Theor Appl Genet (2012) 125:297–309
DOI 10.1007/s00122-012-1834-6

ORIGINAL PAPER

Partial resistance to powdery mildew in German spring wheat 'Naxos' is based on multiple genes with stable effects in diverse environments

Qiongxian Lu · Åsmund Bjørnstad · Yan Ren · Muhammad Azeem Asad · Xianchun Xia · Xinmin Chen · Fang Ji · Jianrong Shi · Morten Lillemo

Received: 14 August 2011 / Accepted: 17 February 2012 / Published online: 21 March 2012

Abstract Powdery mildew is one of the most important wheat diseases in temperate regions of the world. Resistance breeding is considered to be an economical and environmentally benign way to control this disease. The German spring wheat cv. 'Naxos' exhibits high levels of partial and race non-specific resistance to powdery mildew in the field and is a valuable source in resistance breeding. The main objective of the present study was to map the genetic factors behind the resistance in Naxos, based on a population of recombinant inbred lines (RIL) from a cross with the susceptible CIMMYT breeding line SHA3/CBRD. Powdery mildew severity was evaluated in six field trials in Norway and four field trials in China. The major quantitative trait locus (QTL) with resistance from Naxos was detected close to the *Pm3* locus on 1AS in all environments, and explained up to 35% of the phenotypic variation. Naxos was shown to carry another major QTL on 2DL and minor ones on 2BL and 7DS. QTL with resistance from SHA3/CBRD were detected on 1RS, 2DLc, 6BL and 7AL. The QTL on the 1B/1R translocation showed highly variable effects across environments corresponding to known virulence differences against *Pm8*. SHA3/CBRD was shown to possess the *Pm3* haplotype on 1AS, but none of the known *Pm3a-g* alleles. The RIL population did not provide any evidence to suggest that the *Pm3* allele of SHA3/CBRD acted as a suppressor of *Pm8*.

Communicated by J. Snape.

Q. Lu · Å. Bjørnstad · M. Lillemo (✉)
Department of Plant and Environmental Sciences,
Norwegian University of Life Sciences,
P.O. Box 5003, 1432 Aas, Norway
e-mail: morten.lillemo@umb.no

Q. Lu · F. Ji · J. Shi
Jiangsu Academy of Agricultural Sciences (JAAS),
Nanjing 210014, China

Y. Ren · M. A. Asad · X. Xia · X. Chen
National Wheat Improvement Center/The National Key
Facility for Crop Gene Resources and Genetic Improvement,
Institute of Crop Science, Chinese Academy of Agricultural
Sciences (CAAS), 12 Zhongguancun South Street,
Beijing 100081, China

Introduction

Powdery mildew (PM), caused by *Blumeria graminis* (syn. *Erysiphe graminis*) f. sp. *tritici* (*Bgt*), is one of the most important wheat diseases in many regions of the world with temperate climate. It can cause significant yield losses, in the range of 5–34% (Griffey et al. 1993; Conner et al. 2003). Fungicides are extensively applied to prevent epidemics and maintain high yields when susceptible cultivars are grown.

Breeding for resistance is a more economical and environmentally benign way to control this disease and could reduce the dependence on fungicides. So far, at least 43 loci for resistance to wheat powdery mildew (*Pm1–Pm45*) have been identified and assigned to specific chromosomes or chromosome arms (Hua et al. 2009; Huang and Röder 2004; Zhu et al. 2005; Miranda et al. 2006, 2007; Liu et al. 2001; Blanco et al. 2008; Xu et al. 2010; Lillemo et al. 2008; Ma et al. 2011). However, most of them are race-specific and likely of short durability (McDonald and Linde 2002; Skinnes 2002). Most race-specific resistance genes tend to lose their effect within a few years when cultivars are widely grown, due to the occurrence of matching virulence in the pathogen population.

Partial or race non-specific resistance is inherited in a quantitative manner and has been shown to be more

durable. Breeding cultivars with partial resistance is therefore a more sustainable way to control this disease. Identification of germplasm with partial resistance is, however, hampered by symptom similarity with incomplete effects of race-specific resistance (Lillemo et al. 2010a). Breeding for resistance can therefore be greatly enhanced by the use of molecular markers.

Several major quantitative trait loci (QTL) for partial resistance have been identified in the North American winter wheats 'Massey' (Liu et al. 2001) and 'USG3209' (Tucker et al. 2007), the Swiss winter wheat 'Forno' (Keller et al. 1999), the French winter wheats RE714 (Mingeot et al. 2002; Chantret et al. 2000) and RE9001 (Bougot et al. 2006), the Japanese wheat 'Fukuho-komugi' (Liang et al. 2006), in the CIMMYT spring wheats 'Opata 85', W7984 (Börner et al. 2002) and Saar (Lillemo et al. 2008), in the Chinese cultivars 'Lumai 21' (Lan et al. 2010) and 'Bainong 64' (Lan et al. 2009), and the Swedish winter wheat cultivar 'Folke' (Lillemo et al. 2012). The QTL on 1BL, 2BL and 7DS were the most frequently detected. All these QTL showed stable performance across environments and explained considerable proportions of the phenotypic variance. Recently, two leaf rust and yellow rust loci of partial resistance, *Lr34/Yr18* and *Lr46/Yr29* have been demonstrated to be associated with partial PM resistance (Spielmeyer et al. 2005; Lillemo et al. 2008; Singh et al. 2000) and given the gene designations *Pm38* and *Pm39*, respectively (Lillemo et al. 2008). Both genes are present in diverse germplasm and have contributed to effective partial resistance over several decades (Lillemo et al. 2008; William et al. 2003; Kolmer 1996; Singh et al. 1998).

