD) score for declaring QTLs was determined by permutation tests. The parameters used for QTL mapping and characterization were the same as those described in a previous study (Wu et al. 2017). The effects of each QTL and all of the detected QTLs were estimated as the phenotypic variance explained using ICIM.

Conversion of SNPs to KASP markers. The flanking SNP markers linked to the stable QTLs were converted into KASP markers for use in MAS during wheat breeding to obtain stripe rust-resistant cultivars. Briefly, the sequences of the flanking SNP markers were uploaded to PolyMarker (http://polymarker.tgac.ac.uk/) (Ramirez-Gonzalez et al. 2015), and the sequences of only the chromosome-specific markers were synthesized and used as KASP primers. All of the KASP markers with polymorphisms between AvS and QN142 were used to genotype the RIL population and the panel of 150 wheat cultivars or breeding lines according to the procedure described by Wu et al. (2017).

Haplotype analysis. To obtain the genomic locations of all the SNPs, BLASTn (E value 1e-10) was performed using the newest Chinese Spring sequence (IWGSC RefSeq version 1.0) as a reference, with an E value threshold of 10^{-5}. According to the deduced regions of *QYrqin.nwafu-2AL* and *QYrqin.nwafu-6BS*, the 660K SNP

genotype data in the target regions were used for haplotype analysis. Owing to limitations caused by various factors, we only tested AvSYr32NIL carrying *Yr32* on chromosome 2AL (Eriksen et al. 2004) for comparison with *QYrqin.nwafu-2AL*, and wheat cultivars Stephens (*Yr78*) (Santra et al. 2008), RSL65 (*Yr36*) (Fu et al. 2009), and Pingyuan 50 (*QYrpi.caas.3*) (Lan et al. 2010) with the genes or QTLs on chromosome 6BS for comparison with *QYrqin.nwafu-6BS*.

Results

Race-specific all-stage resistance. When race CYR23 was tested at the seedling stage, QN142 exhibited resistance (IT = 1) and AvS was susceptible (IT = 9). The RIL population segregated with various ITs (Supplementary Table S1). The classification of the lines into resistant (IT = 0–6) and susceptible (IT = 7–9) groups suggested the presence of two genes for resistance to CYR23. Because two genes were indicated, QTL analysis was used to map the genes that conferred race-specific ASR. Two QTLs were detected on chromosome arms 1DS and 4AL by ICIM analysis.

Adult-plant responses to stripe rust. In all of the field experiments, QN142 was always resistant (IT = 1–3, MDS < 20%) and AvS was susceptible (IT = 9, DS > 90%) (Table 1, Fig. 1). The mean IT values for the RIL population ranged from 4.2 to 5.6%, and the mean MDS values ranged from 39.7 to 56.9% in the three locations during 2015–2017 (Table 1). The frequency distributions of the IT and MDS classes among the RILs exhibited continuous variation with approximately normal distributions across the six environments (Fig. 1), thereby suggesting the quantitative inheritance of APR to stripe rust. The narrow-sense heritability of the stripe rust severity ranged from 0.86 to 0.93 in the different environments. The calculations using Wright's method (Wright 1968) indicated the presence of three to four APR genes (Table 1). The Pearson's correlation coefficients (*r*) for IT and MDS in the RILs among the six field environments ranged from 0.76 to 0.89, and they were all significant ($P < 0.001$, Table 2). These findings indicate that the expression of these four QTLs was consistent among the different environments with different weather conditions and *P. striiformis* f. sp. *tritici* races.

Table 1. Summary of the adult-plant stripe rust responses by the Avocet S (AvS) × Qinnong 142 (QN142) recombinant inbred line (RIL) population during the 2015–2016 and 2016–2017 cropping seasons in Yangling, Tianshui, and Jiangyou[a]

Environment[b] (location, year)	AvS	QN142	RIL population			σ_g^2	h_b^2	Genes[c]
			No. of RILs	Range	Mean			
Infection type								
YL2016	9.0	0.5	165	0.8–8.5	4.3	3.2	0.89	–
TS2016	9.0	3.0	164	1.8–8.8	5.2	2.4	0.80	–
JY2016	9.0	2.0	165	1.8–8.9	5.1	1.6	0.86	–
YL2017	8.7	1.0	165	1.0–8.3	4.2	3.5	0.93	–
TS2017	9.0	2.2	165	1.7–8.2	5.6	1.3	0.90	–
JY2017	8.0	2.0	165	1.3–8.5	5.2	2.2	0.88	–
Maximum disease severity								
YL2016	90.0	1.0	165	1.0–95.0	39.7	420.7	0.92	4.2
TS2016	90.0	7.5	164	5.5–100.0	50.2	423.4	0.89	3.9
JY2016	100.0	10.0	165	5.0–100.0	53.8	480.3	0.91	3.6
YL2017	93.3	3.3	165	5.0–98.3	46.7	447.8	0.93	3.9
TS2017	100.0	18.3	165	10.0–95.5	56.6	340.4	0.90	4.1
JY2017	100.0	16.7	165	10.0–91.7	56.9	321.7	0.90	3.9

[a] σ_g^2 represents genetic variance, and h_b^2 represents broad sense heritability.
[b] YL, TS, and JY denote Yangling, Tianshui, and Jiangyou, respectively, and 2016 and 2017 represent the RIL populations grown during the 2015–2016 and 2016–2017 cropping seasons, respectively.
[c] A dash (–) indicates gene numbers were not estimated with the infection type data using Wright's method (Wright 1968).

