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ORIGINAL PAPER

QTL mapping of adult-plant resistances to stripe rust and leaf rust in Chinese wheat cultivar Bainong 64

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Abstract Stripe rust and leaf rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici* Erikss. and *P. triticina*, respectively, are devastating fungal diseases of common wheat (*Triticum aestivum* L.). Chinese wheat cultivar Bainong 64 has maintained acceptable adult-plant resistance (APR) to stripe rust, leaf rust and powdery mildew for more than 10 years. The aim of this study was to identify quantitative trait loci/locus (QTL) for resistance to the two rusts in a population of 179 doubled haploid (DH) lines derived from Bainong 64 × Jingshuang 16. The DH lines were planted in randomized complete blocks with three replicates at four locations. Stripe rust tests were conducted using a mixture of currently prevalent *P. striiformis* races, and leaf rust tests were performed with *P. triticina* race THTT. Leaf rust severities were scored two or three times, whereas maximum disease severities (MDS) were recorded for stripe rust. Using bulked segregant analysis (BSA) and simple sequence repeat (SSR) markers, five independent loci for APR to two rusts were detected. The QTL on chromosomes 1BL and 6BS contributed by Bainong 64 conferred resistance to both diseases. The loci identified on chromosomes 7AS and 4DL had minor effects on stripe rust response, whereas another locus, close to the centromere on chromosome 6BS, had a significant effect only on leaf rust response. The loci located on chromosomes 1BL and 4DL also had significant effects on powdery mildew response. These were located at the same positions as the *Yr29/Lr46* and *Yr46/Lr67* genes, respectively. The multiple disease resistance locus for APR on chromosome 6BS appears to be new. All three genes and their closely linked molecular markers could be used in breeding wheat cultivars with durable resistance to multiple diseases.

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Introduction

Stripe rust (or yellow rust, YR) and leaf rust (LR), caused by *Puccinia striiformis* Westend. f. sp. *tritici* Erikss. and *P. triticina*, respectively, are major foliar diseases of common wheat (*Triticum aestivum* L.) in many wheat-growing regions of the world. Rust epidemics are recurrent events

that cause significant grain yield losses and reduced quality (Samborski 1985; Line and Chen 1995). Yield losses caused by YR range from 10 to 70 % depending upon the cultivar, earliness of the initial infection, rate of disease development and duration of the disease (Chen 2005; Afzal et al. 2007), whereas, LR can cause yield losses of up to 40 % in favorable conditions (Knott 1989; Zhao et al. 2008). Although fungicides can provide adequate control of rusts, resistant cultivars are a more economic and effective approach to control these diseases, as it has no cost to growers and is environmentally friendly (Line 2002; Chen 2005).

Currently, at least 49 YR and 68 LR resistance loci are cataloged in wheat and assigned to specific chromosomes or chromosome arms (McIntosh et al. 2011; Herrera-Foessel et al. 2012). Most of these resistance genes are race-specific and are conferred by single or a few major genes (Kilpatrick 1975; Zhao et al. 2008; Lu et al. 2009). Race-specific resistance has been often used by wheat breeders because of its high level of effectiveness throughout the entire growth cycle of the crop. Unfortunately, it is readily overcome by mutation and/or selection in the pathogen population (Chen and Line 1995a, b; Carter et al. 2009). Currently, only a few named YR and LR resistance genes (including *Yr5*, *Yr10*, *Yr15* and *Yr24/Yr26*; *Lr9*, *Lr19*, *Lr24* and *Lr38*) are effective against prevalent Chinese *P. striiformis* and *P. triticina* races, respectively (Yang et al. 2003; Yuan et al. 2007). In contrast, non race-specific or adult-plant resistance (APR) is generally quantitatively inherited. This type of resistance is often characterized by lower frequencies of infections, longer latent periods, smaller uredinial size and less urediniospore production (Caldwell 1968; Chen and Line 1995a, Liang et al. 2006, Lu et al. 2009; Li et al. 2010). Although individual genes of this type do not confer adequate levels of resistance, combinations of four or five slow-rusting genes may confer near immunity (Singh et al. 2000; Herrera-Foessel et al. 2011).

To date, several important genes for slow rusting have been identified in wheat (Herrera-Foessel et al. 2011, 2012; Hiebert et al. 2010; Singh et al. 2011). Evidence suggests that *Yr18/Lr34* on chromosome 7DS (Suenaga et al. 2003; Lagudah et al. 2006) and *Yr29/Lr46* on 1BL (William et al. 2003) have been effective since the early twentieth century (Krattinger et al. 2009). Both loci also confer partial resistance to powdery mildew (PM) (Lillemo et al. 2008) and stem rust (SR) (Bhavani et al. 2011) and are associated with leaf tip necrosis (LTN) (Singh 1992; Rosewarne et al. 2006). *Yr18/Lr34* was cloned and shown to encode a putative ATP-binding cassette (ABC) transporter (Krattinger et al. 2009). Gene-based DNA markers derived from the sequence enable precise marker-assisted breeding (Lagudah et al. 2009). A third locus on 4DL contains *Yr46* (Herrera-Foessel et al. 2011), *Lr67* (Hiebert et al. 2010) and the recently named *Pm46* (McIntosh et al. 2012). These genes can be utilized in combination with other slow-rusting or slow-mildewing genes to develop high levels of durable APR to YR, LR and PM. A fourth slow-rusting resistance gene, *Lr68*, located on chromosome 7BL of CIMMYT cv. Parula, is also available for breeding durable and stable APR to LR (Herrera-Foessel et al. 2012). This gene is likely to be widely distributed in CIMMYT spring bread wheat germplasm (Singh et al. 2011).

