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ORIGINAL PAPER

QTL mapping for adult-plant resistance to stripe rust in Italian common wheat cultivars Libellula and Strampelli

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Abstract Italian common wheat cultivars Libellula and Strampelli, grown for over three decades in Gansu province of China, have shown effective resistance to stripe rust. To elucidate the genetic basis of the resistance, F_3 populations were developed from crosses between the two cultivars and susceptible Chinese wheat cultivar Huixianhong. The F_3 lines were evaluated for disease severity in Beijing, Gansu and Sichuan from 2005 to 2008. Joint- and single-environment analyses by composite interval mapping identified five quantitative trait loci (QTLs) in Libellula for reduced stripe rust severity, designated *QYr.caas-2DS*, *QYr.caas-4BL*, *QYr.caas-5BL.1*, *QYr.caas-5BL.2* and *QYr.caas-7DS*, and explained 8.1–12.4, 3.6–5.1, 3.4–8.6, 2.6 and 14.6–35.0%, respectively, of the phenotypic variance across four environments. Six interactions between different pairs of QTLs explained 3.2–7.1% of the phenotypic variance. The QTLs *QYr.caas-4BL*, *QYr.caas-5BL.1* and *QYr.caas-7DS* were also detected in Strampelli, explaining 4.5, 2.9–5.5 and 17.1–39.1% of phenotypic variance, respectively, across five environments. Three interactions between different pairs of QTLs accounted for 6.1–35.0% of the phenotypic variance. The QTL *QYr.caas-7DS* flanked by markers *csLV34* and *Xgwm295* showed the largest effect for resistance to stripe rust. Sequence analyses confirmed that the lines with the *QYr.caas-7DS* allele for resistance carried the resistance allele of the *Yr18/Lr34* gene. Our results indicated that the adult-plant resistance gene *Yr18* and several minor genes confer effective durable resistance to stripe rust in Libellula and Strampelli.

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Introduction

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is an important disease of common wheat (*Triticum aestivum* L.) worldwide (Stubbs 1985; Chen 2005). The most effective approach to control the disease is growing resistant wheat cultivars (Line 2002; Chen 2005). However, most of the resistance genes used in the past few decades were race-specific, eliciting hypersensitive responses in host plants and easily overcome when new virulent strains increased in the pathogen population. In China, the vulnerability of this type of stripe rust resistance has occurred repetitively since the 1950s with consequent high yield losses (Wan et al. 2004).

The development of wheat cultivars with adult-plant resistance (APR) has been given increasing emphasis in

recent years because of its higher durability (Line 2002). Cultivars conferring APR often show susceptible responses at the seedling stage, but have low disease severities at the adult-plant stage in the field. APR is characterized by a lower frequency of infections, a longer latent period, less urediniospore production, a smaller uredinial size and its polygenic nature (Chen and Line 1995; Liang et al. 2006). It may be identified in cultivars with defeated race-specific genes, or in those lacking known race-specific resistance genes (Singh and Rajaram 1994; Santra et al. 2008).

Genetic analysis of cultivars with APR indicated that this type of resistance was conferred by the additive effects of several minor genes (Singh and Rajaram 1994; Navabi et al. 2004; Singh et al. 2005). This conclusion was further confirmed by studies of an increasing number of cultivars with APR, such as Kariega (Ramburan et al. 2004), Camp Rémy (Mallard et al. 2005), Pavon 76 (William et al. 2006), Attila (Rosewarne et al. 2008), and Express (Lin and Chen 2009). Singh et al. (2000a) demonstrated that the combination of four to five slow rusting genes with small to intermediate effects, but acting additively provides up to near-immune levels of APR.

Since APR is quantitatively inherited, molecular markers can be employed to determine the number, genomic location and effect of the individual resistance genes (Young 1996). Molecular markers closely linked to those resistance loci can then be used in gene pyramiding in facilitating wheat breeding programs (Young 1996). Many quantitative trait loci (QTLs) in wheat for reducing stripe rust severity at adult-plant stage have been identified with molecular markers (Bariana et al. 2001; Boukhatem et al. 2002; Mallard et al. 2005; Santra et al. 2008; Lin and Chen 2009). Of them, the most important slow stripe rusting and designated loci are *Yr18* (Suenaga et al. 2003), *Yr29* (William et al. 2003, 2006), and *Yr30* (Singh et al. 2000b, 2005). These three genes are widely distributed in CIMMYT wheat germplasm (Singh et al. 2005). More recently, *Yr36* (Uauy et al. 2005) and *Yr39* (Lin and Chen 2007) were identified. Due to the significant contributions of these genes for stripe rust resistance, several studies were conducted to map them more precisely (William et al. 2003; Uauy et al. 2005; Lagudah et al. 2006; Spielmeyer et al. 2008), and this resulted in the recent cloning of *Yr18* and *Yr36* (Fu et al. 2009; Krattinger et al. 2009). Both genes differed from the NBS-LRR structures that characterize most of the specific resistance genes cloned to date.