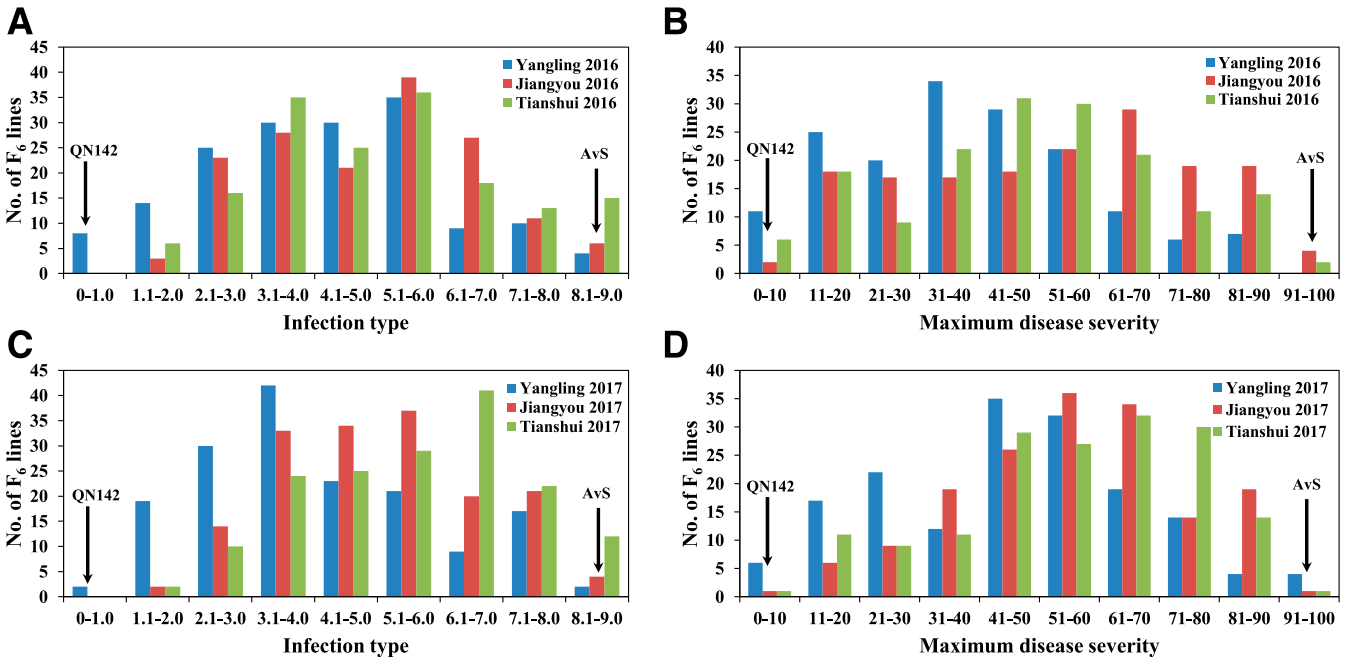

Fig. 1. Frequency distributions of the mean infection type and maximum disease severity for the 165 recombinant inbred lines from Avocet S (AvS) × Qinnong 142 (QN142) evaluated in Yangling, Jiangyou, and Tianshui during 2015–2016 (**A** and **B**) and 2016–2017 (**C** and **D**). The values for the parents AvS and QN142 are indicated by arrows.

Linkage groups. The parental lines and 165 RILs were genotyped with SSR markers and the 35K SNP array. Among the 1,375 SSR markers, 89 were polymorphic. Among the 35,143 SNPs, 7,996 were polymorphic between the parents. Among the polymorphic SNPs, 2,819 were removed because more than 10% of the data were missing and they exhibited severe segregation distortion for a single locus ($P < 0.001$) in the RIL population. The remaining 5,177 SNPs (Supplementary Table S2) fell into 1,456 bins, and 3,731 SNPs were redundant. Similarly, 35 SSR markers had distorted segregation ratios ($P < 0.05$) according to the χ^2 tests based on the expected 1:1 ratio. The 1,456 SNPs and 54 SSR markers that satisfied a ratio of 1:1 and that represented different bins were used for constructing linkage groups. In total, 18 SSR markers and 45 SNP markers were unlinked. The final 36 linkage groups comprised 1,411 SNP and 34 SSR markers, and they spanned a total of 3,303.9 cM. The linkages in the A, B, and D genomes included 515 (35.6%), 714 (49.4%), and 216 (15.0%) markers, respectively, which covered lengths of 1,194.4, 1,274.5, and 835 cM, with average marker densities of 2.3, 1.8, and 3.9 cM. Chromosomes 1B, 2A, 2B, 3B, 3D, 4D, 5A, 6B, and 7A each had one linkage group; 1D, 3A, 4A, 4B, 6A, 6D, and 7D each had two linkage groups; 1A and 5D each had three linkage groups; 2D had four linkage groups; and 5BL/7BL and 5BS/7BS had translocations (Supplementary Tables S3 and S4). Only the linkage groups with significant stripe rust resistance QTLs are shown in Figure 2.