Bainong 64 was a leading winter wheat cultivar in the Yellow-Huai wheat region of China at the end of 1990s and beginning of 2000s, and it occupied about 700,000 ha on average annually for 8 years since its release in Henan Province in 1998 (Wang et al. 2005b, 2006). This cultivar continues to exhibit resistance to YR, LR and PM in the field. Because it is susceptible to Chinese *P. striiformis* race CYR32, *P. triticina* race THTT and *Blumeria graminis* f. sp. *tritici* isolate E20 at the seedling stage, it carries APR to these three diseases. Although Lan et al. (2009) conducted an analysis of APR to PM in Bainong 64, little is known about the genetics of resistance to YR and LR in this cultivar. The aim of the present study was to detect genetic loci for resistance to YR and LR in a doubled haploid (DH) population derived from a Bainong 64 × Jingshuang 16 cross.

Materials and methods

Plant materials

A DH population of 179 lines was developed from Bainong 64 × Jingshuang 16 by the wheat × maize method. Bainong 64 was derived from the cross Bainong 8717/3/Yeda 72-629-52/Shi 82-5594//Bainong 84-4046-1. Jingshuang 16 (Lovrin 10 × Youmanghong 7), released in Beijing in 1985 (Wang et al. 2006), is susceptible to both YR and LR at seedlings.

Field trials

Bainong 64, Jingshuang 16 and 179 DH lines were evaluated for YR response in Tianshui, Gansu Province, and Chengdu, Sichuan Province, during the 2009–2010 and 2010–2011 cropping seasons. They were also evaluated for LR in Baoding, Hebei Province, and Zhoukou, Henan Province, in 2010–2011. The population was planted in randomized complete blocks with three replicates at each location. Trials were managed according to local practices in the respective regions.

YR tests

Both Tianshui and Chengdu are hotspots for YR in China and experience severe epidemics almost every year. Plots

consisted of single rows in 1.5 m length and 25 cm between rows. Approximately 50 seeds were sown in each row. The highly susceptible control Mingxian 169 was planted every tenth row and also perpendicular and adjacent to the test rows to ensure ample inoculum. Inoculations were carried out using mixtures of *P. striiformis* races CYR31, CYR32, CYR33, Shui4, Shui6, Hy6 and Hy7 in Chengdu around January 3, and mixtures of *P. striiformis* races CYR29, CYR31, CYR32, CYR33, Shui4, Shui5 and Hy8 were used for inoculation in Tianshui around April 20. The maximum disease severities (MDS) were assessed (Peterson et al. 1948) when the disease severities on Mingxian 169 reached a maximum level around the 15th of April in Chengdu and the 10th of June in Tianshui, respectively.

LR tests

Leaf rust was tested in Baoding and Zhoukou with ideal conditions for rust infection and spread. Fifty seeds of each line were sown in single-row plots of 1.5 m length and 30 cm between rows. Spreader rows of Zhengzhou 5,389 were planted perpendicular and adjacent to the test rows. LR epidemics were initiated by spraying aqueous suspensions of urediniospores of *P. triticina* pathotype THTT, to which a few drops of Tween 20 (0.03 %) were added, onto the spreader rows at the tillering stage. Disease severities were assessed two or three times at weekly intervals with the first scoring 4 weeks after inoculation based on the modified Cobb scale (Peterson et al. 1948). Areas under the disease progress curve (AUDPC) were calculated according to Bjarko and Line (1988).

Statistical analysis

Analysis of variance was performed with PROC GLM in the Statistical Analysis System (SAS Institute, V8), with genotype as a fixed effect, and environments, a combination of locations and years and replicates as random effects. The information in the ANOVA table was used to calculate the broad sense heritabilities (h_b^2) of resistance to the two diseases reactions based on the formula $h_b^2 = \sigma_g^2/(\sigma_g^2 + \sigma_{ge}^2/e + \sigma_\varepsilon^2/re)$ (Allard 1960), where σ_g^2 is $(MS_f–MS_{fe})/re$, σ_{ge}^2 is $(MS_{fe}–MS_e)/r$ and σ_ε^2 is MS_e; in this formula, σ_g^2 = genetic variance, σ_{ge}^2 = genotype × environment interaction variance, σ_ε^2 = error variance, MS_f is mean square of genotype, MS_{fe} is mean square of genotype × environment interaction, MS_e = mean square of error, r is number of replicates and e is number of environments. Field data from Tianshui in 2011 were excluded from the statistical analysis and QTL detection due to the low YR development caused by the dry weather condition in the spring.