The Italian wheat cultivars Libellula and Strampelli, introduced into China in 1973 (Zheng 1993), have been grown in Gansu province, a hot spot for stripe rust, for over 30 years. In spite of the occurrence of many new pathogenic races, they continued to confer effective APR to stripe rust, justifying their classification as durable resistance (Zhou et al. 2003a). Inheritance of the APR in these two cultivars was previously reported based on the reaction patterns of F_1 and F_2 progenies derived from crosses Libellula/Huixianhong and Strampelli/Huixianhong using conventional quantitative genetic analysis (Yin et al. 2005, 2006). However, the precise positions of the resistance loci in the two cultivars remained unknown. Accordingly, the objective of the present study was to identify and locate the QTLs for APR to stripe rust in the two cultivars using molecular markers.

Materials and methods

Plant materials

F_3 populations used for QTL mapping were derived from the crosses Libellula/Huixianhong and Strampelli/Huixianhong, totaling 244 and 252 lines, respectively. The two cultivars have been grown in Gansu province, a hot spot for stripe rust, for over 30 years, exhibiting high APR expressed as longer latent period, lower disease severity and lower damage to kernel weight (Zhou et al. 2003a), whereas Huixianhong is highly susceptible to almost all isolates of *Pst* at both the seedling and adult-plant stages. The F_3 lines generated from individual F_2 plants were planted and harvested as bulks with over 50 plants of each line to produce F_3 populations that can be maintained as bulk populations, each deriving from a single F_2 plant.

Field trials

The two populations were evaluated for disease severity to stripe rust in Beijing, Gansu and Sichuan provinces from 2005 to 2008, providing data for the populations of Libellula/Huixianhong and Strampelli/Huixianhong for four and five environments, respectively. Field trials were conducted in randomized complete blocks with three replicates. Each plot consisted of two 1.5 m rows spaced 25 cm apart. Approximately 100 seeds were sown in each plot. The highly susceptible line, Tiaogan 601, was used as a susceptible check in Gansu and Beijing and was planted after every ten plots, and Mingxian 169 was used as a susceptible check in Sichuan. Infection rows of Tiaogan 601 or Mingxian 169 were planted perpendicular and adjacent to the test rows to ensure adequate inocula. Artificial inoculation was performed with the prevalent *Pst* race CYR32 at the three-leaf stage in the spring. Stripe rust severities were assessed for the first time 4 weeks after inoculation, and then at weekly intervals for two further weeks using the modified Cobb scale (Peterson et al. 1948) in Beijing and Gansu. In Sichuan, stripe rust severities were visually rated, when the disease severities on Mingxian 169 reached a maximum level around 20 April 2008.

Statistical analysis of variance was conducted by PROC GLM in the Statistical Analysis System (SAS Institute 1997), with genotype as a fixed effect, and environments, a combination of locations and years, and replicates as random effects. Broad-sense heritability (h^2) for stripe rust reaction was calculated using the formula $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_{ge}^2/e + \sigma_\varepsilon^2/re)$, where σ_g^2, σ_{ge}^2, and σ_ε^2 were estimates of genotypic, genotype × environment interaction and error variances, respectively, and e and r were the numbers of environments and replicates per environment, respectively. Phenotypic correlation coefficients between maximum disease severities (MDS) in different environments were calculated on a mean basis using the Microsoft Excel analytical tool.

Microsatellite marker analysis and gene sequencing

Genomic DNA was extracted from young leaves of the parents and F_3 lines (40–50 plants per F_3 line as a bulk) using the CTAB method (Sharp et al. 1988). Simple sequence repeat (SSR) markers were screened for polymorphism between the two parents by polyacrylamide gel electrophoresis. PCR and gel staining were conducted as described by Li et al. (2006) and Bassam et al. (1991). The SSR primers were from the GWM (Röder et al. 1998), BARC (developed by P. Cregan, Q. Song and associates at the USDA-ARS Beltsville Agricultural Research Center), WMC (developed by a team led by P. Isaac, IDnagenetics, Norwich, UK), and CFD (Guyomarc'h et al. 2002) marker series and one STS marker, *csLV34* (Lagudah et al. 2006). Resistant and susceptible bulks were established by mixing equal amounts of DNA from the five most resistant and the five most susceptible lines, respectively, based on the averaged stripe rust severity across environments. SSR markers showing polymorphisms between the resistant and susceptible bulks, as well as between the parents, were used to genotype 15–20 most resistant and most susceptible lines, respectively. Subsequently, the SSRs showing linkage with stripe rust resistance were used to genotype the entire population. Additional markers for enriching the chromosome regions linked to resistance genes were selected from published wheat consensus maps (http://www.shigen.nig.ac.jp/wheat; http://wheat.pw.usda.gov; Somers et al. 2004) and tested for polymorphisms between the parents and bulks. Those showing polymorphism were also used to genotype the population for linkage analysis. The PCR primers used for sequencing the *Yr18* gene in Libellula and Strampelli were kindly provided by Dr. Evans Lagudah, at CSIRO Plant Industry, Canberra, Australia. All the sequencings were performed by Beijing Augct Biological Technology Co., Ltd (http://www.augct.com) and Shanghai Sangon Biological Engineering Technology & Service Co., Ltd (http://www.sangon.com). Sequence alignments were performed using the software DNAMAN (http://www.lynnon.com).