Identified QTLs. For the field tests, the QTLs that could be detected based on both IT and MDS data across the six environments and in the combined analysis were considered stable. Four stable QTLs were identified on 1BL, 2AL, 2BL, and 6BS, which were designated as *QYrqin.nwafu-1BL*, *QYrqin.nwafu-2AL*, *QYrqin.nwafu-2BL*, and *QYrqin.nwafu-6BS*, respectively. All of the QTLs were significant and contributed by the resistant parent QN142. On average, *QYrqin.nwafu-1BL* explained 15.7 and 19.7% of the phenotypic variation in IT and MDS, respectively. This QTL was mapped to a 10.04-cM region flanked by SNP markers *AX-95139868* and *AX-94885318*. *QYrqin.nwafu-2AL* was significant at a threshold LOD value of 3.0, and it explained 8.3 to 19.8% of the phenotypic variation in all environments. This QTL was mapped to a 7.9-cM region flanked by SNP markers *AX-94655393* and *AX-94895021* with SSR marker *Xwmc170* within this region. The largest effect QTL, *QYrqin.nwafu-2BL*, located in a 1.4-cM interval flanked by SNP markers *AX-94507002* and *AX-94562871*, explained 18.3 to 39.1% of the phenotypic variation in all environments. The second largest effect QTL, *QYrqin.nwafu-6BS*, explained 13.5 to 31.0% of the phenotypic variance, and it was located in the SNP marker interval of 0.3 cM between *AX-95235011* and *AX-95188301* (Table 3, Fig. 2).

Stripe rust responses of RILs with different QTL combinations. To investigate the effects of the QTLs on the stripe rust response, the 165 RILs were divided into genotypic groups based on the presence of the closest markers to the four QTLs for APR (Supplementary Table S6). These genotypes were further grouped into five groups based on the number of potential QTLs. The mean IT and MDS values for the five groups are shown in Figure 3. The lines with four QTLs had IT values of 1.3 to 2.4 and MDS values ranging from 8.4 to 18.2% across the six environments, which were similar to those of the resistant parent (Fig. 3B; Supplementary Table S5). In general, the lines with three QTLs were more resistant than those with two QTLs, which had better resistance than those with one QTL. This result was consistent with the three to four QTLs that conferred resistance in the RIL population estimated using Wright's method (Table 1). The RILs without any QTLs were highly susceptible (IT = 8–9, DS = 85 to 95%) but not significantly different from the susceptible parent. This was also supported by the significant additive effects ($P < 0.01$) among the QTLs obtained using the BIP function with the QTL IciMapping version 4.1 software.

KASP markers. Among the 47 SNP markers close to the four QTLs for APR with flanking sequences submitted to PolyMarker, 16 chromosome-specific SNPs were selected for conversion into KASP markers. After screening in the parents to confirm their polymorphisms, 8/16 markers failed to distinguish between the parents. Each QTL was flanked by two KASP markers, and the primer sequences are shown in Table 4. When tested in 165 RILs and 150 cultivars and breeding lines, these markers produced the same genotypes as the original SNP probes. In the 150 wheat accessions, Taihemai 5 carried the same alleles of *AX-94588421* and *AX-94482474* flanking the *QYrqin.nwafu-2BL*, and Xinong 364 had the same alleles as QN142 in the *QYrqin.nwafu-6BS* region. Pedigree analysis showed that Taihemai 5 (Luo 5418/Zhengmai 7698), QN142 (Zhengmai 8329/Zhi 87135-2-1-2-9), Zhengmai 8329 (Xinong 78[6]9-2/Xinong 80[6]5-6-10), and Xinong 364 may have common wheat genotypes in their pedigrees.

Comparisons with other genes/QTLs. To compare *QYrqin.nwafu-2AL* with *Yr32*, 48 SNP markers that covered a 6.5-Mb (from the 711.08 to 717.58 Mb positions) genomic region encompassing the *QYrqin.nwafu-2AL* locus were used to genotype AvSYr32NIL (*Yr32*). Among the 48 markers, 43 had different genotypes, thereby indicating that *QYrqin.nwafu-2AL* is different from *Yr32* (Supplementary Table S7). Similarly, 35 SNP markers that covered a 20.5-Mb (92.47 to 112.98 Mb) genomic region harboring *QYrqin.nwafu-6BS* were used to genotype Stephens, RSL65, and Pingyuan 50, for which 21, 22, and 35 markers were different, respectively. These results indicated that *QYrqin.nwafu-6BS* is a distinct QTL.

Discussion

Wheat cultivar QN142 was found to have high-level APR to stripe rust in fields despite its susceptible reactions to the predominant *P. striiformis* f. sp. *tritici* races such as CYR32 and CYR33, but it was resistant to some old races such as CYR23 at the seedling stage when tested in a greenhouse (Zeng et al. 2014). In the present study, we mapped two genes for ASR to CYR23, but they were not detected in the field experiments, apparently because races such as CYR32 and CYR33 circumvent the race-specific resistance genes. Based on the field data, four genes were estimated using Wright's formula. The estimated number of genes was consistent with the QTL mapping results for the four QTLs identified by ICIM analysis across the six environments. Lan et al. (2015) reported that the numbers of genes estimated using the χ^2 method and Wright's method were similar to the number of QTLs detected by QTL mapping, although with a slight difference. However, previous studies have reported that qualitative methods normally underestimate the number of resistance

Table 2. Correlation coefficients (*r*) of the mean maximum disease severity (MDS) and infection type (IT) for the Avocet S × Qinnong 142 recombinant inbred line (RIL) population in six environments

	r values based on MDS (IT)[b]					
Environment[a] (location, year)	YL2016	TS2016	JY2016	YL2017	TS2017	JY2017
YL2016	1					
TS2016	0.87 (0.86)	1				
JY2016	0.85 (0.84)	0.84 (0.83)	1			
YL2017	0.85 (0.89)	0.86 (0.83)	0.87 (0.81)	1		
TS2017	0.87 (0.85)	0.86 (0.82)	0.88 (0.84)	0.89 (0.80)	1	
JY2017	0.82 (0.78)	0.81 (0.76)	0.83 (0.80)	0.87 (0.79)	0.85 (0.78)	1

[a] YL, TS, and JY denote Yangling, Tianshui, and Jiangyou, respectively, and 2016 and 2017 represent the RIL populations grown during the 2015–2016 and 2016–2017 cropping seasons, respectively.