SSR marker assay and bulked segregant analysis

The parental lines Bainong 64 and Jingshuang 16 and the contrasting bulk for powdery mildew were screened for polymorphism with 406 simple sequence repeat (SSR) markers by Lan et al. (2009). Then 375 more SSR markers were used for screening the two parents to enable more saturated linkage maps in the present study; these included BARC (Song et al. 2002), CFA and CFD (Sourdille et al. 2004), GDM (Pestsova et al. 2000), GWM (Röder et al. 1998) and WMC (Gupta et al. 2002) markers. Based on results of 2 years of field data for YR, equal amounts of DNA from the five most resistant and five most susceptible lines, respectively, were mixed to form resistant and susceptible bulks. SSR markers that showed similar patterns of polymorphism between the bulks and parents were used to genotype individual lines in the population. Additional SSRs around the QTL for resistance to YR or LR were also selected to genotype the DH lines based on several wheat consensus maps (Somers et al. 2004; http://wheat.pw.usda.gov).

Map construction and QTL analysis

Linkage groups were constructed using Map Manager QTX20 (Manly et al. 2001). Genetic distances between markers were calculated based on the Kosambi mapping function (Kosambi 1944). The information on a publicly available wheat consensus map (Somers et al. 2004) was used to assign linkage groups to chromosomes. Cartographer 2.5 was used to detect QTL by composite interval mapping (CIM) (Wang et al. 2005a). A logarithm of odds (LOD) threshold of 2.0 was set to declare QTL as significant. A walk speed of 2.0 cM was chosen for all QTL detections. QTL effects were estimated as the proportion of phenotypic variance (R^2) explained by the QTL.

Results

Phenotypic evaluation

Stripe rust and LR developed well across environments, except for YR at Tianshui in 2011. The frequency distributions of YR MDS for the 179 DH lines in three environments and LR response (MDS and AUDPC) of the DH lines in two environments revealed continuous distributions (Supplementary Figs. 1 and 2), indicating polygenic inheritance. For YR, the averaged MDS of the DH lines across three environments over 2 years was 35.4 %, ranging from 3.7 to 86.1 %. Bainong 64 was rated with a mean MDS of 8.3, 35.0 and 8.7 % in Chengdu 2010, Chengdu 2011 and Tianshui 2010, respectively, whereas Jingshuang

16 had mean MDS of 12.7, 57.5 and 27.5 % in three environments, respectively. For LR, the mean MDS of Bainong 64 and Jingshuang 16 were 56.7 and 58.3 % in Baoding 2011, respectively, whereas their mean MDS were 13.3 and 18.3 % in Zhoukou 2011, respectively. The averaged MDS of the DH lines in Baoding 2011 was 41.0 % ranging from 2.3 to 85 %, and 18.3 % in Zhoukou ranging from 2.3 to 53.3 %. In addition, the averaged AUDPC of the DH lines across two environments were 302.6, ranging from 42.3 to 675.0.

Stripe rust MDS were significantly correlated among three environments, with correlation coefficients of 0.50–0.57 ($P < 0.0001$), and the heritability of YR MDS was 0.76. Significant correlations for LR MDS or AUDPC were also detected in two environments ($r = 0.57$ and 0.64, respectively, $P < 0.0001$), and the heritabilities of LR MDS and AUDPC were 0.63 and 0.52, respectively. Furthermore, the LR MDS and LR AUDPC were significantly correlated in Baoding 2011 and Zhoukou 2011 ($r = 0.95$ and 0.97, respectively, $P < 0.0001$). ANOVA of the two traits revealed significant differences ($P = 0.01$) in MDS and AUDPC among RILs, environments, replicates within environments and line × environment interactions (Table 1).

QTL for YR resistance

Three QTL for resistance to YR were identified on chromosomes 4DL, 6BS and 7AS based on CIM using the MDS in Chengdu 2010, Chengdu 2011 and Tianshui 2010 and averaged MDS from all three environments (Fig. 1; Table 2). They were designated *QYr.caas-4DL*, *QYr.caas-6BS.3* and *QYr.caas-7AS*, respectively. *QYr.caas-6BS.3*, flanked by *Xwmc487-Xcfd13* and located in the telomeric region, explained from 3.8 to 6.2 % of the phenotypic variance. The resistance allele of this QTL was contributed by Bainong 64. *QYr.caas-4DL* was located between *Xwmc331* and *Xgwm165*. This QTL was detected in Chengdu 2011 and explained 8.0 % of the phenotypic variance. It also came from Bainong 64. The third QTL, *QYr.caas-7AS*, was identified between *Xbarc127* and *Xbarc174* and explained 6.0 and 6.1 % of the phenotypic variances in Chengdu 2011 and the averaged MDS, respectively. This gene came from Jingshuang 16.

