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ORIGINAL PAPER

QTL mapping of adult-plant resistance to leaf rust in a RIL population derived from a cross of wheat cultivars Shanghai 3/Catbird and Naxos

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Abstract

Key message **Six QTL for adult plant resistance to leaf rust, including two QTL effective against additional diseases, were identified in a RIL population derived from a cross between Shanghai 3/Catbird and Naxos.**

Abstract Leaf rust is an important wheat disease and utilization of adult-plant resistance (APR) may be the best approach to achieve long-term protection from the disease. The CIMMYT spring wheat line Shanghai 3/Catbird (SHA3/CBRD) showed a high level of APR to Chinese *Puccinia triticina* pathotypes in the field. To identify APR genes in this line, a mapping population of 164 recombinant inbred lines (RILs) was developed from a cross of this line and Naxos, a moderately susceptible German cultivar. The RILs were evaluated for final disease severity (FDS) at Baoding, Hebei province, and Zhoukou, Henan province, in the 2010–2011 and 2011–2012 cropping seasons. QTL analysis detected one major QTL derived from SHA3/CBRD on chromosome 2BS explaining from 15 to 37 % of the phenotypic variance across environments. In addition one minor resistance QTL on chromosome 1AL from SHA3/CBRD and four minor QTL from Naxos on chromosomes 2DL, 5B, 7BS, and 7DS were also detected. SHA3/CBRD also possessed seedling resistance gene *Lr26*, and Naxos contained *Lr1* based on gene postulation following tests with an array of *P. triticina* pathotypes and molecular marker assays. These seedling resistance and APR genes and their closely linked molecular markers are potentially useful for improving leaf rust resistance in wheat breeding programs.

Introduction

Leaf rust (LR), caused by *Puccinia triticina*, is one of the most important wheat diseases worldwide. It is adapted to a wide range of environments, occurs wherever wheat is grown, and can cause significant yield losses (Knott 1989).

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During the past decades, leaf rust has periodically caused significant yield losses in various Chinese wheat growing regions, but particularly in the Yellow and Huai valleys (Zhao et al. 2008). In 2012, significant yield losses were recorded in the provinces of Gansu, Sichuan, Shaanxi, Henan and Anhui (Zhou et al. 2013). Resistant cultivars are the most efficient, economic and environmentally friendly means to reduce losses caused by leaf rust.

Much progress has been made in searching for resistance to leaf rust in wheat. To date, 72 leaf rust resistance genes have been catalogued (McIntosh et al. 2013; Li et al. 2014). Most of these genes confer race-specific resistance that generally lacks durability. Therefore, it is important to identify and use slow rusting types of adult-plant resistance (APR) in breeding programs as current evidence suggests that such sources are more likely to be durable. Cultivars with slow rusting non-hypersensitivity types of APR develop rust symptoms at later growth stages, but at levels that do not lead to significant losses, or losses that are much lower than in susceptible controls. This type of resistance is less likely to be overcome by changes in the pathogen population and is selected in greater durability (Bjarko and Line 1988). In epidemiological terms, slow rusting APR is characterized by slow disease development in the field despite high infection types, longer latent periods, low infection frequencies, smaller uredinial size and reduced duration of sporulation, and less spore production (Caldwell 1968). The Chinese landrace Pingyuan 50, the Californian CIMMYT-derived cultivar Anza, and the Mexican line Pavon 76 with these characteristics have shown high, stable and durable resistance to leaf rust (Zhang et al. 2009). Three of the 72 cataloged leaf rust resistance genes, namely, *Lr34* (Dyck 1977), *Lr46* (Singh et al. 1998) and *Lr67* (Herrera-Foessel et al. 2011), are APR or slow leaf rusting genes that also confer resistance to stripe rust and powdery mildew (Li et al. 2014). Dyck and Samborski (1982) first reported slow rusting resistance gene *Lr34* in the Brazilian wheat cultivar Frontana. Dyck (1987) located *Lr34* on chromosome 7D. McIntosh (1992) and Singh (1992) independently discovered that lines possessing *Lr34* also conferred resistance resistance to stripe rust and the name *Yr18* was given to it. *Lr34* confers a moderate level of leaf rust resistance when present alone; however, combinations with additional slow rusting genes generally result in higher resistance levels (Singh et al. 2000a). Nelson et al. (1997) identified two QTL for leaf rust resistance, one located on chromosome 7DS (*Lr34*), the other on 2BS, and together they explained 45 % of the phenotype variation. Krattinger et al. (2009) showed that the *Lr34* protein resembled ABC (ATP-binding cassette) transporters. Singh et al. (1998) identified gene *Lr46* for slow leaf rusting on chromosome 1B in the cultivar Pavon 76. William et al. (2003) mapped *Lr46* to the terminal bin of 1BL, closely linked to AFLP marker *PstAAgMseCTA-1*, and it co-located with a slow stripe rusting gene later named *Yr29*. Lillemo et al. (2008) reported that *Lr46*/*Yr29* co-located with a powdery mildew resistance gene later named *Pm39*, and that *Lr34*/*Yr18* co-located with *Pm38*. Mutation studies have now confirmed the pleiotropy of these genes (Krattinger et al. 2011). Herrera-Foessel et al. (2011) mapped a third slow rusting resistance gene *Lr67*/*Yr46* on chromosome 4DL, closely linked to SSR markers *gwm165* and *gwm192*. This gene is also associated with partial resistance to powdery mildew (Herrera-Foessel et al. 2014). Herrera-Foessel et al. (2012) found at least three APR QTL for leaf rust in wheat line Parula, including *Lr34* and *Lr46*, and a third gene on chromosome 7BL designated as *Lr68*. At present, around 80 leaf rust APR QTL are located on 16 wheat chromosomes (Li et al. 2014), but further characterization of most of them is still required.