QTL analysis

Quantitative trait loci mapping was based on the averaged MDS of three replicates in each environment, and also the averaged data across all environments. Linkage groups were established with the software Map Manager QTXb20 (Manly et al. 2001). Recombination values were converted to genetic distances using the Kosambi mapping function (Kosambi 1944). The positions of the detected QTLs were determined by composite interval mapping (CIM) using the software Cartographer 2.5 (Wang et al. 2005). A logarithm of odds (LOD) of 2.5 was set to declare QTL as significant. Each QTL was represented by a 20 cM interval with the local LOD maximum at its center. QTL with overlapping 20 cM intervals among different environments were considered as being in common. QTL effects were estimated as the proportion of phenotypic variance (R^2) explained by the QTL. Digenic interactions between non-allelic QTLs were analyzed by inclusive composite interval mapping (ICIM) method, using the software IciMapping 2.2 (Li et al. 2007a, 2008). The chromosomal assignments of the linkage groups were based on published wheat maps (Somers et al. 2004), and the Graingenes (http://wheat.pw.usda.gov) and Komugi integrated wheat consensus maps (http://www.shigen.nig.ac.jp/wheat).

Results

Distribution of MDS and correlation analysis

Libellula and Strampelli were susceptible to the prevalent races CYR31 and CYR32 of *Pst* at the seedling stage, but were highly resistant at the adult-plant stage, and they displayed a MDS of less than 10% in all environments, whereas the susceptible parent, Huixianhong, showed an MDS ranging from 30 to 80% across different environments (Fig. 1). This indicates typical APR to stripe rust in these two cultivars. Both populations exhibited a continuous distribution of stripe rust severities, indicating the polygenic characteristics of the slow rusting resistance.

Correlations for MDS in the Libellula/Huixianhong population ranged from 0.58 to 0.70 ($P < 0.0001$) among different environments, and the heritability of MDS was 0.95. Significant correlations ($r = 0.58$–0.74, $P < 0.0001$) for MDS were also detected in the Strampelli/Huixianhong population among different environments, and the heritability was 0.97. ANOVA of the two populations revealed significant differences ($P < 0.0001$) in MDS among lines

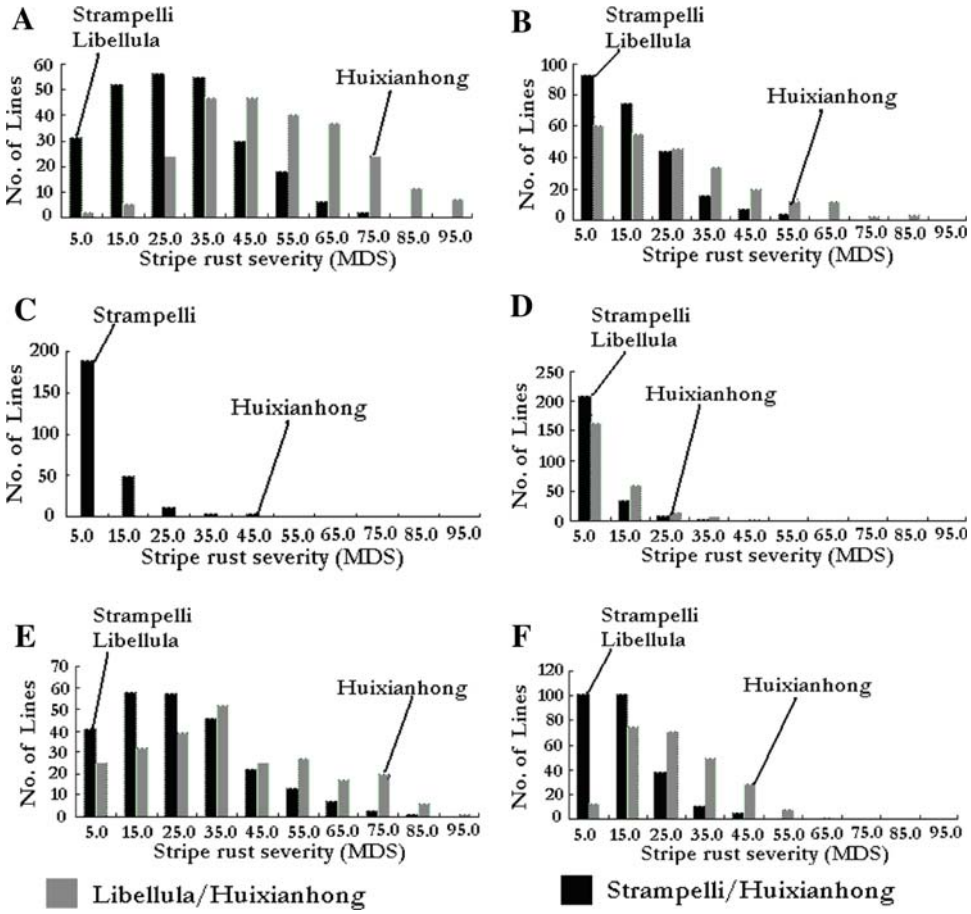

Fig. 1 Frequency distribution of stripe rust maximum disease severities (MDS) for F_3 populations from two wheat crosses in four or five environments. **a** Gansu 2005, **b** Gansu 2006, **c** Beijing 2006, **d** Gansu 2007, **e** Sichuan 2008, **f** Average MDS in four or five environments; *gray* and *black* columns indicate the Strampelli/Huixianhong and Libellula/Huixianhong populations, respectively

in the two populations. Highly significant differences ($P < 0.0001$) were also observed among different environments and for genotype × environment interactions (Table 1).