The German spring wheat cv. 'Naxos' exhibits high levels of partial resistance to powdery mildew in the field. The race non-specificity of its resistance was recently confirmed in a seedling test with differential isolates (Lillemo et al. 2010b). It is a promising source of resistance, but little is known about the genetic basis of its resistance. The objectives of this study were: (1) to detect QTL responsible for partial resistance in a population of recombinant inbred lines (RIL) from a cross with Shanghai3/Catbird (SHA3/CBRD); (2) to assess the stability of detected QTL across different environments; (3) to identify closely linked markers for resistance breeding.

Materials and methods

Plant materials

A RIL population of 181 F_6 lines was developed by single seed descent from the cross SHA3/CBRD × Naxos. Naxos was developed by Strube GmbH & Co.KG from the cross 'Tordo/St.Mir808-Bastion//Minaret'. It does not show any race-specific resistance at the seedling stage but high levels of partial resistance to powdery mildew at the adult plant stage (Lillemo et al. 2010b). SHA3/CBRD is a breeding line from CIMMYT with the pedigree 'Shanghai 3//Chuanmai 18/Bagula' and selection history "-0SHG-6GH-0FGR-0FGR-0Y". It is moderately susceptible to powdery mildew, carrying *Pm8* on the 1B/1R translocation and the *Pm3* haplotype on chromosome 1AS based on the UP3B/UP1A primers, but none of the *Pm3a-g* alleles based on allele-specific PCR markers (Tommasini et al. 2006). The spring wheat cultivar 'Prins' (NGB 6688) and its near-isogenic line (NIL) with *Pm8* (Weique/8*Prins, NGB 6099) were obtained from the Nordic Genetic Resource Center (Alnarp, Sweden).

Field trials

Norway

Powdery mildew resistance was evaluated over 3 years (2008, 2009 and 2010) at two locations in southeastern Norway that represent powdery mildew populations with different virulence composition (Skinnes 2002): Vollebekk Research Farm at the Norwegian University of Life Sciences, Aas (59°N, 90 m above sea level) and Staur research farm close to Hamar (60°N, 153 m above sea level). Both locations experienced severe natural powdery mildew epidemics during experimental seasons. Field trials were carried out in a randomized complete block design with two replications. The RIL population, parents and *Pm8* NILs were planted as hillplots to provide favourable conditions for disease development. The planting was delayed compared with the normal planting to ensure sufficient natural mildew inoculum, and the highly susceptible line Avocet-S was planted as spreader rows on each side of the experiments. Disease severity was scored on penultimate leaves as the percentage of infected leaf area at three times during the disease development with about 1 week intervals (Lillemo et al. 2008). The area under the disease progress curve (AUDPC) was calculated according to Bjarko and Line (1988). Heading date was recorded every year in the same trials at Aas. In 2008 heading date in a separate Fusarium head blight (FHB) experiment was also scored. The days to heading (DH) were calculated for further analysis.

China

Powdery mildew severity was evaluated during cropping seasons 2010 and 2011 at Chinese Academy of Agricultural Sciences (CAAS), Beijing (39°N, 43.5 m above sea level) and in 2010 at CAAS Cotton Research Institute, Anyang, Henan province (36°N, 70–80 m above sea level). The field trials were laid out in randomized complete

blocks with three replications. Fifty seeds of each genotype were sown together in a circle (diameter, 8 cm) and circle to circle distance was maintained by 0.4 m in a row of 2 m length, and row to row distance was 30 cm apart to produce a conductive environment for disease development. The susceptible cultivar 'Jingshuang16' was planted as a check at each tenth row and around the experimental plot to ensure plenty of powdery mildew inoculum in spring. Artificial inoculation was carried out using a highly virulent *Blumeria graminis* f. sp. *tritici* isolate E20 prior to stem elongation in Beijing. Disease severity was scored first time 5 weeks after inoculation based on percentage of leaf area covered by powdery mildew on penultimate leaves (F-1 leaf) and then 1 week later when disease appeared at its maximum level around May 20. In Anyang, powdery mildew disease severity was evaluated once under natural inoculum conditions when the infection level on the susceptible check Jingshuang16 reached its maximum around the third week of May.