Although quantitative resistance may be more durable than qualitative resistance, it could be overcome by slow evolution in the pathogen population (McDonald and Linde 2002). It is therefore very important to identify further sources of slow rusting genes for breeding cultivars with durable resistance. Ren et al. (2012b) detected three QTL for resistance to leaf rust on chromosomes 1BL and 6BS (2 genes) in Chinese wheat cultivar Bainong 64; those QTL explained 14.9–21.2, 10.0–11.2 and 9.0–9.7 % of the phenotypic variance, respectively.

The CIMMYT spring wheat line Shanghai 3/Catbird has a high level of APR to Chinese *P. triticina* pathotypes in the field, but is susceptible to some pathotypes at the seedling stage. QTL mapping of APR to powdery mildew and stripe rust in a SHA3/CBRD//Naxos RIL population was recently reported by Lu et al. (2012) and Ren et al. (2012a), respectively. However, similar studies on APR to LR in this population have not been reported. Thus, the objectives of this study were to undertake a QTL study of APR to LR in the SHA3/CBRD//Naxos population, and to assess the stability of the identified QTL across environments.

Materials and methods

Plant materials and *P. triticina* pathotypes

One hundred and sixty-four F_6 RILs developed by single-seed descent from the cross SHA3/CBRD//Naxos were used for QTL mapping of APR to leaf rust. Naxos (Tordo/St.Mir808-Bastion//Minaret), a spring wheat cultivar from Germany, is susceptible at the seedling stage to some Chinese *P. triticina* pathotypes, and usually displays susceptibility in the field. SHA3/CBRD (Shanghai 3//Chuanmai 18/Bagula), developed by the International Maize and Wheat Improvement Center (CIMMYT), is susceptible to

some Chinese *P. triticina* pathotypes at the seedling stage, but is highly resistant in the field. SHA3/CBRD has the 1B.1R translocation (Ren et al. 2012a). Both parents are adapted to growing conditions in China. A set of 36 differential lines, mostly near-isogenic lines in the background of Thatcher, with known leaf rust resistance genes, and 14 Chinese *P. triticina* pathotypes were included in the seedling tests (Table 1). The 14 pathotypes were designated following the coding system of Long and Kolmer (1989), with addition of a fourth letter for the reactions to a fourth quartet of differentials (http://www.ars.usda.gov/SP2UserFiles/ad_hoc/36400500Cerealrusts/pt_nomen.pdf).

Seedling tests in the greenhouse

Naxos, SHA3/CBRD and 36 wheat lines with known leaf rust resistance genes were tested with 14 Chinese *P. triticina* pathotypes (Table 1). Seedlings were grown in a growth chamber. When the first leaf was fully expanded, inoculations were performed by brushing urediniospores from fully infected susceptible genotype Zhengzhou 5389 onto the new seedlings. Inoculated seedlings were placed in plastic-covered cages and incubated at 15 °C and 100 % relative humidity (RH) for 24 h in darkness. They were then transferred to a growth chamber programmed with 12 h light/12 h darkness at 18–22 °C and 70 % RH. Infection types (IT) were scored 10–14 days after inoculation according to the Stakman scale as modified by Roelfs et al. (1992). Plants with IT 0–2+ were considered to be resistant and those with IT 3–4 were susceptible. Resistance genes were postulated following Dubin et al. (1989).

Field trials

The 164 F_6 RILs and their parents were evaluated for leaf rust reaction in field nurseries at Baoding in Hebei and Zhoukou in Henan in the 2010–2011 and 2011–2012 cropping seasons. Both locations are hotspots for leaf rust with ideal conditions for rust infection and spread. Field trials were conducted in randomized complete blocks with three replicates at each location. Each plot consisted of a single 1.5 m row with 30 cm between rows. Approximately 100 seeds were sown in each row. Every tenth row was planted with the highly susceptible line Zhengzhou 5389 as a control and to aid the spread of the spores within the trial. Additional rows of Zhengzhou 5389 were planted perpendicular and adjacent to the test rows. Epidemics were initiated by spraying aqueous suspensions of urediniospores containing equal amounts of *P. triticina* pathotypes MHJS, THJL, and PHGP to which a few drops of Tween 20 (0.03 %) were added, onto the spreader rows at tillering. These pathotypes were virulent on SHA3/CBRD and Naxos at the seedling stage (Table 1). Disease severities were scored two or three times at weekly intervals according to the modified Cobb scale (Peterson et al. 1948), with the first scoring 4 weeks after inoculation. The final disease severity (FDS) was recorded for each line in each environment as a percentage leaf area infected when the susceptible check Zhengzhou 5389 displayed its FDS around 25 May in Zhoukou and 10 June in Baoding.

Genotyping

Nine hundred and fifty-two simple sequence repeat (SSR) markers were screened on SHA3/CBRD and Naxos, including the series designated BARC (Song et al. 2002), CFA, CFD and GPW (Sourdille et al. 2004), CNL and KSUM (Yu et al. 2004), GDM (Pestsova et al. 2000), GWM (Röder et al. 1998), MAG (Xue et al. 2008), SWM (Bossolini et al. 2006; Krattinger et al. 2009), UGWM (Parida et al. 2006) and WMC (Gupta et al. 2002). Polymorphic SSR markers were then used to genotype all 164 RILs from the cross of SHA3/CBRD//Naxos by polyacrylamide gel electrophoresis. The population was also screened with diversity array technology (DArT) markers. In addition, a rye (*Secale cereale* L.) 1RS-specific marker (Chai et al. 2006) was included for detection of the 1B.1R translocation.