QTL for LR resistance

Based on the mean MDS in Baoding 2011, Zhoukou 2011 and MDS averaged from the two environments, three QTL for resistance to LR were detected on chromosomes 1BL and 6BS (2 QTL) (Fig. 2; Table 2). The most stable locus with the largest effect across environments was *QLr.caas-1BL*, located on 1BL between *Xgwm153.2* and *Xwmc44*. This QTL explained 15.6, 14.9 and 21.1 % of the phenotypic variance in Baoding 2011, Zhoukou 2011 and averaged MDS, respectively. *QLr.caas-6BS.1,* flanked by *Xwmc487* and *Xcfd13*, explained 11.2 and 10.0 % of the phenotypic variance in Baoding 2011 and averaged MDS, respectively. The third QTL, *QLr.caas-6BS.2*, in the interval *Xgwm518-Xwmc398* close to the centromere, was approximately 22 cM from *QLr.caas-6BS.1* based on the wheat consensus map (Somers et al. 2004). This QTL explained from 9.0 to 9.7 % of the phenotypic variance

Table 1 Analysis of variance of maximum disease severities (MDS) and the area under the disease progress curve (AUDPC) values for YR and LR responses on doubled-haploid (DH) lines derived from Bainong 64 × Jingshuang 16

Phenotype	Source of variance	*df*	Mean square	*F* values
YR	MDS			
	Lines	178	3,101	4.2**
	Environments	2	222,737	304.3**
	Replicates	2	5,578	31.3**
	Lines × environments	356	732	4.1**
	Error	1,071	178	
LR	MDS			
	Lines	178	1,065	2.7**
	Environments	1	138,835	348.8**
	Replicates	2	870	5.5**
	Lines × environments	178	398	2.5**
	Error	714	158	
	AUDPC			
	Lines	178	112,680	2.1**
	Environments	1	34,221,842	629.1**
	Replicates	2	412,481	37.0**
	Lines × environments	178	54,396	4.9**
	Error	714	11,142	

** Significant at $P = 0.01$

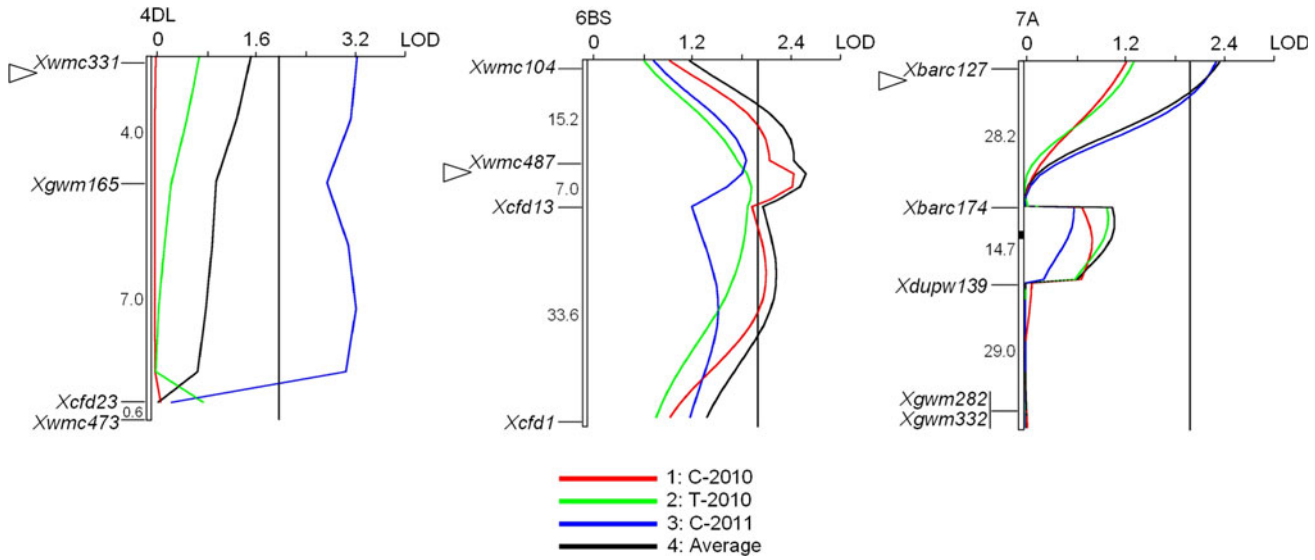

Fig. 1 LOD contours obtained by CIM analysis for QTL on chromosomes 4DL, 6BS and 7AS affecting YR MDS in Bainong 64 × Jingshuang 16 DH lines. The approximate positions of centromeres are indicated by *solid squares* in the *vertical axis*. Genetic distances are shown in centiMorgans to the *left* of vertical axis. The approximate positions of the QTL are indicated by *arrowheads* to the *left* of markers. LOD thresholds of 2.0 are indicated by a *dashed vertical line* in *graphs*. *C-2010* and *C-2011* MDS in Chengdu 2010 and 2011, respectively, and *T-2010* MDS in Tianshui 2010. *Average* average of MDS across three environments

across environments. *QLr.caas-1BL* and *QLr.caas-6BS.1* were contributed by Bainong 64, whereas *QLr.caas-6BS.2* was from Jingshuang 16.