Another powdery mildew testing in China was carried out at Jiangsu Academy of Agricultural Sciences (JAAS), Nanjing (32°N, 15 m above sea level), Jiangsu province in 2009. The field trial was laid out in a randomized complete block design with two replications, and sown in late October in 150 cm rows at 33 cm distance. On the bilateral of experiment blocks, Sumai 3, a highly susceptible local cultivar, was planted as spreader rows. PM inoculum was prepared from the pathogen mixture collected from the local area in the previous season, and inoculated onto the Sumai 3 spreader rows in the spring when plants returned green. Disease severity was scored on penultimate leaves as the percentage of infected leaf area at the stage of flowering.

Statistical analysis

The distribution of each trait in each year and location was tested for normality using the PROC UNIVARIATE procedure of the SAS software package (SAS Institute Inc.,Version 9.1). Analysis of variance was performed using the PROC GLM procedure of SAS. Heritability was estimated from the ANOVA information using the formula $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_E^2/r)$ within an experiment and the formula $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_{g\times y}^2/y + \sigma_E^2/ry)$ across experiments, where σ_g^2 is genetic variance, $\sigma_{g\times y}^2$ is genotypes-by-experiments interaction, σ_E^2 is error variance, y is number of experiments, r is number of replications. The Pearson correlation coefficients were calculated using the PROC CORR procedure of SAS.

Genetic map construction and QTL analysis

Initially, 283 polymorphic DArT markers covering all the chromosomes were used for mapping of the RIL population. Based on the SSR consensus map of Somers et al. (2004), the gap regions were supplemented with 105 polymorphic SSR markers. After initial QTL detection, the genetic map was refined with more SSR markers in the QTL regions. In addition, SCM9 for detection of the 1B/1R wheat-rye translocation (Schneider and Molnár-Láng 2009) and the *Pm3* haplotype-specific marker UP3B/UP1A (Tommasini et al. 2006; Yahiaoui et al. 2006) were also included. The genotypic data of 181 lines including 283 DArT and 271 SSR loci were finally used to construct a genetic linkage map with the software JoinMap v. 3.0 (Van Ooijen and Voorrips 2001). Consensus map information from Graingenes (http://wheat.pw.usda.gov/GG2/index.shtml) was used to assign linkage groups to chromosomes.

QTL analysis was performed with PLABQTL v. 1.2 (Utz and Melchinger 1996). Simple interval mapping (SIM) was conducted first to detect the major QTL for PM. The markers most closely linked to each QTL across environments were then used as cofactors in composite interval mapping (CIM). Significant QTL in single environments and for the overall mean were set at a LOD threshold of 3.0, which corresponded to a significance level of $p = 0.05$ based on 1,000 permutations for each phenotypic trait. QTL reaching this level in one environment were also reported for other environments even though their LOD scores were lower. The additive-by-additive epistasis effect was estimated using the Model AA command. Genetic map drawing and QTL marking were conducted by the software MapChart v.2.1 (Voorrips 2002).

AUDPC was calculated and used together with maximum PM severity for correlation analysis and QTL mapping. The results were consistent with those for PM severity. Hence, all the tables and figures only present the results from PM severity.

Results

Phenotypic evaluation

Histograms of mean PM severity of the 181 RILs in each environment are shown in Fig. 1. Powdery mildew developed well in Norway both in 2008 and 2010 with some extreme lines exceeding 90% disease severity. The 2009 season in Norway experienced less favourable conditions, and the powdery mildew symptoms ceased to develop due to a leaf blotch epidemic promoted by rainy weather. The distributions were therefore skewed towards low severity. In China, a broad variation was observed in Nanjing in 2009 with maximum severity around 80%. PM epidemics at Anyang and Beijing developed less with maximum severities around 15%.

Despite unevenly developed symptoms, the correlation coefficients among PM scores remained highly significant