QTL analyses for APR to stripe rust

Libellula/Huixianhong population

A total of 943 SSR markers were screened for polymorphism between Libellula and Huixianhong. Of them, 133 showing polymorphisms between the two parents were used to test the resistant and susceptible bulks. Subsequently, 39 markers producing polymorphic bands between the two bulks were used to genotype the entire population. Based on the mean MDS in each environment and that averaged from all environments, five QTLs were detected on chromosomes 2DS, 4BL, 5BL (two QTLs) and 7DS (Table 2; Fig. 2).

The most consistent locus with the largest effect found in all environments was *QYr.caas-7DS*, located between *csLV34* and *Xgwm295* on the short arm of chromosome 7D. This QTL explained 28.2, 35.0, 26.8 and 14.6% of the phenotypic variances (R^2) in Gansu 2005, Gansu 2006, Gansu 2007 and Sichuan 2008, respectively (Table 2; Fig. 2e). R^2 was as high as 32.2% for the QTL computed by the averaged MDS of all environments. The locus with the second largest effect, *QYr.caas-2DS*, was located in the marker interval *Xcfd51–Xgwm261* on chromosome 2DS. This gene was detected in two environments as well as for the averaged MDS, explaining from 8.1 to 12.4% of the

Table 1 Analysis of variance of MDS scores for F_3 lines of crosses Libellula/Huixianhong and Strampelli/Huixianhong populations

Population	Source of variation	df	MS	*F* value
Libellula/ Huixianhong	Line	243	2,415	10.71**
	Environment	3	236,950	1,050.99**
	Replicate	2	3,043	13.50**
	Line × environment	729	398	1.76**
	Error	1,947	225	
Strampelli/ Huixianhong	Line	251	1,222	15.65**
	Environment	4	80,728	1,033.53**
	Replicate	2	1,504	19.26**
	Line × environment	990	182	2.33**
	Error	2,444	78	

** Significant at $P < 0.0001$

Table 2 Summary of QTLs for MDS to stripe rust detected by CIM in Libellula/Huixianhong population across four environments

Environment	QTL[a]	Marker interval	LOD	AE	R^2 (%)	Total R^2 (%)
Gansu 2005	*QYr.caas-4BL*	*Xgwm165–Xgwm149*	2.92	4.96	3.6	31.8
	QYr.caas-7DS	*csLV34–Xgwm295*	11.33	14.15	28.2	
Gansu 2006	*QYr.caas-2DS*	*Xcfd51–Xgwm261*	4.94	6.81	8.1	46.8
	QYr.caas-5BL.1	*Xwmc415–Xwmc537*	4.00	5.08	3.7	
	QYr.caas-7DS	*csLV34–Xgwm295*	15.17	15.36	35.0	
Gansu 2007	*QYr.caas-5BL.1*	*Xwmc415–Xwmc537*	3.95	1.96	3.4	30.2
	QYr.caas-7DS	*csLV34–Xgwm295*	9.06	5.41	26.8	
Sichuan 2008	*QYr.caas-2DS*	*Xcfd51–Xgwm261*	10.01	10.08	12.4	43.3
	QYr.caas-4BL	*Xgwm165–Xgwm149*	3.57	7.22	5.1	
	QYr.caas-5BL.1	*Xwmc415–Xwmc537*	9.35	9.54	8.6	
	QYr.caas-5BL.2	*Xbarc142–Xgwm604*	2.53	5.11	2.6	
	QYr.caas-7DS	*csLV34–Xgwm295*	8.20	11.64	14.6	
Average in four environments	*QYr.caas-2DS*	*Xcfd51–Xgwm261*	8.00	5.92	9.9	52.4
	QYr.caas-4BL	*Xgwm165–Xgwm149*	3.19	3.61	3.1	
	QYr.caas-5BL.1	*Xwmc415–Xwmc537*	8.19	5.53	7.2	
	QYr.caas-7DS	*csLV34–Xgwm295*	16.16	11.37	32.2	

For each QTL the corresponding marker interval, individual explained phenotypic variances R^2 (%), additive effect and LOD value are given

AE additive effect of resistance allele

[a] Only QTL with LOD > 2.5 are shown

phenotypic variance (Table 2; Fig. 2a). One QTL, *QYr.caas-4BL*, in the interval *Xgwm165–Xgwm149* on chromosome 4BL, explained from 3.1 to 5.1% of the phenotypic variances in two environments as well as the averaged data over four environments (Table 2; Fig. 2b). Two QTLs, *QYr.caas-5BL.1* and *QYr.caas-5BL.2*, identified on chromosome 5BL, explained 3.4–8.6 and 2.6% of the phenotypic variance, respectively (Table 2; Fig. 2c, d). All five QTLs for APR to stripe rust came from the resistant parent Libellula (Table 2). These QTLs accounted for 30.2–46.8% of the total phenotypic variance in a simultaneous fit across four environments (Table 2), suggesting a significant effect of the QTLs in reducing disease severity.