The three loci for LR resistance were also detected using the mean AUDPC in each environment and when averaged from both environments (Fig. 2, Table 2). *QLr.caas-1BL*, derived from Bainong 64, explained 26.4, 14.4 and 28.5 % of the phenotypic variance in Baoding 2011, Zhoukou 2011 and the averaged AUDPC. *QLr.caas-6BS.1* from Bainong 64 explained 11.4 and 10.8 % of the phenotypic variance in Baoding 2011 and the overall mean, respectively. The third locus, *QLr.caas-6BS.2*, was contributed by Jingshuang 16 and explained 8.2–8.7 % of the phenotypic variance.

Pleiotropic effects of detected QTL

The same population of Bainong 64 × Jingshuang 16 was earlier used for QTL mapping of PM resistance and four QTL for APR to PM were identified on chromosomes 1A, 4DL, 6BS and 7A (Lan et al. 2009). In comparison with that study, *QYr.caas-4DL* co-located with a QTL for PM resistance with the allele for resistance contributed by Bainong 64. Therefore, *QYr.caas-4DL* may be used in breeding for resistance to both YR and PM. Based on the same study *QYr.caas-7AS* and *QLr.caas-6BS.2* also co-located to similar positions to PM resistance QTL, but with resistance contributed by opposite parents. The parental lines and two bulks had been screened with 406 SSR markers by Lan et al. (2009), and in subsequent QTL mapping 73 SSR markers were used to genotype all DH lines. However, in the present study, a total of 99 SSR markers were used to genotype individual lines after detection of polymorphisms between the two parents and contrasting bulks. Because more markers were selected to genotype the DH lines in the present study, we identified a new PM gene (*QPm.caas-1BL*) in Bainong 64 based on CIM using the PM data from Lan et al. (2009). This QTL was located on chromosome 1BL between *Xgwm153.2* and *Xwmc44* and corresponded with a LR resistance QTL from the same parent (Table 2; Fig. 3). Thus, the gene located on chromosome 1BL could be used to simultaneously improve resistance to LR and PM. Furthermore, *QYr.caas-6BS.3* with YR resistance contributed by Bainong 64 coincided with a LR resistance QTL from the same parent. *QYr.caas-6BS.3/QLr.caas-6BS.1* is therefore a valuable gene for resistance to both YR and LR.

Discussion

Leaf rust MDS was significantly associated with LR AUDPC across environments in this study ($r = 0.95$–0.97, $P < 0.0001$). This is in agreement with previous reports (Wang et al. 2005b; Liang et al. 2006; Lan et al. 2009) indicating that it is feasible to replace AUDPC with MDS. In addition, the use of MDS reduces the labor and time for field investigations, as it is only assessed once when the disease severities on susceptible controls reach maximum levels.

In the present study, all QTL for resistance to YR, LR and PM in the Bainong 64 × Jingshuang 16 population

Table 2 Quantitative trait loci (QTL) for adult-plant resistance (APR) to YR, LR and PM detected by composite interval mapping (CIM) in the Bainong 64 × Jingshuang 16 DH population across environments