[b] *r* values calculated with IT are shown in parentheses. All of the *r* values are significant at $P < 0.001$.

genes in an $F_{2:3}$ population compared with QTL analysis (Qiu et al. 2014). This may be caused by the assumptions of additive effects of polygenes and equal contributions of different QTLs to phenotypic variation in disease resistance (Lan et al. 2017; Ren et al. 2017; Wright 1968).

QYrqin.nwafu-1BL was detected near the distal end of chromosome arm 1BL. Several stripe rust APR QTLs have been reported on chromosome 1BL, most of which corresponded to the *Yr29/Lr46* locus (Rosewarne et al. 2013; Singh et al. 1998). Based on the consensus 35K and 90K map (Allen et al. 2017; Maccaferri et al. 2015), *AX-94642355* and *IWB12335* are the same SNP locus, and the identified QTL region adjoins *Yr29*. In addition, QN142 and Pavon 76 share the same alleles at the *wmc44* and *bac17R* SSR marker loci, which are linked to *Yr29* (Rosewarne et al. 2006). *Yr29* is known to be present in many wheat lines such as Pavon 76 developed by CIMMYT, and this locus confers APR to three rust diseases (Herrera-Foessel et al. 2011) and powdery mildew (Lillemo et al. 2008). In previous studies, *Yr29* explained 7 to 65% of the phenotypic variation in the stripe rust response depending on environmental conditions and genetic background (Lan et al. 2015; Singh et al. 1998). In the present study, *QYrqin.nwafu-1BL* explained 15.7 to 19.7% of the variation in stripe rust severity, which was similar to the previously observed results (Ponce-Molina et al. 2018; Ren et al. 2017). Therefore, it is necessary to combine this gene with other APR genes to achieve adequate levels of resistance in the field, as also found with QN142.

The *QYrqin.nwafu-2AL* QTL was located on chromosome arm 2AL with the flanking markers *AX-94655393* and *AX-94895021*. Based on the integrated genetic map, six genes or QTLs were reported previously in this region: *Yr32* (Eriksen et al. 2004), *IWA7339_APR* (Zegeye et al. 2014), *IWA544_APR* (Jighly et al. 2015), *QYrns.orz-2AL* (Vazquez et al. 2015), *QYr.wpg-2A.5* (Naruoka et al. 2015), and *QYr.caas-2AL* (Liu et al. 2015) (Fig. 2D). In our previous study, wheat line AvSYr32NIL (*Yr32*) was resistant to *P. striiformis* f. sp. *tritici* race CYR32 at both the seedling and adult plant stages (DS = 0 to 40%), whereas QN142 was susceptible to CYR32 (Zeng et al. 2014, 2015). In the seedling test, QN142 was resistant to CYR23, but the resistance loci were mapped to 1DL and 4AL, thereby suggesting that the seedling resistance genes in QN142 are different from *Yr32*. Moreover, haplotype comparisons between QN142 and AvSYr32NIL confirmed that the two genes are different. QTL *IWA7339_APR* derived from synthetic hexaploid wheat, *QYrns.orz-2AL* in U.S. Pacific Northwest winter wheat cultivar Tubbs, *IWA544_APR* from a wheat cultivar of ICARDA (International Center for Agricultural Research in Dryland Areas), and *QYr.wpg-2A.5* in U.S. Pacific Northwest winter wheat cultivars are all from other countries. *QYr.caas-2AL* was contributed by Chinese wheat cultivar Zhong 892 with the pedigree of 786-11// Ourou/Beijing 8///LK338/730-04 (Liu et al. 2015). The pedigrees of these gene sources did not indicate obvious relatedness, but further studies are needed to clearly determine the genetic relationships among these genes.

QYrqin.nwafu-2BL mapped to chromosome arm 2BL and it was flanked by SNP markers *AX-94507002* and *AX-94562871*, and it was the largest effect QTL in QN142. Several permanently named genes (*Yr3*, *Yr5*, *Yr7*, *Yr43*, *Yr44*, and *Yr53*) have been reported on