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, locations × years, and replicates as random effects. The information in the ANOVA (Table 2) was used to calculate broad sense heritabilities $\left(h_b^2\right)$ based on: $h_b^2 = \sigma_g^2 / \left(\sigma_g^2 + \sigma_{ge}^2/e + \sigma_\varepsilon^2/\mathrm{re}\right)$ (Allard 1960), where $\sigma_g^2 = (\mathrm{MS_f} - \mathrm{MS_{fe}})/re$, $\sigma_{ge}^2 = (\mathrm{MS_{fe}} - \mathrm{MS_e})/r$ and $\sigma_\varepsilon^2 = \mathrm{MS_e}$; σ_g^2 is the genetic variance, σ_{ge}^2 is the genotype × environment interaction variance, σ_ε^2 is the error variance, $\mathrm{MS_f}$ is the genotype mean square, $\mathrm{MS_{fe}}$ is the mean square for genotype × environment interaction, $\mathrm{MS_e}$ is the mean square for error, r is the number of replicates and e is the number of environments.

Map construction and QTL analysis

The genotypic data for markers were used to construct genetic linkage maps with the software MapManager QTX20 (Manly et al. 2001). Genetic distances between markers were estimated using the Kosambi mapping function (Kosambi 1944). The assignment of linkage groups on chromosomes was checked according to previously published wheat consensus maps (Somers et al. 2004; http://www.wheat.pw.usda.gov).

Table 1 Seedling infection types[a] on 36 wheat lines with known leaf rust resistance genes when tested with 14 pathotypes of *Puccinia triticina* collected from China

Tester	*Lr* gene	Infection types to *Puccinia triticina* pathotypes[a]													
		PHKS	MHJS	FHDQ①	FGBQ	FHBQ	FHDQ②	THJL	FHDR	FGDQ	FHDS	THJP	TGTT	PHGP	THJC
RL6003	*Lr1*	4	4	;	;	;	;	4	0	;	0	4	4	4	4
Naxos	*Lr1*	4	4	;	0	0;	0	3	;	0	0	3	4	3	1
RL6016	*Lr2a*	;	;	1+	;	;	1	3	;	;	2	3	3	;	4
RL6047	*Lr2c*	4	1	4	4	4	4	4	4	4	4	4	4	4	4
RL6002	*Lr3*	4	4	4	4	4	4	4	4	4	4	4	4	4	4
RL6010	*Lr9*	;	;	;	;	0;	0	;	0	0	;	;	;	0	;
RL6005	*Lr16*	4	4	4	4	4	4	3+	4	4	4	4	4	3	4
RL6064	*Lr24*	;1	;	;	;	;	;	;	;	;	;	;	;	;	;
RL6078	*Lr26*	4	4	4	1	4	4	4	4	1	4	4	;1	4	4
SHA3/CBRD	*Lr26+*	1	4	3	0	3	3	4	3	0	;	1	;	3+	3+
RL6007	*Lr3 ka*	X	X	;	;	;	;	1	;	;	;	1	4	;	X
RL6053	*Lr11*	4	4	1	;	;	1+	3+	1	1	2	4	3+	4	4
RL6008	*Lr17*	4	3+	3+	2	2	3+	4	3+	4	4	4	4	2+	4
RL6049	*Lr30*	3C	1	1	;	;	;	1	;	;	;	;	4	;	1
RL6051	*LrB*	3+	4	4	4	3+	4	3+	4	4	4	4	4	4	X
RL6004	*Lr10*	3	3	4	4	4	4	2	4	4	4	2+	4	1	X
RL6013	*Lr14a*	4	4	X	X	X	X	X	X	2	4	4	4	3+	X
RL6009	*Lr18*	1	1+	2	2	2	2	1+	4	2+	2	4	3+	3C	3
RL6019	*Lr2b*	1	0;	4	;	3	3+	4	3	3+	3+	2	4	3C	4
RL6042	*Lr3bg*	4	4	4	4	4	3+	4	4	4	4	4	4	4	4
Manitou	*Lr13*	3	4	4	3	3	4	3	3	2	3+	4	4	4	4
RL6006	*Lr14b*	4	4	4	4	4	4	4	4	4	4	X	4	X	4
RL6052	*Lr15*	1	;	;	;	;	;	4	1	;	;	4	3+	4	4
RL6040	*Lr19*	0	0	;	0	0	;	0	0	0	;	0	0	0	;
RL6092	*Lr20*	4	4	;	;	;	;	;	;	;	;	4	1	4	;
RL6043	*Lr21*	4	2	2	;	2+	3	;	1	;	1+	;	3	1	1
RL6012	*Lr23*	4	4	4	3+	3+	4	1	4	4	4	4	4	3+	4
RL6079	*Lr28*	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RL6080	*Lr29*	0	0	0	0	;	0	;	;	0	3+	4	;	0	0
RL6057	*Lr33*	3	4	3+	3+	3+	4	3C	3+	3	4	4	4	3+	3+
E84018	*Lr36*	4	2	1+	;	2	2	1	2	2+	3	2+	3+	2+	3+
KS86 NGRC02	*Lr39*	;	;1	;	;	;	;	;	;	;	;	;	;	;	;
KS91 WGRC11	*Lr42*	;	;	;	0	0	;	;	1	;	;	0	;	0	1
RL6147	*Lr44*	1	;	4	4	4	4	1	4	4	4	;	1+	;1	1
RL6144	*Lr45*	4	4	4	4	4	4	;	4	4	4	4	;	;	;
PAVON + Lr47	*Lr47*	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C78.5	*Lr51*	;	;	;	;	1	;	;	;	;	;	0	;	;	;
98M71	*Lr53*	;	0	0	0	0	0	;	0	0	0	0	0	0	0