	Location and year	QTL[a]	Marker interval	LOD[b]	Add[c]	R^2 (%)[d]
YR	MDS					
	Chengdu 2010	*QYr.caas-6BS.3*	*Xwmc487-Xcfd13*	2.4	4.8	6.2
	Chengdu 2011	*QYr.caas-4DL*	*Xwmc331-Xgwm165*	3.3	8.3	8.0
		QYr.caas-6BS.3	*Xwmc487-Xcfd13*	1.9	5.8	3.8
		QYr.caas-7AS	*Xbarc127-Xbarc174*	2.3	−7.0	6.0
	Tianshui 2010	*QYr.caas-6BS.3*	*Xwmc487-Xcfd13*	1.9	5.6	4.5
	Averaged MDS in 2 environments	*QYr.caas-6BS.3*	*Xwmc487-Xcfd13*	2.6	5.2	6.1
		QYr.caas-7AS	*Xbarc127-Xbarc174*	2.4	−5.0	6.1
LR	MDS					
	Baoding 2011	*QLr.caas-1BL*	*Xgwm153.2-Xwmc44*	5.3	8.7	15.6
		QLr.caas-6BS.1	*Xwmc487-Xcfd13*	5.0	7.9	11.2
		QLr.caas-6BS.2	*Xgwm518-Xwmc398*	3.7	−6.7	9.7
	Zhoukou 2011	*QLr.caas-1BL*	*Xgwm153.2-Xwmc44*	5.4	4.2	14.9
	Averaged MDS in 2 environments	*QLr.caas-1BL*	*Xgwm153.2-Xwmc44*	7.1	6.8	21.1
		QLr.caas-6BS.1	*Xwmc487-Xcfd13*	4.6	5.0	10.0
		QLr.caas-6BS.2	*Xgwm518-Xwmc398*	3.3	−4.4	9.0
	AUDPC					
	Baoding 2011	*QLr.caas-1BL*	*Xgwm153.2-Xwmc44*	8.1	130.7	26.4
		QLr.caas-6BS.1	*Xwmc487-Xcfd13*	5.5	91.6	11.4
		QLr.caas-6BS.2	*Xgwm518-Xwmc398*	3.2	−72.6	8.7
	Zhoukou 2011	*QLr.caas-1BL*	*Xgwm153.2-Xwmc44*	4.6	27.7	14.4
	Averaged AUDPC in 2 environments	*QLr.caas-1BL*	*Xgwm153.2-Xwmc44*	8.8	82.0	28.5
		QLr.caas-6BS.1	*Xwmc487-Xcfd13*	5.2	54.2	10.8
		QLr.caas-6BS.2	*Xgwm518-Xwmc398*	3.1	−42.7	8.2
PM[e]	Averaged MDS	*QPM.caas-1BL*	*Xgwm153.2-Xwmc44*	2.4	2.4	5.7

[a] QTL were detected with a minimum LOD score of 2.0 in at least one environment

[b] LOD, logarithm of odds score

[c] Add, additive effect of resistance allele

[d] R^2, percentages of the phenotypic variance explained by individual QTL

[e] Four QTL for APR to PM were identified in Bainong 64 by Lan et al. (2009); in addition, *QPm.caas-1BL* contributed by Bainong 64 was detected in the present study

were integrated into linkage maps. As shown in Fig. 3, linkage groups on chromosomes 1BL, 4DL and 6BS showed significant associations of resistance with two or three diseases. Lillemo et al. (2008) concluded that resistance to YR, LR and PM might be under common genetic control as in the present study. Two loci located on chromosomes 1BL and 4DL were in the same position as the *Yr29/Lr46* and *Yr46/Lr67* loci, respectively, and we report here a new resistance locus conferring APR to YR and LR on chromosome 6BS.

Yr29/Lr46 located at the distal region of chromosome 1BL with significant effects on response to YR and LR was detected in several mapping populations (Suenaga et al. 2003; Rosewarne et al. 2006; William et al. 2003, 2006). However, Zhang et al. (2009) suggested that *Lr46* was significantly affected by environment. Lillemo et al. (2008) conducted QTL mapping for resistance to PM, LR and YR in the same population and found that *Yr29/Lr46* was linked with a major PM resistance QTL (designated as *Pm39*) in wheat line Saar. In the present study, the allele on 1BL derived from Bainong 64 significantly reduced LR and PM severities, but the effect on YR response was very weak. This was likely due to the different expression levels of QTL in different genetic backgrounds and environments. Considering that the LOD peak for *QLr.caas-1BL*, near *Xwmc44*, was in the same position as *Yr29/Lr46*, these two genes might be either at the same locus or very closely linked. The slow-rusting gene *Yr29/Lr46* has provided APR to YR and LR for almost 30 years (William et al. 2006) and is widely distributed in CIMMYT germplasm (Singh et al. 2005). Because the linked markers for *Yr29/Lr46* are often population-specific or parent related, the molecular