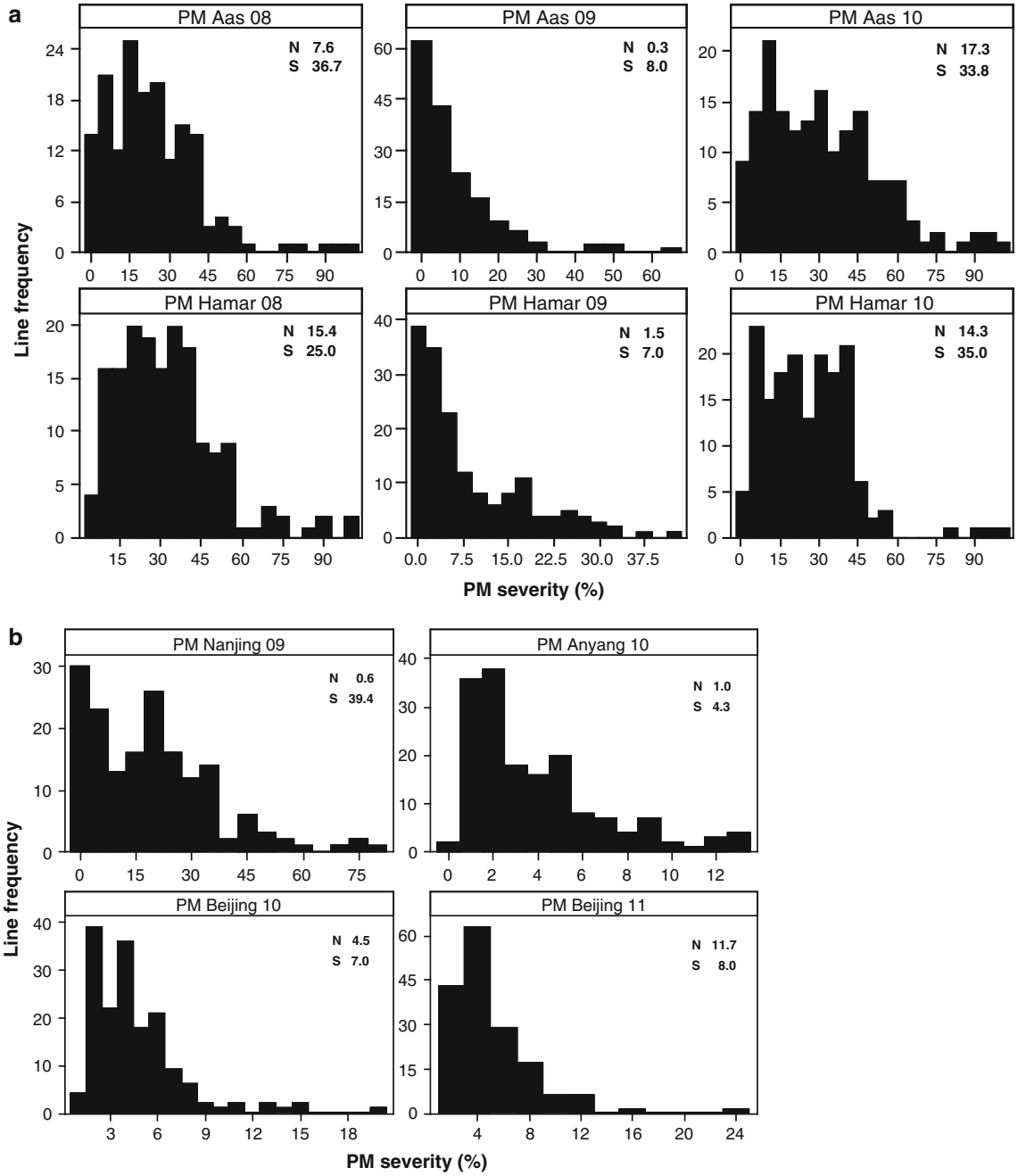

Fig. 1 Frequency distribution of powdery mildew (PM) severity in the SHA3/CBRD × Naxos RIL population in **a** Norway and **b** China. *N* mean severity of Naxos, *S* mean severity of SHA3/CBRD

across environments, ranging from 0.35 to 0.87 (Table 1). The heritabilities across environments were 0.92 in Norway and 0.60 in China (Table 2).

Continuous variation with transgressive segregation was present in all environments (Fig. 1), which indicates that both parents carry resistance genes to powdery mildew. This was confirmed later in the QTL mapping. The ANOVA analysis confirmed significant variation among RILs both within and across locations (Table 2).

PM mapping

From the total of 554 polymorphic marker loci, 422 loci were assembled into 29 linkage groups, while the rest of the markers remained unlinked or were discarded due to poor quality. The genetic map spanned a total of 2,192.3 cM and represented all chromosomes.

QTL for PM severity were detected on 1AS and 2DL in most environments with SIM. These two consistent QTL

Table 1 Pearson correlation coefficients among powdery mildew severities for individual environments and mean days to heading, and heritability estimated for each environment of the SHA3/CBRD × Naxos RIL population

	Aas 08	Aas 09	Aas 10	Hamar 08	Hamar 09	Hamar 10	Nanjing 09	Anyang 10	Beijing 10	Beijing 11
Aas 09	0.82***									
Aas 10	0.81***	0.74***								
Hamar 08	0.81***	0.67***	0.80***							
Hamar 09	0.51***	0.63***	0.57***	0.48***						
Hamar 10	0.81***	0.74***	0.83***	0.87***	0.58***					
Nanjing 09	0.71***	0.77***	0.65***	0.60***	0.62***	0.59***				
Anyang 10	0.55***	0.50***	0.44***	0.44***	0.42***	0.44***	0.51***			
Beijing 10	0.58***	0.57***	0.57***	0.54***	0.56***	0.55***	0.64***	0.59***		
Beijing 11	0.45***	0.43***	0.41***	0.38***	0.36***	0.35***	0.49***	0.58***	0.66***	
Mean days to heading	−0.09	0.01	−0.04	−0.07	0.39**	−0.06	0.18	0.09	0.12	0.10
Heritability	0.94	0.95	0.89	0.93	0.87	0.94	0.70	0.79	0.77	0.83

** 0.01 level, *** 0.0001 level

Table 2 Analysis of variance for powdery mildew severity in the SHA3/CBRD × Naxos RIL population