[a] According to the 0–4 Stakman scale as modified by Roelfs et al. (1992)

+Uredinia somewhat larger than normal for the IT

QTL for leaf rust resistance were detected using the mean FDS of three replicates in each environment. QTL analysis was conducted using the ICIM-ADD function with the software QTL IciMapping 3.1 (Li et al. 2007). A logarithm of odds (LOD) threshold of 2.5, calculated from 1,000 permutations at a probability of 0.05, was used for declaring definitive

Table 2 Analysis of variance of FDS scores for RILs generated from the cross SHA3/CBRD//Naxos

Source of variation	*df*	Mean square	*F* value	*P*
Lines	163	2,853	8.29**	<0.0001
Environments	3	15,160	44.07**	<0.0001
Replicates	2	14	0.5	0.5848
Lines × environments	489	344	13.3**	<0.0001
Error	984	26		

QTL. A walk speed of 1.0 cM was chosen for all QTL estimations. QTL effects were estimated as the proportion of phenotypic variance (R^2) explained (PVE) by the QTL.

Results

Phenotypic evaluation

Leaf rust developed well in all trials. The frequency distribution of leaf rust FDS for the 164 RILs in each environment revealed a continuous distribution skewed towards resistance (Fig. 1), indicating polygenic inheritance. The FDS of the susceptible control Zhengzhou 5389 ranged from 70 to 100 %, from 90 to 100 %, from 80 to 90 %, and from 80 to 90 % in Baoding 2011, Baoding 2012, Zhoukou 2011 and Zhoukou 2012, respectively. SHA3/CBRD had a mean FDS of 0–5 % across the four environments, whereas Naxos was rated with a mean FDS of 15–70 % (Fig. 1). Similar results were obtained across locations and years (Fig. 1). The broad-sense heritability of FDS was 0.88. ANOVA showed significant differences ($P = 0.01$) in FDS among RILs, environments and line × environment interactions (Table 2).

Construction of linkage maps

A total of 952 SSR markers was tested for polymorphism between SHA3/CBRD and Naxos, and 279 (29.3 %) were polymorphic. The latter were used to genotype individual RILs from the population for construction of genetic linkage maps. Additionally, 283 polymorphic DArT markers covering all chromosomes and 6 molecular markers specific for the 1B.1R translocation were used for mapping the population.

Resistance genes postulated from seedling reactions and molecular markers

Variation in IT and IT arrays conferred by 36 known leaf rust genes in differential lines, inoculated with 14 *P. triticina* pathotypes (Table 1), provided an ability to postulate 17 resistance genes (*Lr1*, *Lr2a*, *Lr2b*, *Lr3ka*, *Lr10*, *Lr11*, *Lr14a*, *Lr15*, *Lr17a*, *Lr18*, *Lr20*, *Lr21*, *Lr26*, *Lr30*, *Lr36*, *Lr44* and *Lr45*). Resistance genes *Lr9*, *Lr19*, *Lr24*, *Lr28*, *Lr29*, *Lr39*, *Lr42*, *Lr47*, *Lr51* and *Lr53* conferred low ITs to all pathotypes. Postulation of genes *Lr2c*, *Lr3*, *Lr3bg*, *Lr13*, *Lr14b*, *Lr16*, *Lr23*, *Lr33*, and *LrB* was not possible because high infection types (ITs) were recorded with most pathotypes. SHA3/CBRD displayed low ITs with three *Lr26*-avirulent pathotypes (FGBQ, FGDQ and TGTT) and three *Lr26*-virulent pathotypes (PHKS, FHDS and THJP) (Table 1), indicating SHA3/CBRD possessed *Lr26*, which was confirmed by the 1BL.1RS specific markers (Chai et al. 2006), plus other unknown genes. Naxos was postulated to contain *Lr1* because it gives low ITs to seven *Lr1*-avirulent pathotypes. The presence of *Lr1* in Naxos was also confirmed by molecular marker WR003 (Qiu et al. 2007).

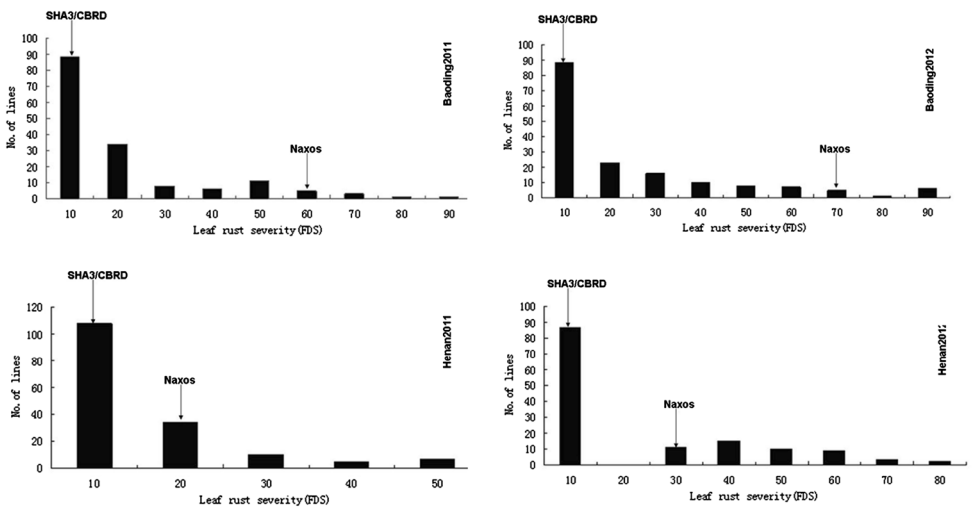


Fig. 1 Distribution for FDS in the SHA3/CBRD//Naxos F_6 RIL population across four environments