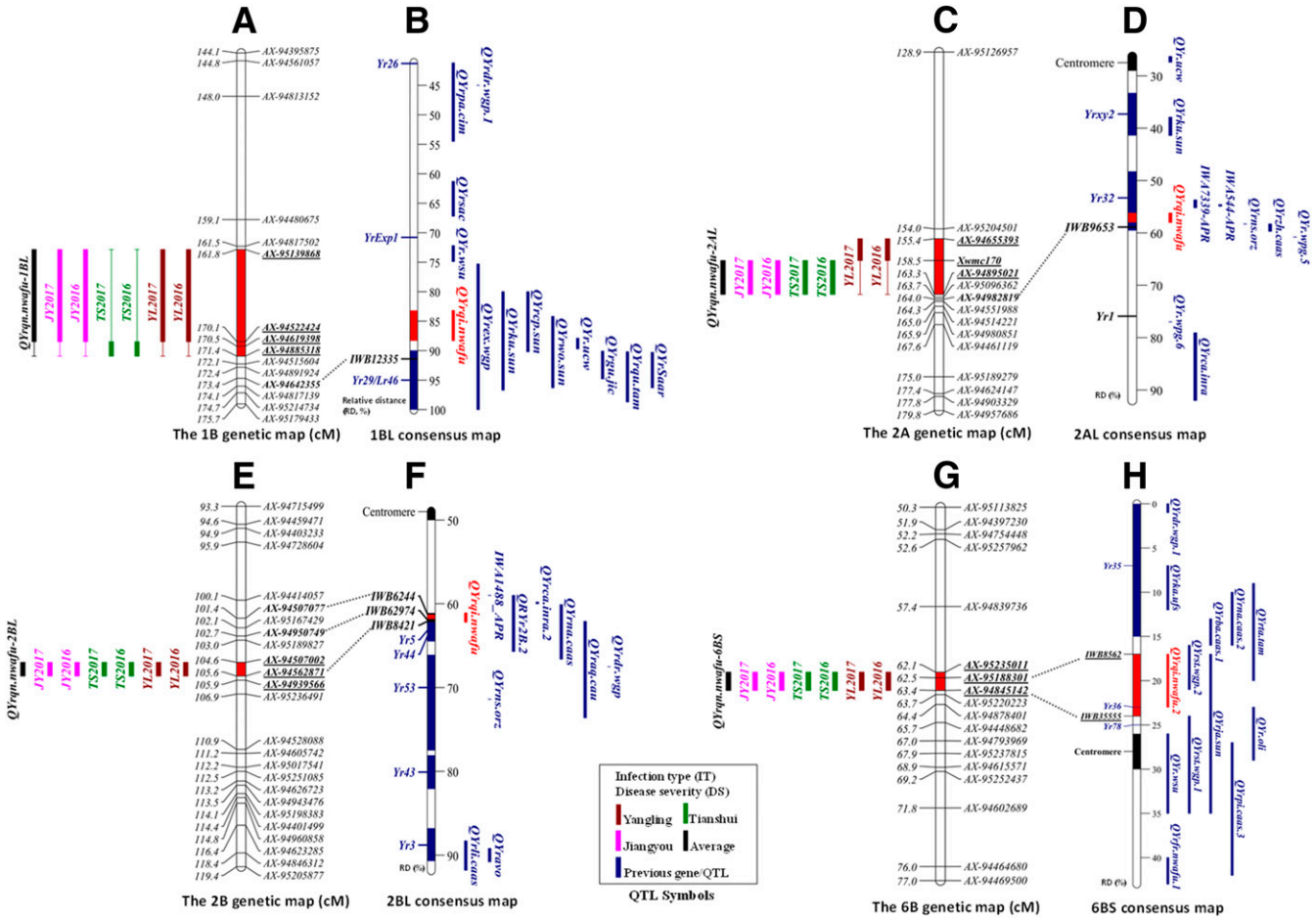

Fig. 2. Locations on wheat chromosome arms of quantitative trait loci (QTLs) for stripe rust response in six individual environments. Four linkage groups covering four QTLs for resistance to stripe rust were constructed with simple sequence repeat and single-nucleotide polymorphism markers (**A, C, E,** and **G**). The names of the marker loci and the QTLs identified in this study are listed on the left of the linkage groups. The QTLs were identified by inclusive composite interval mapping, and the QTL intervals were associated with a logarithm of odds score (LOD) > 2.0, with LOD peak values of more than 2.5 determined by permutation tests. The environments where the corresponding QTLs were detected are shown in different colors (**B, D, F,** and **H**). The QTLs detected in the present study (colored in red on the corresponding chromosomes) and previously mapped stripe rust resistance genes or QTLs (colored in blue) were positioned based on integrated genetic maps published by Maccaferri et al. (2015) and Bulli et al. (2016).

chromosome arm 2BL, and they all confer ASR to stripe rust (Maccaferri et al. 2015; Wang and Chen 2017). These genes are ineffective against Chinese *P. striiformis* f. sp. *tritici* races, except for *Yr5* and *Yr53* (Zeng et al. 2015). *Yr5* is derived from *Triticum aestivum* ssp. *spelta* var. *album* (Macer 1966), and it was mapped onto the interval between SNP markers *IWA6121* and *IWA4096* (Naruoka et al. 2016), which contains the region of *QYrqin.nwafu-2BL*. *Yr53* is originally from durum wheat PI 480148, and it was flanked by *Xwmc441* and *XLRRrev/NLRRrev350* (Xu et al. 2013). However, *QYrqin.nwafu-2BL* confers APR despite having a small effect on the seedling response. In addition, *QYrqin.nwafu-2BL* shared no marker alleles with *Yr5* and *Yr53* (data not shown). Based on the resistance types and markers, *QYrqin.nwafu-2BL* should be different from *Yr5* and *Yr53*. In addition, several stripe rust QTLs on chromosome arm 2BL have been reported and located in a similar region (Rosewarne et al. 2013), including *QYrdr.wgp-2BL* in Druchamp (Hou et al. 2015), *QYr.caas-2BL* in Naxos (Ren et al. 2012b), *QYraq.cau-2BL* in Aquileja (Guo et al. 2008), and *QYr.inra-2BL* in Camp Remy (Mallard et al. 2005). *QYrdr.wgp-2BL* was a minor QTL that explained 4.0 to 10.8% of the phenotypic variance, and its linked SNP marker was *IWA7583* (Hou et al. 2015). *QYr.caas-2BL* also had a minor effect, and it was detected in a larger interval between SSR markers *Xwmc441* and *Xwmc361* (Ren et al. 2012b). *QYraq.cau-2BL* accounted for 61.5% of the phenotypic variance, and it was located in the marker interval *Xwmc175* to *Xwmc332* (Guo et al. 2008). *QYr.inra-2BL* accounted for up to 61% of the phenotypic variance, and it was flanked by *Xbarc101* and *Xgwm120* (Mallard et al. 2005). Based on the consensus 35K and 90K maps, *IWA7583* linked to *QYrqin.nwafu-2BL* was more