Source	DF	Mean square	*F* value	*P*	Heritability
Norway 6 exp.					
Genotype	167	2,295.01	13.14	<0.0001	0.92
Experiment	5	36,091.62	206.63	<0.0001	
Genotype × experiment	832	174.66	3.55	<0.0001	
Rep (Experiment)	6	212.83	4.33	0.0003	
Error	966	49.14			
China 4 exp.					
Genotype	167	322.60	2.43	<0.0001	0.60
Experiment	3	19,873.06	149.79	<0.0001	
Genotype × experiment	495	132.67	4.41	<0.0001	
Rep (Experiment)	7	327.18	10.87	<0.0001	
Error	1,092	30.11			
Total 10 exp.					
Genotype	167	2,250.50	10.54	<0.0001	0.91
Experiment	9	45,879.74	214.83	<0.0001	
Genotype × experiment	1,494	213.56	5.47	<0.0001	
Rep (Experiment)	13	274.40	7.03	<0.0001	
Error	2,058	39.04			

were used as cofactors in CIM. QTL regions with lower resolution or partial peaks were then supplemented with more SSR markers based on consensus maps (Somers et al. 2004; GrainGenes: http://wheat.pw.usda.gov/GG2/index.shtml). The final QTL analysis from CIM is presented in Table 3 and Fig. 2. Eight QTL for resistance were identified across 10 environments. Two major QTL with resistance from Naxos were detected on 1AS and 2DL in most environments, while two other minor QTL with resistance from Naxos were detected on 2BL and 7DS. SHA3/CBRD contributed a total of four QTL on 1RS, 2DLc (2DL near centromere), 6BL and 7AL, which showed more consistent effects in Norway.

The most frequently detected major QTL was mapped on 1AS about 6 cM from the *Pm3* locus (Table 3; Fig. 2). It explained from 12 to 35% of the phenotypic variation across all environments with the resistance contributed by Naxos, which does not have the *Pm3* haplotype based on the functional markers UP3B/UP1A and Pm3MF/Pm3ER1. Another major QTL mapped to the distal region of 2DL in the interval *Xwmc817–Xcfd50*. This QTL was highly consistent across eight environments and explained from 9 to

Table 3 QTL results from composite interval mapping (CIM) in the SHA3/CBRD × Naxos RIL population, showing the percentage of explained phenotypic variation (R^2) of individual QTL effect in multiple regression and for the whole model

QTL	PM severity in Hamar				PM severity in Aas				PM severity in China				Overall mean	Resistance source
	08	09	10	Mean	08	09	10	Mean	Nanjing 09	Anyang 10	Beijing 10	Beijing 11		
1AS	**15.4****	**17.2****	**11.5****	**19.2****	**33.9****	**30.8****	**21.3****	**31.4****	**34.5****	**20.1****	**17.8****	**14.6****	**37.7****	Naxos
1RS	**6.9****		**10.7****	**7.5****	**8.5****	**11.4****	**10.4****	**12.4****	3.7*				**4.5***	SHA3/CBRD
2BL	**11.5****		**13.6****	**11.4****	**4.5***		**6.5****	**4.3***					**8.7****	Naxos
2DLc	**9.4****		**9.9****	**6.8****	**5.9****		**7.7****	**5.0****		6.3**			3.2*	SHA3/CBRD
2DL	**20.8****	**16.9****	**19.4****	**23.5****	**8.9****	**17.7****	**16.4****	**17.2****	**14.5****		**13.7****		**14.3****	Naxos
6BL	**5.6****	5.6**				**10.3****	**3.3***	**0.5**					4.8*	SHA3/CBRD
7AL					**6.4****	**11.7****		**5.3****					3.3*	SHA3/CBRD
7DS		5.3**			**4.8****	**6.2****		3.0*	**9.3****		6.0**		3.9*	Naxos
1AS × 2DL	6.3**				3.6*	8.1**								
1AS × 6BL				5.8**		8.9**	5.2**	7.2**					9.7**	
1AS × 7AL					5.8**	11.2**		5.6**					8.1**	
1RS × 2DL			3.1*	2.8*		9.3**								
2BL × 2DL			2.6*				2.6*							
2BL × 6BL	2.7*													
2BL × 7DS								2.9*						
2DLc × 2DL			4.4**	4.5*	8.6**		3.4*	8.0**					5.2**	
2DL × 6BL						4.8**								
2DL × 7AL						3.3*								
2DL × 7DS						4.3*								
6BL × 7AL						4.8**								
Total R^2	54.7	31.7	49.7	55.3	56.6	67.4	55.1	63.4	42.5	21.4	26.7	13.6	62.1	

The table indicates all the QTL that were detected with a minimum LOD score of 3 (highlighted in bold) in at least one environment. Other QTL are also listed if they were detected above the threshold in other environments and showed significant contribution in multiple regression