Strampelli/Huixianhong population

A total of 1,136 SSR markers were screened for polymorphism between Strampelli and Huixianhong. Thirty-four markers showing polymorphisms between the resistant and susceptible bulks were used to genotype the entire population. Based on the mean MDS in each environment and that averaged from five environments, three QTLs were identified on chromosomes 4BL, 5BL and 7DS (Table 3; Fig. 2). All three were located at similar chromosomal positions to those detected in the Libellula/Huixianhong population. Similarly, the largest and most consistent resistance locus was mapped on chromosome 7DS, again designated *QYr.caas-7DS*, explaining from 17.1 to 39.1% of the phenotypic variance across five environments (Table 3; Fig. 2h). The second QTL, *QYr.caas-5BL.1*, on chromosome 5BL in the interval *Xwmc415–Xwmc537* detected in two individual environments as well as the overall mean, explained 2.2–5.5% of the phenotypic variances (Table 3; Fig. 2g). The third QTL, *QYr.caas-4BL*, located on chromosome 4BL was detected only in Gansu 2006, explaining 4.5% of the phenotypic variance (Table 3; Fig. 2f). No QTL was detected on chromosome 2DS in this population, due either to the lack of polymorphic markers between the two parents in this region, or because of the absence of a QTL in this chromosome region in Strampelli. The total phenotypic variance explained by the three QTLs ranged from 17.1 to 43.6% in a simultaneous fit across five environments (Table 3).

Epistasis between non-allelic QTLs

Among the five QTLs for APR to stripe rust in Libellula/Huixianhong population, six significant interactions between different pairs of QTLs were detected in four environments, explaining from 3.2 to 7.1% of the phenotypic variance (Table 4). In Strampelli/Huixianhong population, three interactions between different pairs of QTLs

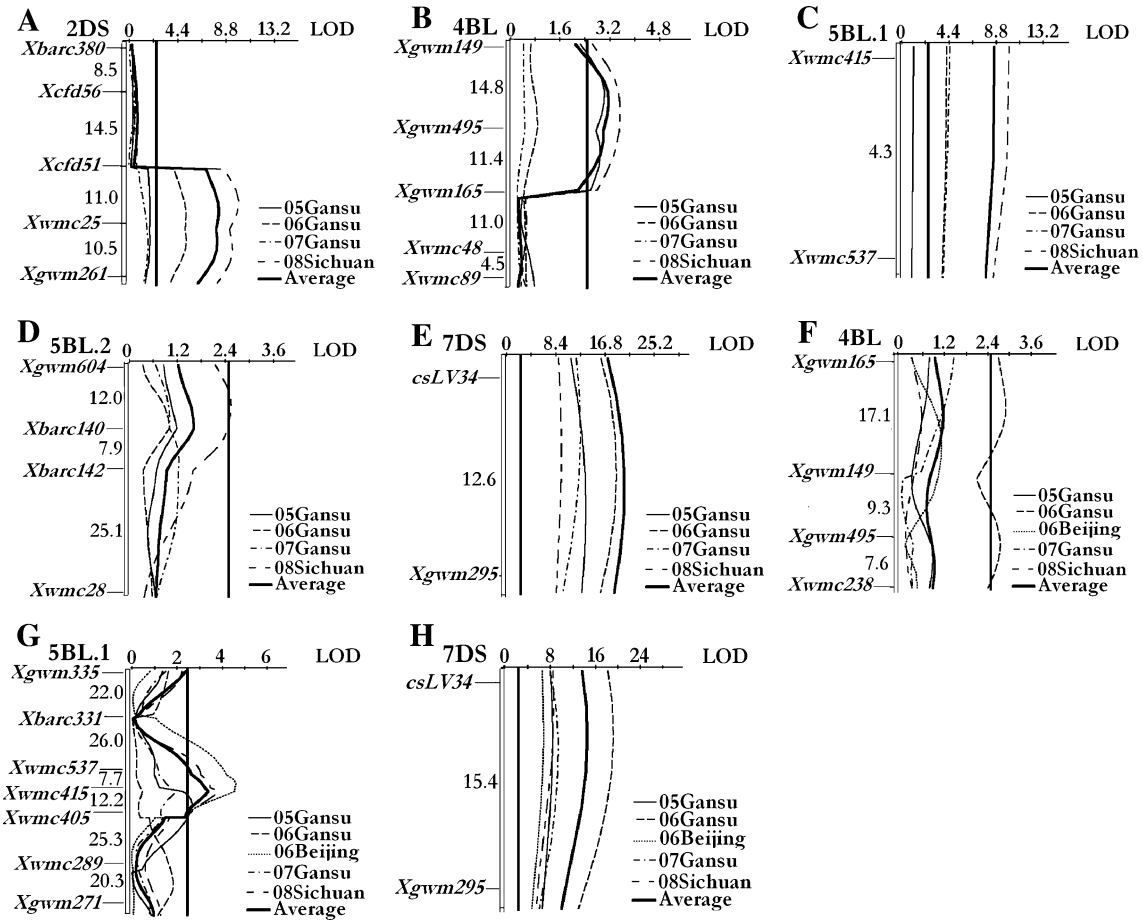

Fig. 2 Likelihood plots of QTL for stripe rust APR on chromosomes 2DS, 4BL, 5BL and 7DS identified by composite interval mapping in cross Libellula/Huixianhong (**a**–**e**), and on chromosomes 4BL, 5BL and 7DS in cross Strampelli/Huixianhong (**f**–**h**). The LOD threshold for significance is 2.5. Positions (in cM) of the molecular markers along chromosomes are shown on the *vertical axes*; genetic distances between markers are shown