QTL for LR resistance

Using inclusive composite interval mapping (ICIM) analysis, 6 QTL for APR were identified on chromosomes 1AL, 2BS, 2DL, 5B, 7BS and 7DS based on the mean FDS in Baoding 2011, Baoding 2012, Zhoukou 2011 and Zhoukou 2012 (Fig. 2; Table 3). The resistance alleles of the QTL on 1AL and 2BS were contributed by SHA3/CBRD, whereas those on 2DL, 5B, 7BS and 7DS were from Naxos.

A major QTL for LR located on chromosome 2BS, designated *QLr.hebau-2BS*, was detected in all four environments. Flanked by *XwPt8548* and *XwPt2314*, it explained 19.3, 15.3, 37.4 and 19.6 % of the phenotypic variance in Baoding 2011, Baoding 2012, Zhoukou 2011 and Zhoukou 2012, respectively.

The consistently detected QTL with larger effect, *QLr.hebau-1AL*, flanked by *Xbarc213* and *Xcfa2219*, and explained 5.5, 8.2 and 5.3 % of the phenotypic variance in Baoding 2011, Zhoukou 2011 and Zhoukou 2012, respectively.

QTL *QLr.hebau-7BS*, flanked by *XwPt7653* and *Xgwm573*, was detected in two environments and explained 5.3 and 4.2 % of the phenotypic variance in Zhoukou 2011 and Zhoukou 2012, respectively.

Three QTL, *QLr.hebau-2DL*, *QLr.hebau-5B* and *QLr.hebau-7DS*, were detected in single environments. *QLr.hebau-5B* and *QLr.hebau-7DS* were in marker intervals *Xtpt7755-Xbarc128a* and *Xgwm1220-Xswm10*, and explained 6.0 and 4.4 %, respectively, of the phenotypic variance in Zhoukou 2012. Another QTL *QLr.hebau-2DL* was located between *XwPt2781* and *Xcfd233* on chromosome 2DL, explaining 5.7 % of the phenotypic variance in Zhoukou 2011.

The total phenotypic variance explained by detected QTL ranged from 15.3 to 61.0 % across the four environments in a simultaneous fit, suggesting a significant effect of these QTL in reducing LR severity. Interactions among different QTL across the four environments were not identified using IciMapping V3.1, indicating all six QTL had additive effects (Table 3).

Discussion

Although the wheat line SHA3/CBRD was susceptible to *P. triticina* pathotypes MHJS, THJL and PHGP at the seedling stage (Table 1), it showed high resistance with mean FDS from 0 to 5 % when inoculated with these pathotypes in the field, and two APR QTL, viz. *QLr.hebau-2BS* and *QLr.hebau-1A*, for leaf rust were detected in SHA3/CBRD. The other parent Naxos was also susceptible to the three *P. triticina* pathotypes at the seedling stage. Although it showed moderately resistant to moderately susceptible reaction with FDS from 15 to 70 % in field tests, four of the APR QTL, viz. *QLr.hebau-7BS*, *QLr.hebau-2DL*, *QLr.hebau-5B* and *QLr.hebau-7DS*, were derived from this parent.

Fig. 2 LOD contours for QTL on chromosomes 1AL, 2BS, 2DL, 5B, 7BS and 7DS that reduce leaf rust severities in the SHA3/CBRD//Naxos RIL population. Genetic distances are shown in centiMorgans (cM) to the left of the vertical axis. The approximate positions of QTL are indicated by oblong *black squares* to the left of markers and their lengths represent the interval distances of flanking markers. LOD threshold of 2.5 is indicated by a *dashed vertical line* in each graph

Environmental influence on APR QTL

Expression of minor genes is often affected by environment, including temperature, light and moisture (Roelfs et al. 1992). It is therefore necessary to evalute segregating populations across environments in order to identify stable QTLs. For example, two leaf rust APR QTLs on chromosome 6BS were identified in a RIL population from Bainong 64/Jingshuang 16; they explained 11.2 and 9.7 % of phenotype variance, respectively, in Baoding 2011, but they were not detected in Zhoukou 2011 (Ren et al. 2012b). In the present study, ANOVA showed significant line × environment interaction differences ($P < 0.0001$) in FDS (Table 2), indicating that environments had significant effects on expression of APR genes. Three APR QTL, viz. *QLr.hebau-2DL*, *QLr.hebau-7BS*, *QLr.hebau-7DS* and two APR QTL, viz. *QLr.hebau-5B*, *QLr.hebau-7BS*, from Naxos were detected in Zhoukou 2011 and Zhoukou 2012, respectively, but they were not identified in both Baoding 2011 and Baoding 2012 (Fig. 1).