* 0.05 level, ** 0.01 level

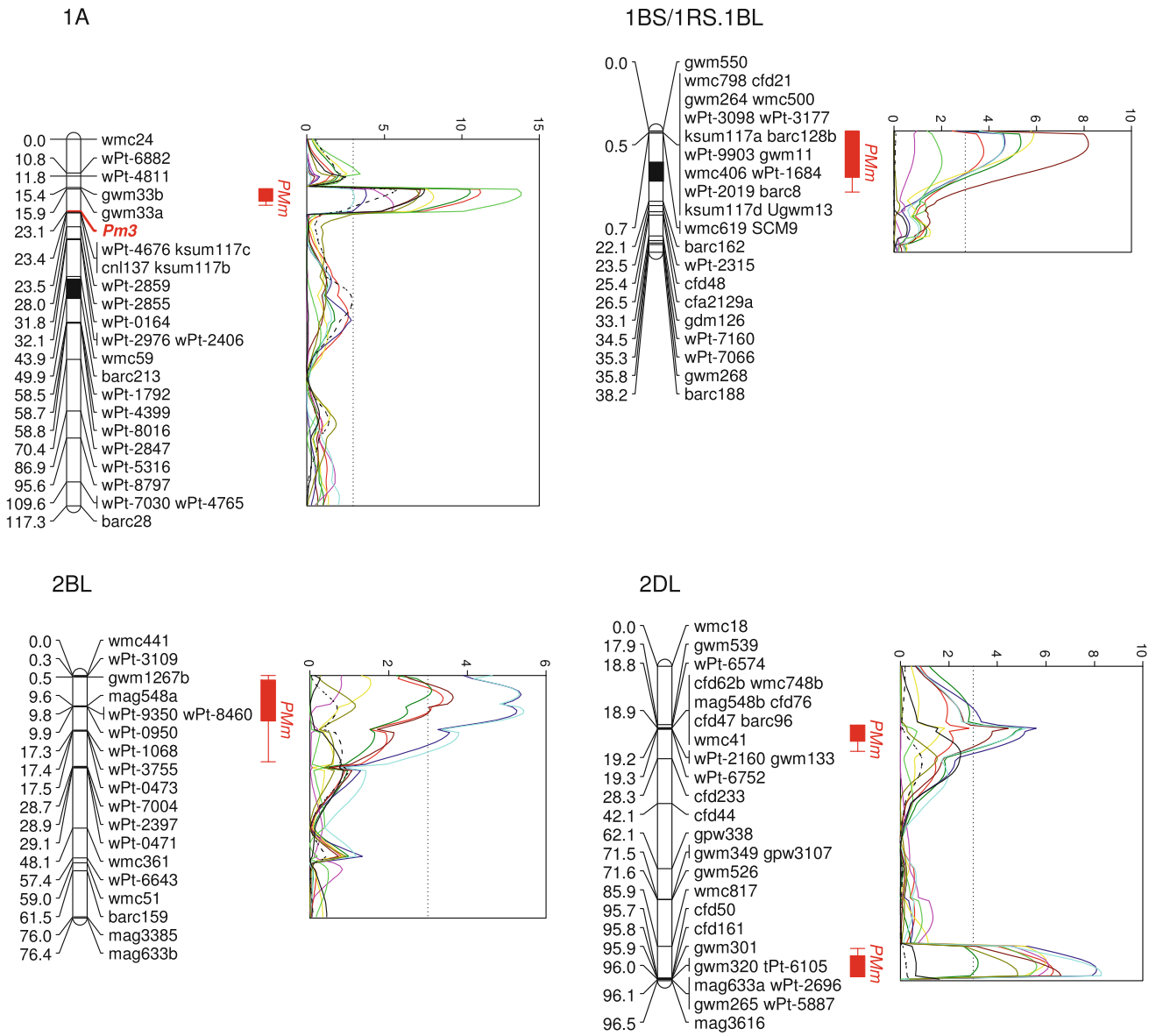

Fig. 2 Chromosomes with significant QTL, with corresponding LOD curves obtained from CIM. Genetic distances are shown in centimorgans to the *left* of the chromosomes. A threshold of 3.0 is indicated by a *dashed vertical line* in the LOD graphs. The approximate positions of centromeres are indicated by *solid squares*

21% of the phenotypic variation. The third consistent QTL with favourable allele from Naxos was detected near *Xwmc463* and *Xwmc438* on 7DS, and explained up to 9% of the phenotypic variation. The QTL on 2BL with resistance from Naxos was detected at both locations in Norway in 2008 and 2010, but not in the 2009 season which had less disease development. The QTL was located near *Xwmc441* and explained up to 14% of the phenotypic variation in single environments.

The QTL on 1RS was detected in five of the six environments in Norway (except Hamar 2009) with the explained phenotypic variation ranging from 7 to 11%, however, it was less effective in Nanjing and showed no effect in Beijing and Anyang. The 2DLc QTL with resistance from SHA3/CBRD was detected in Norway in 2008 and 2010 and in Anyang. It mapped near the centromere close to *Xgwm539* and explained from 6 to 10% of the phenotypic variation in detectable environments. The other two minor QTL close to the centromeres on 6BL and 7AL with resistance from SHA3/CBRD were only detected in Norway.

Generally, the epistasis effect of detected QTL was much less compared to their additive effects. The most common epistasis across environments was observed between 1AS and 6BL, 1AS and 7AL, and 2DLc and 2DL (Table 3; Fig. 3).