Table 3 Summary of the QTLs for MDS to stripe rust detected by CIM in Strampelli/Huixianhong population across five environments

Environment	QTL[a]	Marker interval	LOD	AE	R^2 (%)	Total R^2 (%)
Gansu 2005	*QYr.caas-7DS*	*csLV34–Xgwm295*	8.46	8.68	17.1	17.1
Gansu 2006	*QYr.caas-4BL*	*Xgwm165–Xgwm149*	2.82	2.92	4.5	43.6
	QYr.caas-7DS	*csLV34–Xgwm295*	17.03	9.42	39.1	
Beijing 2006	*QYr.caas-5BL.1*	*Xwmc415–Xwmc537*	4.34	2.37	5.5	30.4
	QYr.caas-7DS	*csLV34–Xgwm295*	6.77	3.60	24.9	
Gansu 2007	*QYr.caas-7DS*	*csLV34–Xgwm295*	6.78	3.29	19.8	19.8
Sichuan 2008	*QYr.caas-5BL.1*	*Xwmc415–Xwmc537*	3.00	5.23	2.9	21.5
	QYr.caas-7DS	*csLV34–Xgwm295*	7.30	9.92	18.6	
Average in five environments	*QYr.caas-5BL.1*	*Xwmc415–Xwmc537*	2.54	2.12	2.2	30.5
	QYr.caas-7DS	*csLV34–Xgwm295*	13.07	6.91	28.3	

For each QTL the corresponding marker interval, individual explained phenotypic variances R^2 (%), additive effect and LOD value are given

AE additive effect of resistance allele

[a] Only QTL with LOD > 2.5 are shown

Table 4 Summary of significant (LOD > 2.5) epistatic interactions between pairs of QTLs in two F_3 populations of Libellula/Huixianhong and Strampelli/Huixianhong across different environments

Population	Environment	$QTL_1 \times QTL_2$	LOD	R^2 (%)[a]
Libellula/Huixianhong	Gansu 2005	*QYr.caas-2DS* × *QYr.caas-4BL*	2.81	4.6
	Gansu 2006	*QYr.caas-2DS* × *QYr.caas-4BL*	3.39	7.1
		QYr.caas-2DS × *QYr.caas-7DS*	4.17	6.5
		QYr.caas-4BL × *QYr.caas-7DS*	2.99	3.7
	Gansu 2007	*QYr.caas-2DS* × *QYr.caas-4BL*	3.07	4.5
		QYr.caas-2DS × *QYr.caas-5BL.2*	2.62	6.4
		QYr.caas-5BL.1 × *QYr.caas-7DS*	3.49	6.6
	Sichuan 2008	*QYr.caas-4BL* × *QYr.caas-5BL.2*	3.67	6.6
	Average in four environments	*QYr.caas-2DS* × *QYr.caas-4BL*	3.02	3.2
Strampelli/Huixianhong	Gansu 2005	*QYr.caas-4BL* × *QYr.caas-7DS*	2.64	6.1
		QYr.caas-5BL.1 × *QYr.caas-7DS*	3.20	6.6
	Gansu 2006	*QYr.caas-4BL* × *QYr.caas-5BL.1*	4.86	25.5
		QYr.caas-4BL × *QYr.caas-7DS*	13.15	35.0
		QYr.caas-5BL.1 × *QYr.caas-7DS*	11.55	31.6
	Beijing 2006	*QYr.caas-4BL* × *QYr.caas-5BL.1*	5.28	16.8
		QYr.caas-4BL × *QYr.caas-7DS*	4.70	8.5
		QYr.caas-5BL.1 × *QYr.caas-7DS*	6.41	10.0
	Gansu 2007	*QYr.caas-4BL* × *QYr.caas-5BL.1*	6.89	28.2
		QYr.caas-4BL × *QYr.caas-7DS*	11.50	30.5
		QYr.caas-5BL.1 × *QYr.caas-7DS*	17.43	32.9
	Sichuan 2008	*QYr.caas-4BL* × *QYr.caas-7DS*	5.85	10.9
	Average in five environments	*QYr.caas-4BL* × *QYr.caas-5BL.1*	4.69	18.1
		QYr.caas-4BL × *QYr.caas-7DS*	6.13	11.8
		QYr.caas-5BL.1 × *QYr.caas-7DS*	6.07	19.5

[a] R^2 is the percentage of phenotypic variance explained by the QTLs

were stably identified across five environments, explaining from 6.1 to 35.0% of the phenotypic variance.