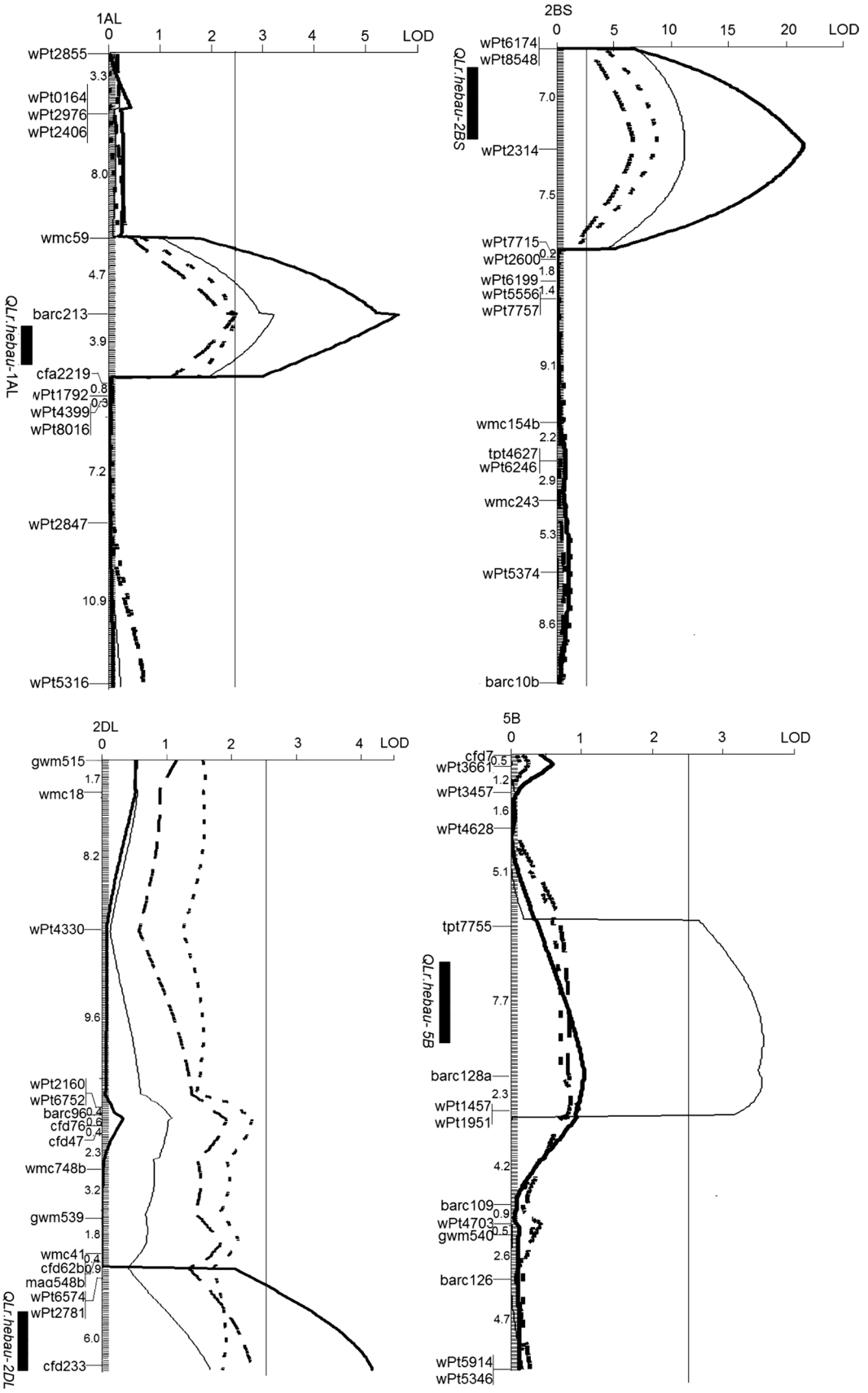
Comparisons with previous reports

QLr.hebau-1AL

This is the first QTL for LR resistance identified on chromosome 1AL and is therefore likely to be a new leaf rust APR QTL. A QTL for stripe rust resistance, *QYr.caas-1AL*, was reported by Ren et al. (2012a) using the same RIL population from SHA3/CBRD//Naxos, but *QYr.caas-1AL* was contributed by Naxos whereas *QLr.hebau-1AL* is from SHA3/CBRD.

A major leaf rust APR QTL on chromosome 2BS

The QTL on chromosome 2BS was derived from SHA3/CBRD. *QLr.hebau-2BS* was stably detected across all four environments, and explained 19.3–37.4 % of the phenotypic variation. Marker *wPt2314* near *QLr.hebau-2BS* is not present on the consensus map (Somers et al. 2004),


1AL
LOD
wPt2855
wPt0164
wPt2976
wPt2406
wmc59
barc213
cfa2219
wPt1792
wPt4399
wPt8016
wPt2847
wPt5316
QLr.hebau-1AL
2BS
wPt6174
wPt8548
wPt2314
wPt7715
wPt2600
wPt6199
wPt5556
wPt7757
wmc154b
tpt4627
wPt6246
wmc243
wPt5374
barc10b
QLr.hebau-2BS
2DL
gwm515
wmc18
wPt4330
wPt2160
wPt6752
barc96
cfd76
cfd47
wmc748b
gwm539
wmc41
cfd62b
mag548b
wPt6574
wPt2781
cfd233
QLr.hebau-2DL
5B
cfd7
wPt3661
wPt3457
wPt4628
tpt7755
barc128a
wPt1457
wPt1951
barc109
wPt4703
gwm540
barc126
wPt5914
wPt5346
QLr.hebau-5B