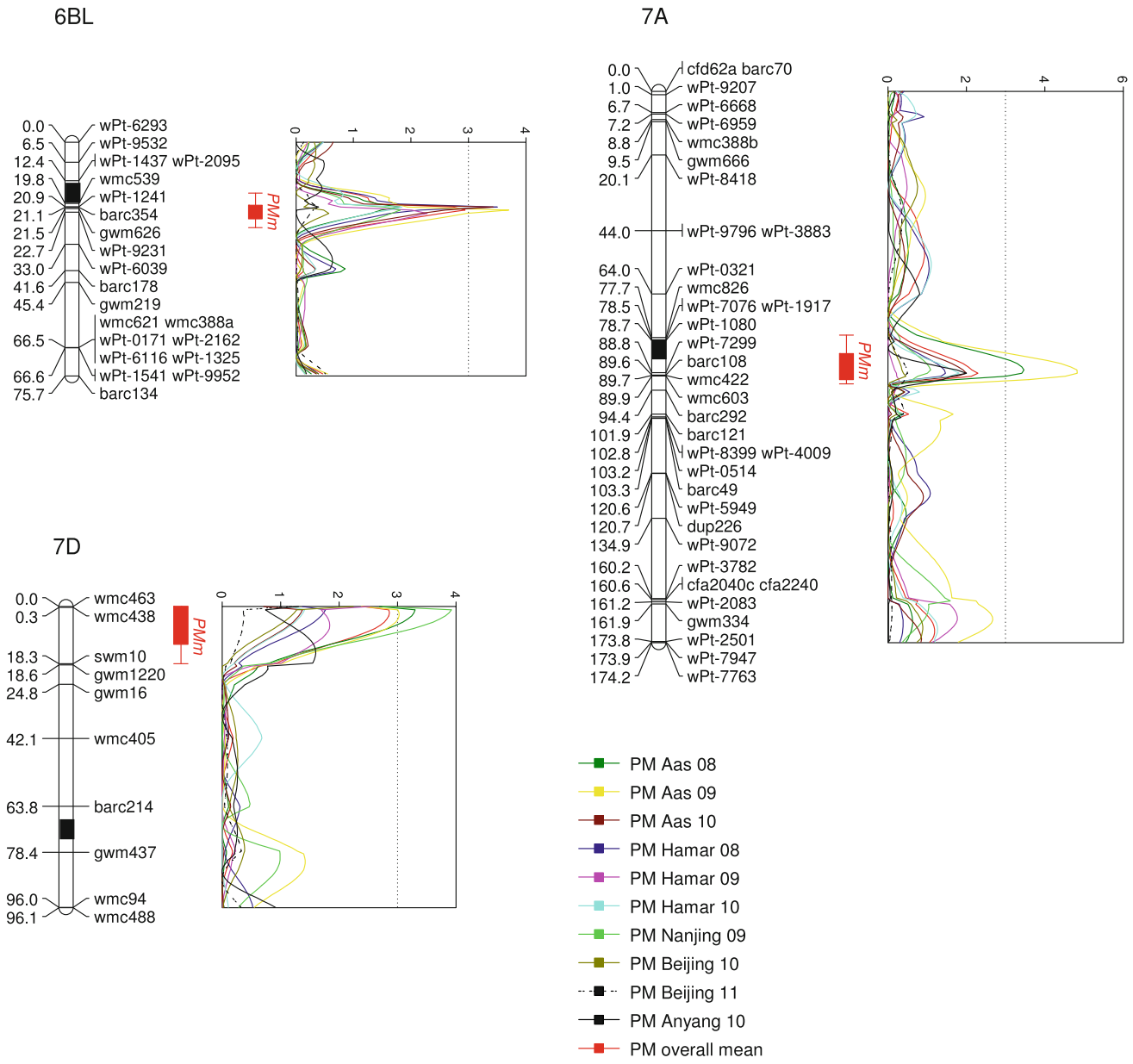

Fig. 2 continued

Effect of race-specific alleles

Pm8 was more effective in Norway than in China (Fig. 4). In Norway, RILs with *Pm8* significantly decreased PM severity by 25–60% compared to their counterparts. In China, *Pm8* decreased PM severity by 22% in Nanjing while the effect was negligible in Beijing and Anyang. The effect of *Pm8* was stronger in the Prins NILs than in the RILs (Fig. 4), but followed the same pattern of environmental variation as in the RILs.