Discussion

During the past decades, Italian wheat cultivars contributed greatly to Chinese wheat improvement and production (Zheng 1993). Libellula and Strampelli have been planted in China for over 30 years and showed effective APR to stripe rust. In the present study, we detected five QTLs for stripe rust resistance in Libellula and three in Strampelli. These results are in agreement with previous reports on the inheritance of APR, indicating that a few additive genes often conferred APR to stripe rust (Bariana et al. 2001; Boukhatem et al. 2002; Suenaga et al. 2003; Mallard et al. 2005; Rosewarne et al. 2008). Using conventional quantitative genetic analysis, Zhang et al. (2001) inferred that two to three stripe rust resistance genes were present in Libellula based on the frequency distribution of the area under the disease progress curve (AUDPC) of F_2 and F_3 lines in the cross of Libellula/Mingxian 169. Yin et al. (2005, 2006) concluded that at least two genes were involved in resistance to stripe rust in the same two cultivars, but their study was based on F_2 plant phenotypes. The greater number of resistance genes found in our study illustrates the advantage of using later generation materials and the greater power of QTL analysis in resolving individual gene (or gene region) effects.

In this study, three QTLs were common to both populations. This was likely because both resistant cultivars had a common ancestor. Libellula has the pedigree Tevere/Giuliari//San Pastore, and Strampelli came from Libero//San Pastore-14/Jacometti-49 (http://genbank.vurv.cz/wheat/pedigree). The common parental cultivar San Pastore was derived from Villa Glori/Balilla. Villa Glori was one of four cultivars selected from the cross Riete/Wilhelmina Tare//Akakomugi made by the Italian breeder Nazareno Strampelli in 1913. The other three cultivars were Ardito, Mentana and Damiano Chiesa (Borojevic and Borojevic 2005). Using diagnostic STS marker *csLV34*, Kolmer et al. (2008) traced the origin of the *Lr34/Yr18* rust resistance region in many current wheat cultivars to the Italian wheat cultivars Ardito and Mentana. In addition,

DNA sequencing revealed the presence of *Lr34/Yr18* gene in Libellula and Strampelli but not in Huixianhong, indicating that *QYr.caas-7DS* identified in Libellula and Strampelli is most likely *Yr18*. The *Lr34/Yr18* gene is an important slow rusting gene and can confer high levels of resistance when combined with other minor genes (Singh and Rajaram 1994; Navabi et al. 2004). Cultivars possessing *Yr18* have been widely used in CIMMYT germplasm (Singh et al. 2005). Chinese landraces may have a relatively high frequency of *Yr18* (Kolmer et al. 2008). Yang et al. (2008) screened 422 Chinese landraces with the marker *csLV34* and found 85.1% of them contained the specific allele for *Yr18*. Field test of the landraces indicated that most of the genotypes with the specific allele for *Yr18* showed moderate to high resistance to stripe rust (data not shown). Therefore, both Italian wheat cultivars and Chinese landraces can be important wheat germplasm with durable resistance gene *Yr18*.

Mallard et al. (2005) identified a QTL, *QYr.inra-5BL.1*, on chromosome 5BL in the French cultivar Camp Rémy within the marker interval *Xgwm499*–*Xgwm639*. This QTL explained 18–26% of the phenotypic variance for AUDPC. It is approximately 3 cM from the *QYr.caas-5BL.1* found in the present study based on the wheat consensus map (Somers et al. 2004). Suenaga et al. (2003) also reported a QTL for stripe rust severity on 5BL in the cultivar Oligoculm near marker locus *Xwmc415*, which falls within the interval carrying *QYr.caas-5BL.1* in our study.

We identified a second QTL, *QYr.caas-5BL.2*, on the telomeric region of chromosome 5BL. This gene was more than 40 cM from *QYr.caas-5BL.1* based on the wheat consensus map (Somers et al. 2004). Mallard et al. (2005) reported *QYr.inra-5BL.2* flanked by *Xgwm234* and *DuPw115a* in this region in the cross Camp Rémy/Récital. Because these two markers were consistently mapped on 5BS in several populations, those authors proposed that *QYr.inra-5BL.2* might be in a translocated region from chromosome 5BS of cultivar Cappelle-Desprez. In our study, the markers flanking the *QYr.caas-5BL.2* were located on 5BL based on several consensus wheat maps (http://www.shigen.nig.ac.jp/wheat/komugi/maps/markerMap.jsp; Somers et al. 2004), indicating no translocation happened in this chromosomal region in our population.

QYr.caas-4BL identified in both crosses examined in this study was in the marker interval *Xgwm165*–*Xgwm149*. William et al. (2006) identified a QTL on 4BL near marker *Xgwm495* in the cross Avocet S/Pavon 76. It was derived from Avocet *S* and reduced the stripe rust response by 7.4–12.7% over 3 years. This QTL coincided with the position of *QYr.caas-4BL* in Libellula and Strampelli. Suenaga et al. (2003) also reported a QTL for stripe rust severity in the cultivar Oligoculm. The LOD peak for this QTL, near *Xgwm538*, and was more than 15 cM away from *QYr.caas-4BL* identified in this study based on the Somers et al.'s (2004) consensus map.

Bariana et al. (2001) identified a QTL flanked by the loci *Xwmc111* and *Xwmc25* on chromosome 2DS for disease severity from the cultivar Katepwa. This QTL was also detected by Suenaga et al. (2003) in a Fukuho-Komugi/Oligoculm population, and was possibly mapped as *QYr.caas-2DS* in the present study. Mallard et al. (2005) identified *QYr.inra-2DS* from cultivar Camp Rémy on chromosome 2DS. However, according to the Somers et al. (2004) map, the distance between the peaks for this QTL and *QYr.caas-2DS* is more than 30 cM. Therefore, the QTL of Camp Rémy is likely to be different from *QYr.caas-2DS*.