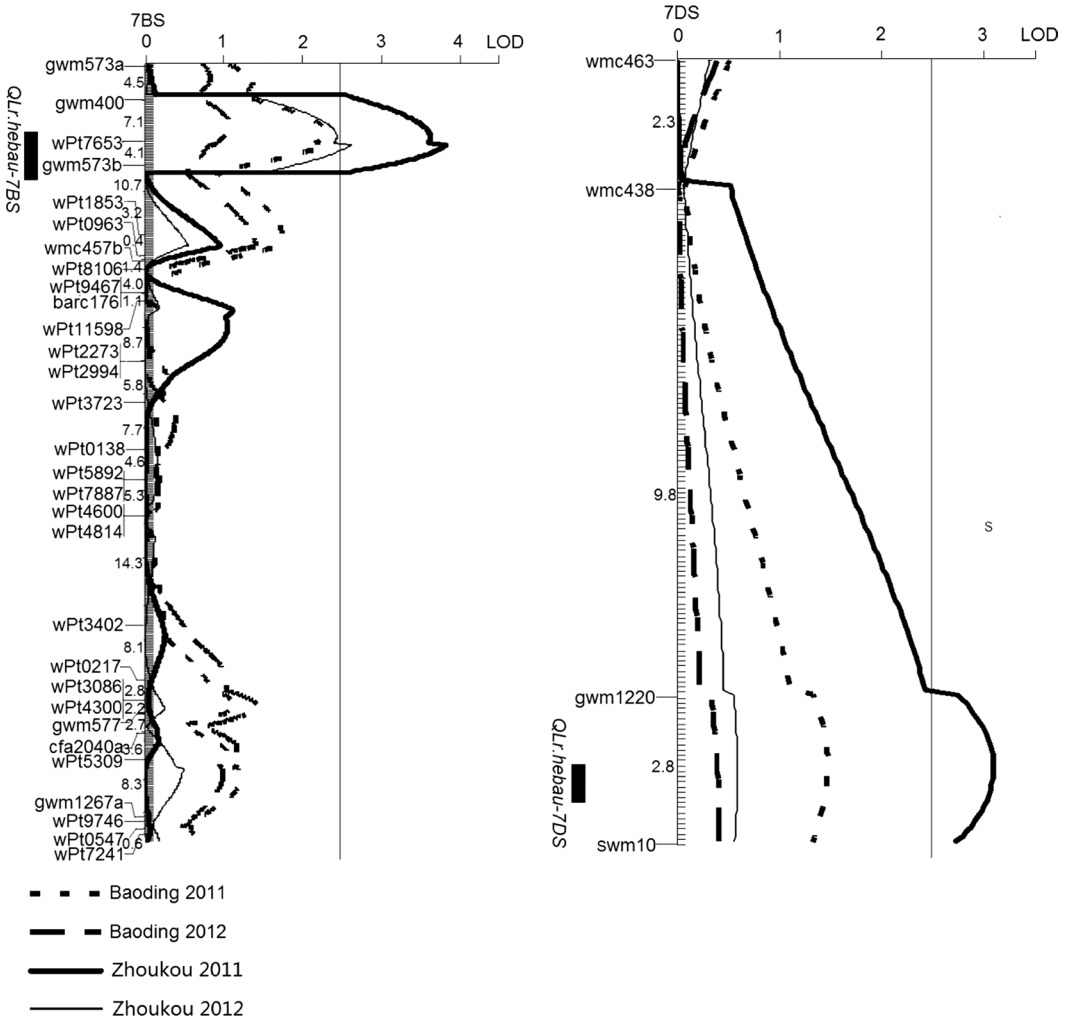


Fig. 2 continued

but its position was inferred to be 15 cM from the distal end of the chromosome based on its linkage (about 15 cM distal) to *wmc154* (Somers et al. 2004). There are five *Lr* genes, *Lr13* (Seyfarth et al. 2000), *Lr16* (McCartney et al. 2005), *Lr23* (McIntosh and Dyck 1975), *Lr35* (Seyfarth et al. 1999) and *Lr48* (Bansal et al. 2008) on 2BS. *Lr13*, *Lr35* and *Lr48* are APR genes. *Lr13* and *Lr35* are estimated to be 44 cM, and *Lr48* 32 cM proximal to *QLr.hebau-2BS* based on the wheat consensus map (Somers et al. 2004), indicating that *QLr.hebau-2BS* is different from *Lr13*, *Lr35* and *Lr48*. Based on the wheat consensus map, *Lr16* is at the terminal of chromosome 2BS (Somers et al. 2004) and linked to *QLr.hebau-2BS* by a genetic distance of 15 cM. In Mexico *Lr16* behaves as a slow rusting gene at the adult plant stage (J. Huerta-Espino, pers. comm.), but in our trials, the reference line with *Lr16* was highly susceptible to all *P. triticina* pathotypes in seedling tests and was also highly susceptible (80S) in the field (data not shown). Thus *QLr.hebau-2BS* is likely different from *Lr16*. It was concluded that *QLr.hebau-2BS* might be a new major APR QTL for leaf rust resistance and could be a potentially important APR gene for wheat breeding.