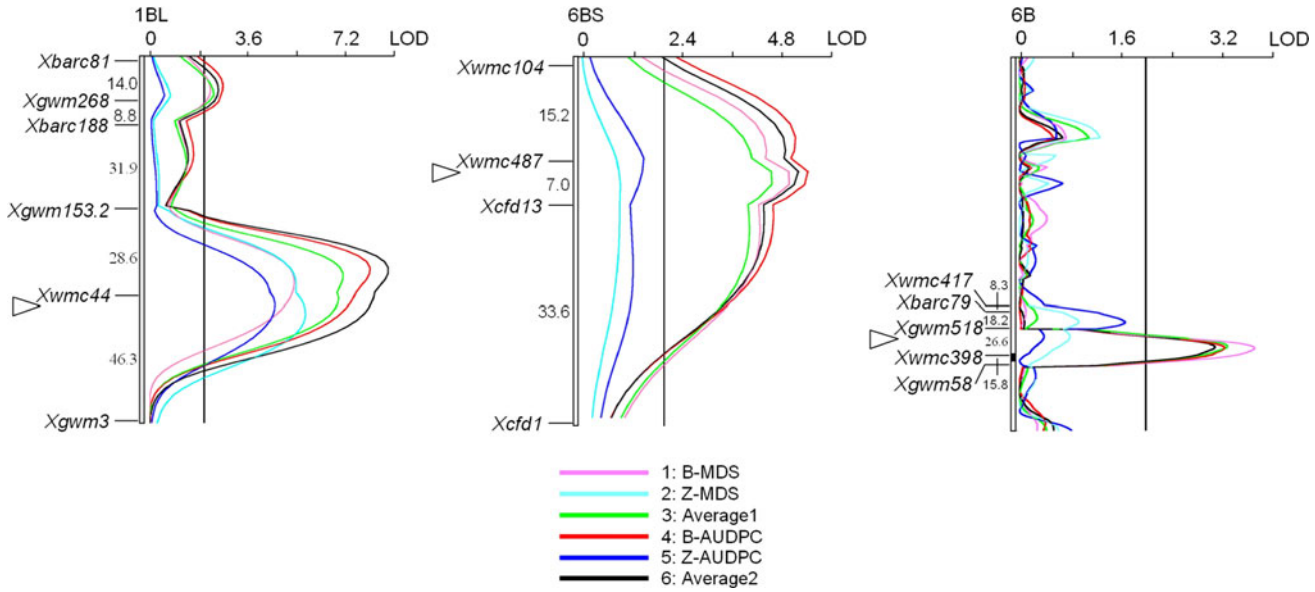

Fig. 2 LOD contours obtained by CIM analysis for QTL on chromosomes 1BL and 6BS (2 QTL) affecting LR MDS and AUDPC in Bainong 64 × Jingshuang 16 DH lines. The *short arms* are toward the *top* and the approximate positions of centromeres are indicated by *solid squares* in the *vertical axis*. Genetic distances are shown in centiMorgans to the *left* of vertical axis. The *arrowheads* indicate the likely positions of the QTL based on LR MDS and AUDPC. LOD thresholds of 2.0 are indicated by a *dashed vertical line in graphs*. *B-MDS* MDS in Baoding 2011, *Z-MDS* MDS in Zhoukou 2011 and *Average 1* averaged MDS across two environments; *B-AUDPC* AUDPC in Baoding 2011, *Z-AUDPC* AUDPC in Zhoukou 2011 and *Average 2* averaged AUDPC across two environments

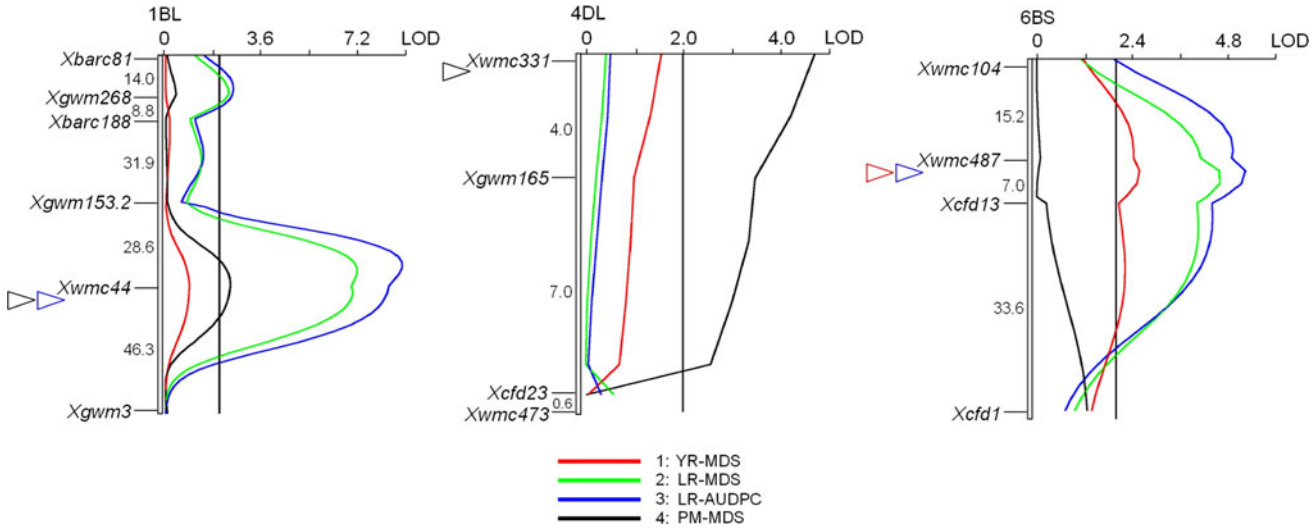

Fig. 3 LOD contours obtained by CIM analysis for QTL associated with averaged YR MDS, LR MDS, LR AUDPC and PM MDS in the Bainong 64 × Jingshuang 16 population. Genetic distances are shown in centiMorgans to the *left* of *vertical axis*. The *red*, *blue* and *black arrowheads* indicate the likely positions of the QTL based on YR MDS, LR MDS and AUDPC and PM MDS, respectively. LOD thresholds of 2.0 are indicated by a *dashed vertical line in graphs*. *YR-MDS* averaged YR MDS across three environments (Tianshui 2010, Chengdu 2010, 2011), *LR-MDS* and *LR-AUDPC* averaged LR MDS and AUDPC across two environments (Baoding 2011 and Zhoukou 2011), respectively, and *PM-MDS* averaged PM MDS across three environments (Anyang 2006, Beijing 2006 and 2008, Lan et al. 2009)

detection of this gene in some wheat genotypes might be difficult. The QTL in this report and its closely linked marker may be helpful for selecting *Yr29/Lr46* in Chinese wheat germplasm.