It has been well known that disease-resistance genes in plant genomes frequently occur in clusters on particular chromosomes (McIntosh et al. 2003; Islam et al. 1989). For example, the resistance gene *Yrns-B1* was found to be at a similar position as the APR genes *Yr30* against stripe rust (Singh et al. 2000b) and *Sr2* against stem rust (Bariana et al. 1998; Spielmeyer et al. 2003). Similarly, the leaf rust resistance gene *Lr27* (Nelson et al. 1997), a QTL for Fusarium head blight resistance (Zhou et al. 2003b) and a QTL for leaf rust resistance (Börner et al. 2002) were also mapped on the same location. Therefore, although a few QTLs identified in this study were mapped on the similar chromosome regions to those of QTLs reported previously, the allelism among them still needs to be investigated. The similar chromosome locations of these QTLs indicate that they are either at one locus or closely linked loci.

Seedling resistance in Libellula was reported by Li et al. (2007b) in the cross Libellula/Mingxian 169 following inoculation with certain *Pst* races. Based on the infection types of F_1 and the segregation ratios of F_2 and BC_1 populations, they found one recessive gene in Libellula conferring resistance to races CYR22 and CYR25, and two genes giving resistance to races CYR30 and Su4. Thus, Libellula also has race-specific seedling resistance genes in addition to APR genes. The combination of APR and seedling resistance in a same genotype is not uncommon. Cultivars Stephens and Druchamp have both high-temperature APR and race-specific seedling resistances (Chen and Line 1995). Camp Rémy contained a major seedling resistance factor, *QYr.inra-2BL* (probably *Yr7*), together with five other QTLs responsible for APR (Mallard et al. 2005). Cultivar Express possessed two seedling resistance genes *YrExp1* and *YrExp2* as well as three QTLs effective at the adult-plant stage (Lin and Chen 2009). In order to avoid seedling resistance genes in the identification of APR, we used a race (CYR32) of *Pst* that was virulent on seedlings of both Libellula and Strampelli. CYR32 is a current predominant race in China. Clearly, the APR genes

identified in Libellula and Strampelli could be a valuable resource to control the disease, but may not be unique.

APR to stripe rust is conferred by the combined effects of several resistance loci (Singh and Rajaram 1994; Navabi et al. 2004; Singh et al. 2005), and the effects of some resistance genes may not be stable across different environments (Singh et al. 2000b; Suenaga et al. 2003; Rosewarne et al. 2008; Lin and Chen 2009). In our study, the effect of *QYr.caas-7DS* was very stable, suggesting that QTLs with major effect are most likely to be detected across environments (Tables 2, 3). Boukhatem et al. (2002) reported that QTLs that contributed less than 10% of the phenotypic variance were difficult to detect across years and environments. However, in the present study, both *QYr.caas-4BL* and *QYr.caas-5BL.1* were detected across different environments, in spite of the fact that they simply explained around 5% of phenotypic variance. Therefore, molecular markers *Xwmc165* and *Xwmc415*, closely linked to *QYr.caas-4BL* and *QYr.caas-5BL.1,* respectively, are likely to be useful for marker-assisted selection in wheat breeding programs.

Additive effects among APR genes have been reported in many studies (Singh and Rajaram 1994; Singh et al. 2000a, b, 2005; Suenaga et al. 2003; Navabi et al. 2004). However, epistasis among them was less stressed. In the present study, several significant epistasis interactions among pairs of APR epistatic QTLs were detected in both the Libellula/Huixianhong and Strampelli/Huixianhong populations, with relatively large effects across different environments (Table 4), this indicates that epistatic interactions among APR genes contribute to the overall resistance of these wheat lines to stripe rust. This is in agreement with a previous report indicating that epistasis often occurred among genes affecting complex traits (Carlborg and Haley 2004).

Previously, bulked segregant analysis (BSA) strategy was widely used for characterizing major resistance genes (Li et al. 2006; Lin and Chen 2007), and it was also employed to map the APR genes for stripe rust and powdery mildew in wheat (Liu et al. 2001; Lin and Chen 2007, 2009). Obviously, it is possible that additional minor QTLs segregating in the population are not detected using this approach, compared with a saturated linkage mapping strategy. However, it is much time consuming and resource intensive to construct a full linkage map of a population. To reduce the possibility of missing some QTLs using BSA method, the bulks were formed simply with the DNA from five resistant and five susceptible lines, respectively, in the present study. The polymorphic markers between the bulks were firstly tested with 15–20 resistant and susceptible lines, respectively, and then used to genotype the entire population. With this approach, five and three QTLs for APR to stripe rust were identified in the populations of Libellula/Huixianhong and Strampelli/Huixianhong, respectively. This was in agreement with the previous reports that the quantitative resistance was often associated with three to five QTLs (Young 1996; Singh et al. 2000a; Liu et al. 2001; Liang et al. 2006), although there are examples of several (>5) QTLs involved in quantitative resistance. In consideration of the possible limitations of BSA strategy in QTL mapping for APR genes, we also used two resistant and two susceptible bulks, each with the DNA from four to five lines, to map the QTLs in other populations (data not shown), to reduce the possibility of missing minor QTLs.

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