Table 3. Summary of stripe rust resistance quantitative trait loci (QTLs) detected in the Avocet S × Qinnong 142 recombinant inbred line population across six environments[a]

			Infection type			Maximum disease severity		
QTL	Environment[b]	Marker interval	LOD	Add	PVE	LOD	Add	PVE
QYrqn.nwafu-1BL	YL2016	*AX-95139868—AX-94522424*	8.9	–0.7	17.0	6.3	–9.2	11.1
	TS2016	*AX-95139868—AX-94522424*	6.7	–0.8	12.0	6.1	–8.0	11.2
	JY2016	*AX-95139868—AX-94522424*	10.5	–0.7	14.3	15.6	–11.6	25.3
	YL2017	*AX-95139868—AX-94522424*	5.3	–0.7	9.2	16.4	–9.6	17.4
	TS2017	*AX-94619398—AX-94885318*	7.9	–0.6	14.7	13.1	–7.9	16.2
	JY2017	*AX-95139868—AX-94522424*	9.0	–0.6	16.7	8.7	–9.2	14.8
	Mean	*AX-95139868—AX-94522424*	8.5	–0.7	15.7	9.3	–9.3	19.7
QYrqn.nwafu-2AL	YL2016	*AX-94655393—Xwmc170*	15.5	–0.8	18.2	14.2	–8.8	19.8
	TS2016	*Xwmc170—AX-94895021*	10.2	–0.7	9.8	11.8	–8.8	13.5
	JY2016	*Xwmc170—AX-94895021*	4.2	–0.4	9.1	7.8	–6.5	10.6
	YL2017	*AX-94655393—Xwmc170*	11.6	–0.7	9.3	10.1	–8.3	12.8
	TS2017	*Xwmc170—AX-94895021*	4.3	–0.5	8.5	8.8	–5.9	13.8
	JY2017	*Xwmc170—AX-94895021*	4.1	–0.4	8.3	7.2	–6.5	18.7
	Mean	*Xwmc170—AX-94895021*	9.8	–0.6	11.4	14.6	–7.5	12.5
QYrqn.nwafu-2BL	YL2016	*AX-94507002—AX-94562871*	17.6	–1.0	31.6	22.6	–10.7	29.7
	TS2016	*AX-94507002—AX-94562871*	18.2	–0.9	25.7	21.1	–11.5	22.9
	JY2016	*AX-94507002—AX-94562871*	26.6	–1.3	39.1	17.6	–11.8	25.9
	YL2017	*AX-94507002—AX-94562871*	20.2	–1.1	24.1	18.9	–12.7	30.0
	TS2017	*AX-94507002—AX-94562871*	13.8	–0.8	27.4	14.6	–8.4	18.3
	JY2017	*AX-94507002—AX-94562871*	8.7	–0.6	15.5	9.8	–7.5	23.1
	Mean	*AX-94507002—AX-94562871*	17.7	–0.9	24.1	14.3	–11.1	27.8
QYrqn.nwafu-6BS	YL2016	*AX-95188301—AX-94845142*	11.0	–0.7	14.3	16.6	–9.3	22.2
	TS2016	*AX-95235011—AX-95188301*	18.4	–0.9	15.4	18.3	–13.1	30.0
	JY2016	*AX-95235011—AX-95188301*	14.7	–0.8	14.2	11.8	–9.9	18.2
	YL2017	*AX-95235011—AX-95188301*	17.8	–1.3	31.0	15.3	–11.6	25.2
	TS2017	*AX-95235011—AX-95188301*	17.1	–0.9	30.6	18.9	–8.5	35.1
	JY2017	*AX-95235011—AX-95188301*	11.5	–1.0	21.2	9.9	–8.8	13.5
	Mean	*AX-95235011—AX-95188301*	14.2	–0.8	20.1	19.3	–9.9	22.2

[a] LOD = logarithm of odds score; Add = additive effect of the resistance allele (negative signs indicate the resistance allele from QN142); and PVE = percentages of the phenotypic variance explained by individual QTL.

[b] YL, TS, and JY denote Yangling, Tianshui and Jiangyou, respectively, and 2016 and 2017 represent the field experiments during the 2015–2016 and 2016–2017 cropping seasons, respectively.