Suenaga et al. (2003) reported a QTL for YR resistance closely linked to *Xwmc399* on chromosome 4DL in Israeli wheat Oligoculm. It accounted for low levels of phenotypic variance ranging from 2.5 to 8.0 %. This QTL was

approximately 26 cM from *QYr.caas-4DL* (Suenaga et al. 2003; He et al. 2011) indicating that it is probably different from *QYr.caas-4DL*. A recently reported multiple disease resistance locus on chromosome 4DL confers APR to YR (*Yr46*), LR (*Lr67*) and PM (*Pm46*) (Hiebert et al. 2010; Herrera-Foessel et al. 2011; McIntosh et al. 2012). The APR gene *Yr46/Lr67/Pm46*, closely linked to *Xgwm165* and *Xgwm192*, was located in a similar position to *QYr.caas-4DL* based on the consensus map of Somers et al. (2004). However, *QYr.caas-4DL* in Bainong 64 was co-located only with a PM resistance QTL *QPm.caas-4DL* (Lan et al. 2009) and had no effect on LR response. This is probably due to the relatively lower heritability of the LR data compared with YR. Further study is needed to test the allelism between *QYr.caas-4DL* and *Yr46/Lr67* to confirm whether they are at the same locus.

To date, a total of six YR APR QTL on chromosome 6BS have been reported, including *QYr.jirc-6B* in Oligoculm (Suenaga et al. 2003), *QYrst.wgp-6BS.1* and *QYrst.wgp-6BS.2* in Stephens (Santra et al. 2008), *Yr36* in wild emmer (*T. turgidum* ssp. *dicoccoides* accession FA15-3) (Fu et al. 2009), *QYr.inra-6B* in Renan (Dedryver et al. 2009) and *QYr.caas-6BS* in Pingyuan 50 (Lan et al. 2010). One of these QTL, *QYrst.wgp-6BS.2* (Santra et al. 2008), flanked by *Xgwm132* and *Xgdm113*, was located in the same region as *QYr.caas-6BS.3* based on the consensus map (Somers et al. 2004). Pedigree analyses showed no relationship between Bainong 64 and Stephens (http://genbank.vurv.cz/wheat/pedigree/pedigree.asp). To date, no LR APR genes were located on chromosome 6BS. In the present study, we detected two LR APR QTL on chromosome 6BS, with one being contributed by each parent. Both QTL are possibly new. *QLr.caas-6BS.1* contributed by Bainong 64 was also associated with *QYr.caas-6BS.3* from the same parent.

QYr.caas-7AS in Jingshuang 16 was close to centromere. Although this QTL was significant only in Chengdu 2011 and averaged MDS, the LOD curves indicate it also had effects on YR response in other environments (Fig. 1). Dedryver et al. (2009) identified an APR QTL on chromosome 7A in wheat cultivar Récital, designated *QYr.inra-7A*. This QTL was located between AFLP markers *Xbcd129b* and *Xfba127c*. Because of different kinds of flanking markers used in these studies, the relationship between *QYr.inra-7A* and *QYr.caas-7AS* can not be determined.

Previous studies indicate that slow-rusting resistance is controlled by genetic factors with moderate heritability and generally with additive gene action or interactions (Bjarko and Line 1988; William et al. 2006). In the present study all five loci for APR to YR or LR showed additive effects (Table 2), whereas interactions among different additive QTL were not identified across environments using IciMapping V3.1 (Li et al. 2008).

Several loci that have effects on multiple disease responses were detected in the present study. The first locus located on chromosome 1BL, at a same chromosome region to *Yr29/Lr46*, showed significant effects on response to LR and PM. The second locus located on 4DL and conferring significant effects on both PM and YR responses was possibly at the same locus as *Yr46/Lr67*. A new multiple resistance locus was detected on chromosome 6BS with significant effects on both YR and LR. These three multiple resistance QTL in Bainong 64, and their corresponding closely linked molecular markers, *Xwmc44*, *Xwmc331* and *Xwmc487*, will be useful for marker-assisted selection in breeding for resistance to YR, LR and PM. *QLr.caas-6BS.2* is likely a new APR gene for resistance to leaf rust. This QTL and its flanking markers might serve to diversify the genetic basis of APR to LR and to accelerate the breeding process.

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