QLr.hebau-2DL

Schnurbusch et al. (2004) identified a QTL flanked by *Xglk302* and *Xgwm539* on chromosome 2DL in a Swiss winter wheat population derived from Arina/Forno. It was approximately 12 cM from *QLr.hebau-2DL* based on the wheat consensus map (Somers et al. 2004), indicating that this locus may be the same as *QLr.hebau-2DL*, and the relationship between these two loci should be further assessed in the future. A QTL for stripe rust resistance on chromosome 2DL, *QYr.caas-2DL*, was reported by Ren et al. (2012a) using the present population of SHA3/CBRD//Naxos, with both of the resistance alleles contributed by Naxos. They are likely to be the same QTL based on their chromosomal locations. Lu et al. (2012)

Table 3 Quantitative trait loci (QTL) for adult-plant resistance (APR) to LR detected by inclusive composite interval mapping (ICIM) in the SHA3/CBRD//Naxos RIL population across four environments on FDS

QTL[a]	Marker interval	Location and year	LOD	Add	R^2 (%)[b]
QLr.hebau-1AL	*Xbarc213-Xcfa2219*	Baoding 2011	2.5	−4.4	5.5
		Zhoukou 2011	5.6	−3.6	8.2
		Zhoukou 2012	3.2	−4.6	5.3
QLr.hebau-2BS	*XwPt8548-XwPt2314*	Baoding 2011	8.7	−8.6	19.3
		Baoding 2012	6.7	−9.6	15.3
		Zhoukou 2011	21.6	−8	37.4
		Zhoukou 2012	11.2	−9.2	19.6
QLr.hebau-2DL	*XwPt2781-Xcfd233*	Zhoukou 2011	4.1	3	5.7
QLr.hebau-5B	*Xtpt7755-Xbarc128a*	Zhoukou 2012	3.6	4.9	6
QLr.hebau-7BS	*XwPt7653-Xgwm573*	Zhoukou 2011	3.8	2.9	5.3
		Zhoukou 2012	2.6	4.2	4.2
QLr.hebau-7DS	*Xgwm1220-Xswm10*	Zhoukou 2011	3	2.7	4.4

LOD logarithm of odds score, *Add* additive effect of resistance allele

[a] QTL were detected with a minimum LOD score of 2.5 in at least one environment

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

mapped a major QTL for powdery mildew (PM) derived from Naxos to the distal *Xwmc817-Xcfd50* region of 2DL. Although the reported maps suggested about 20 cM between the PM QTL and *QLr.hebau-2DL*, when we re-analyzed the data using a combined model for leaf rust and powdery mildew (data not shown), the two QTL were much more closely associated and could be the same gene. Thus, the APR QTL for *QLr.hebau-2DL* was likely to be associated with QTL for stripe rust and powdery mildew.

QLr.hebau-5B

Messmer et al. (2000) identified a QTL for leaf rust resistance on chromosome 5B in winter wheat Forno, closely linked to the markers *Xpsr580b* and *Xpsr143*. This QTL should be different from *QLr.hebau-5B* based on a map distance of about 100 cM separating them on the wheat consensus map (Somers et al. 2004).

QLr.hebau-7BS

Schnurbusch et al. (2004) identified a QTL *QLr.sfr-7BS* flanked by *Xsfr.BE427461* and *Xgwm573b* on chromosome 7BS in a winter wheat population derived from Arina/Forno. It explained 8.8 % of the phenotypic variance in LR severity across environments. In the present study, *QLr.hebau-7BS*, flanked by *XwPt7653* and *Xgwm573*, explained 5.3 and 4.2 % of the phenotypic variance in Zhoukou 2011 and Zhoukou 2012, respectively. *QLr.hebau-7BS* was located on the same chromosome region as *QLr.sfr-7BS*.

QLr.hebau-7DS

Only the pleiotropic APR gene *Lr34/Yr18/Pm38/Sr57* was previously mapped to chromosome 7DS (Singh 1992; Lillemo et al. 2008; Spielmeyer et al. 2005). In the present study, *QLr.hebau-7DS* also mapped to 7DS, and was flanked by *Xgwm1220* and *Xswm10* which are linked to *Lr34* (Somers et al. 2004). However, genotyping with the diagnostic marker *cssfr5* (Lagudah et al. 2009) showed that both parents carry the 523 bp allele associated with susceptibility (results not shown), indicating that *QLr.hebau-7DS* is different from *Lr34*. *Lr34* generally provides a strong effect in decreasing leaf rust severity (Nelson et al. 1997; Singh et al. 2000b), whereas *QLr.hebau-7DS* showed a relatively weak effect and was detected above the significance threshold in only one environment. A powdery mildew APR QTL from Naxos mapped to the same chromosome region using this population. Therefore *QLr.hebau-7DS* is likely a new minor APR QTL with potentially pleiotropic effects on leaf rust and powdery mildew responses.

Breeding application

Six QTL for resistance to leaf rust were detected on chromosomes 1AL, 2BS, 2DL, 5B, 7BS and 7DS (Table 3). All the closely linked markers should be validated in marker-assisted selection (MAS) before usage for improving resistance to leaf rust in wheat breeding. The APR QTL *QLr.hebau-2BS* provided a significant level of stable resistance to leaf rust in all environments, and may be an important APR gene for use in wheat breeding. However, further research should be done to study the resistance mechanism and to determine if it is race specific and therefore more likely to be non-durable.

Two potentially pleiotropic APR QTL were detected in the population and both were derived from Naxos. The first was on 2DL, and evidence from different studies indicated effects on leaf rust, stripe rust and powdery mildew responses. The second was mapped on 7DS at a

similar position to, but evidently different from the pleiotropic *Lr34/Yr18/Pm38/Sr57*. It conferred resistance to leaf rust and powdery mildew, but no effect on stripe rust response was found in a previous study. Known pleiotropic APR genes, such as *Lr34/Yr18/Pm38/Sr57* and *Lr46/Yr29/Pm39/Sr58* (William et al. 2003), can be combined with potentially pleiotropic APR QTL identified in the present study to breed wheat cultivars with durable resistance to multiple diseases.

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Conflict of interest The authors declare that there are no conflicts of interest in the reported research.

Ethical standards The authors note that this research is performed and reported in accordance with ethical standards of the scientifc conduct.

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