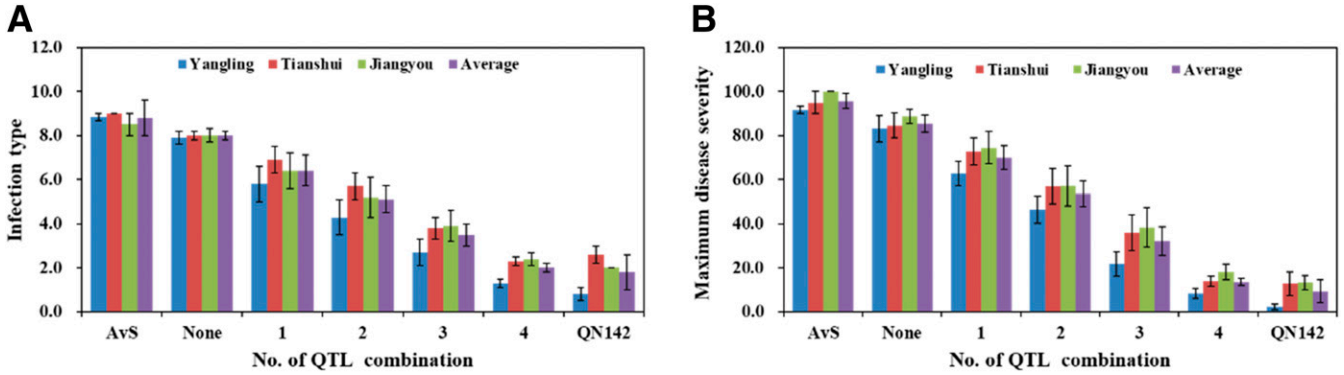

Fig. 3. Effects of quantitative trait locus (QTL) combinations on stripe rust scores according to the mean infection type (**A**) and maximum disease severity (**B**) for the recombinant inbred lines from Avocet S (AvS) × Qinnong 142 (QN142) in Yangling, Tianshui, and Jiangyou.

than 15 cM away from *IWB8421* (=*AX-94562871*), *QYr.caas-2BL* covered the *QYrqin.nwafu-2BL* region, and *QYraq.cau-2BL* and *QYr.inra-2BL* were in a close proximity to *QYrqin.nwafu-2BL*. These findings indicate that *QYrqin.nwafu-2BL* is the same as one or both of the other QTLs in this chromosomal region. Allelism tests are needed to clearly determine their genetic relationships.

Chromosome arm 6BS is a resistance gene-rich region that includes many genes or QTLs for stripe rust resistance (Rosewarne et al. 2013; Wang and Chen 2017). Based on an integrated genetic map (Bulli et al. 2016; Maccaferri et al. 2015; Wang and Chen 2017), most of them were concentrated in the interval of 29.2 to 49.0 cM, such as *QYr.tam-6BS* in TAM 111 (Basnet et al. 2014), *QYr.caas-6BS.2* in Naxos (Ren et al. 2012b), *QYr.caas-6BS* in Bainong 64 (Ren et al. 2012c), *QYrste.wgp-6BS.2* in Stephens (Santra et al. 2008), *QYr.sun-6BS* in Janz (Bariana et al. 2010), *QYr-6B* in Oligoculm (Suenaga et al. 2003), *Yr36* in RSL65 (Uauy et al. 2005), *QYr.wsu-6B.1* (Bulli et al. 2016), *QYr.ucw-6B* in PI 519805 (Dong et al. 2017; Maccaferri et al. 2015), *QYr.wgp-6B.1* in Stephens (Naruoka et al. 2015; Santra et al. 2008), and *QYr.caas-6BS* in Pingyuan 50 (Lan et al. 2010). Some QTLs were identified by genome-wide association analysis (Bulli et al. 2016; Maccaferri et al. 2015; Naruoka et al. 2015), whereas the others were mapped using the biparental mapping approach (Bariana et al. 2010; Basnet et al. 2014; Dong et al. 2017; Ren et al. 2012a, b; Santra et al. 2008; Suenaga et al. 2003; Uauy et al. 2005). After validating *QYr.ucw-6B* in 10 biparental populations, Dong et al. (2017) suggested that *QYr.sun-6BS* in Janz, *QYr.wgp-6B.1* in Stephens, and *QYr.wsu-6B.1* could be designated as synonymous with *QYr.ucw-6B* (named as *Yr78*). *QYrqin.nwafu-6BS* was also located in a similar region, but according to the haplotypes, *QYrqin.nwafu-6BS* was different from the QTLs in Stephens, RSL65, and Pingyuan 50. Both *QYr.tam-6B* and *QYr.caas-6BS* had minor effects, in which they explained less than 10% of the phenotypic variation, but the former was detectable in only some environments, whereas *QYr.caas-6BS* was stable across all the test environments. Based on the different haplotypes of the gene sources and the stability of their effects, *QYrqin.nwafu-6BS* is probably different from the other QTLs on 6BS. Moreover, none of the markers that flanked these QTLs exhibited polymorphisms in the QN142 mapping population. Therefore, *QYrqin.nwafu-6BS* identified in QN142 is a distinct QTL. Further studies are required to dissect the chromosomal region and confirm the genetic relationships among the stripe rust resistance genes or QTLs on 6BS.

The four QTLs identified in QN142 provide different levels of APR. *QYrqin.nwafu-2BL* and *QYrqin.nwafu-6BS*, which each consistently explained up to 30% of the phenotypic variation, are more useful than the other two QTLs with smaller effects for breeding wheat cultivars with resistance to stripe rust. However, the small-effect QTLs identified in this study can enhance resistance when combined with other QTLs. RILs with more QTLs had higher levels of resistance, and those with all four QTLs had a similar level of resistance to QN142. Thus, the high level of APR in QN142 is controlled by the combination of the four stable QTLs. Therefore, combining different QTLs is an effective approach for developing wheat cultivars with an adequate level of resistance. QN142 has been grown widely in the Yellow and Huai River Valley wheat zone, and it has remained resistant since its release in 2005, so its APR appears to be durable. The durability can be determined by continually growing QN142 and its derivative cultivars with similar level of resistance. In addition, the QTLs from QN142 can be used together with other effective stripe rust resistance genes in breeding programs to develop more cultivars with different combinations of diverse genes.

Breeding programs are increasingly using MAS to accelerate the selection process and increase the total genetic gains. In the present study, the SNP markers with tight linkage to four stable QTLs for stripe rust resistance provide an opportunity to pyramid these genes with other genes using MAS. When combined with tests using different wheat cultivars, the designed KASP markers linked to all four QTLs were reliable for MAS. The intervals of the two QTLs with the largest effects, *QYrqin.nwafu-2BL* and *QYrqin.nwafu-6BS*, were only 1.4 and 0.3 cM. Using the flanking markers, we detected these two genes in wheat cultivars related to QN142. We also found that the flanking markers of the four stable QTLs were highly polymorphic among the wheat cultivars and breeding lines, thereby allowing the incorporation of these genes into other backgrounds to develop new cultivars through MAS. All of these markers are codominant, which allows the distinction between heterozygous and homozygous plants. Therefore, the codominant markers can

Table 4. Primer sequences of KASP markers developed based on single-nucleotide polymorphism (SNP) markers with close linkages to stripe rust resistance quantitative trait loci (QTLs)[a]

SNP name	QTL name	Primer sequence (5′–3′)
AX-94509279_A	*QYrqin.nwafu-1BL*	GAAGGTGACCAAGTTCATGCTGGCCAGGAAGAACTGTGAAATT
AX-94509279_B	*QYrqin.nwafu-1BL*	GAAGGTCGGAGTCAACGGATTGGCCAGGAAGAACTGTGAAATG
AX-94509279_C	*QYrqin.nwafu-1BL*	CAAAGCATCACTGTGGCCTC
AX-94673495_A	*QYrqin.nwafu-1BL*	GAAGGTGACCAAGTTCATGCTAAGCTGCATGAGATTGCTACA
AX-94673495_B	*QYrqin.nwafu-1BL*	GAAGGTCGGAGTCAACGGATTAAGCTGCATGAGATTGCTACG
AX-94673495_C	*QYrqin.nwafu-1BL*	ACTACAATGTACCTGATGAATCCCC
AX-94992915_A	*QYrqin.nwafu-2AL*	GAAGGTGACCAAGTTCATGCTGCAAACTCCAACTTCTCCTTG
AX-94992915_B	*QYrqin.nwafu-2AL*	GAAGGTCGGAGTCAACGGATTGCAAACTCCAACTTCTCCTTC
AX-94992915_C	*QYrqin.nwafu-2AL*	AGAGGGAGAGCTCGAAACTC
AX-94757208_A	*QYrqin.nwafu-2AL*	GAAGGTGACCAAGTTCATGCTTGCTTGATAGGTGGGAGTGG
AX-94757208_B	*QYrqin.nwafu-2AL*	GAAGGTCGGAGTCAACGGATTTGCTTGATAGGTGGGAGTGA
AX-94757208_C	*QYrqin.nwafu-2AL*	TGCAACAAAAGAAAGGCAGCT
AX-94588421_A	*QYrqin.nwafu-2BL*	GAAGGTGACCAAGTTCATGCTCGGCGGAGGGATCGAACT
AX-94588421_B	*QYrqin.nwafu-2BL*	GAAGGTCGGAGTCAACGGATTCGGCGGAGGGATCGAACC
AX-94588421_C	*QYrqin.nwafu-2BL*	CTCTCTCATCTCGCCGAAC
AX-94482474_A	*QYrqin.nwafu-2BL*	GAAGGTGACCAAGTTCATGCTTGGAGCTTCCGGTGTAGAAG
AX-94482474_B	*QYrqin.nwafu-2BL*	GAAGGTCGGAGTCAACGGATTTGGAGCTTCCGGTGTAGAAC
AX-94482474_C	*QYrqin.nwafu-2BL*	TCCTCTTTTGGACACGGCAG
AX-95134713_A	*QYrqin.nwafu-6BS*	GAAGGTGACCAAGTTCATGCTAACCCTTGATCACATCAGCA
AX-95134713_B	*QYrqin.nwafu-6BS*	GAAGGTCGGAGTCAACGGATTAACCCTTGATCACATCAGCG
AX-95134713_C	*QYrqin.nwafu-6BS*	GGGAGGATGTAGTACCGAATGT
AX-94839736_A	*QYrqin.nwafu-6BS*	GAAGGTGACCAAGTTCATGCTTCATGTAGAACTGTTGTTGTCGG
AX-94839736_B	*QYrqin.nwafu-6BS*	GAAGGTCGGAGTCAACGGATTTCATGTAGAACTGTTGTTGTCGC
AX-94839736_C	*QYrqin.nwafu-6BS*	AAGGTTGGAAGATGCGAGAATA

[a] KASP = kompetitive allele-specific polymerase chain reaction. KASP markers at the end of the SNP name: A = primers with the added fluorescein amidite adapter; B = primers with the added hexachloro-fluorescein adapter; and C = common primers.

be used for directly selecting homozygous resistant plants in the early generations to speed up the breeding process.

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