Pm3 haplotype analysis showed that the 5′ primer set UP3B/UP1A identified the *Pm3*-specific 900 bp product in SHA3/CBRD, while only the 1.1 kb homoeologue on 1B was amplified in Naxos. The 3′ primer set Pm3MF/Pm3ER1 gave positive amplification in SHA3/CBRD, but not in Naxos. However, none of the *Pm3a-g* allele-specific primers (Tommasini et al. 2006) amplified any product from SHA3/CBRD, indicating that the line has an unknown allele at this locus. RILs were classified into four groups based on the *Pm3* haplotype analysis (Fig. 5). This could also be considered a combination analysis of *Pm3* and *Pm8* since RILs carrying the 1B/1R translocation had the 1B homoeologue replaced by *Pm8*. A similar pattern was observed in all five locations. The combination of *Pm8* from SHA3/CBRD and the 1AS QTL from Naxos showed the highest resistance to PM, while the combination of the

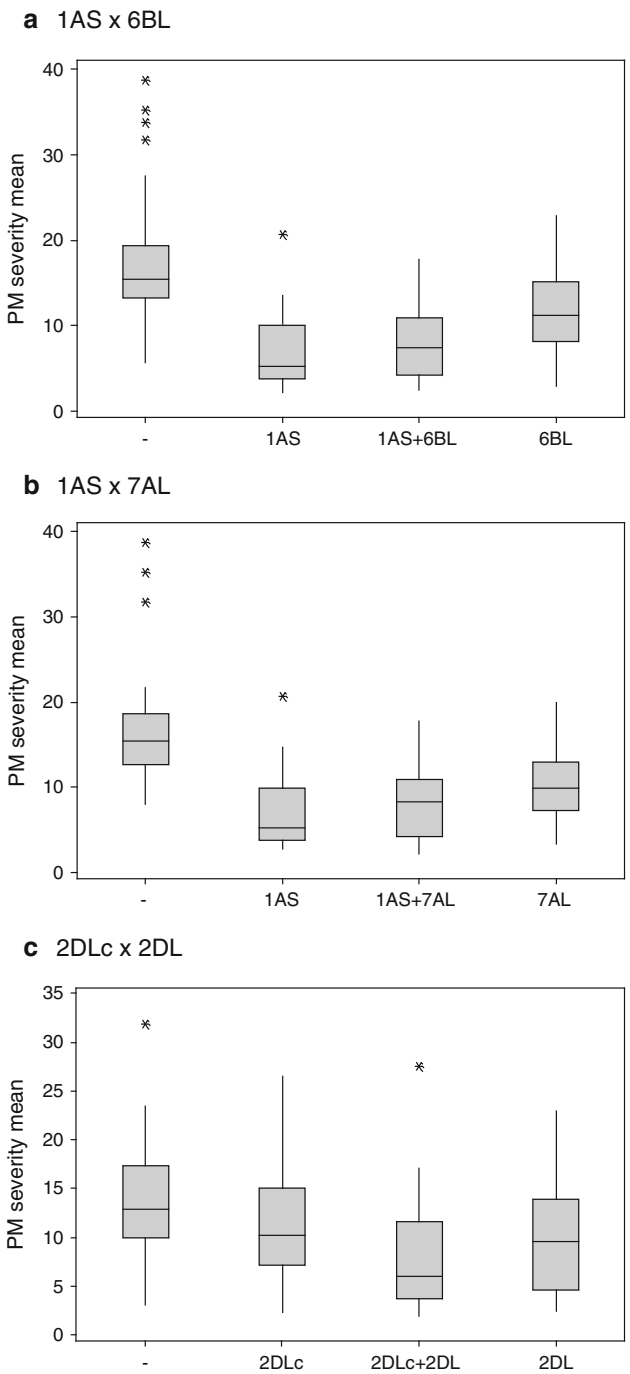

Fig. 3 Epistasis of main QTL pairs. QTL are indicated by chromosome arms, *Hyphen* indicates the absence of QTL. The QTL combinations were determined by the closely linked markers gwm33a and UP3B/UP1A for 1AS, gwm626 and wPt-9231 for 6BL, wPt-7299 and barc108 for 7AL, wmc41 and gwm133 for 2DLc, cfd50 and cfd161 for 2DL

1B homoeologue from Naxos and the *Pm3* haplotype from SHA3/CBRD always provided the highest susceptibility. Whether combined with *Pm8* or the 1B homoeologue of *Pm3*, the 1AS QTL from Naxos always provided better resistance than the *Pm3* haplotype from SHA3/CBRD. The

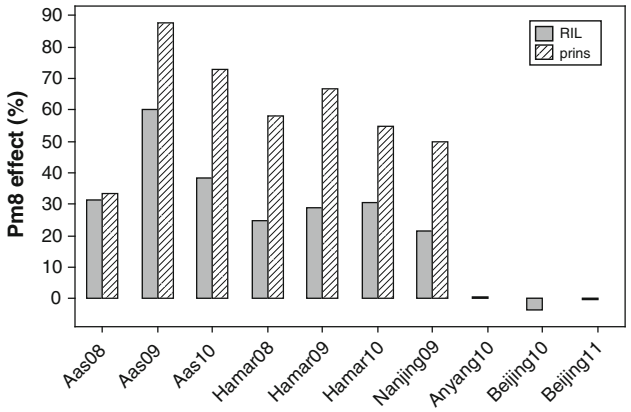

Fig. 4 The effect of *Pm8* in RIL and Prins NIL background in each environment. The effect was charted in terms of relative reduction in PM severity compared with its counterparts

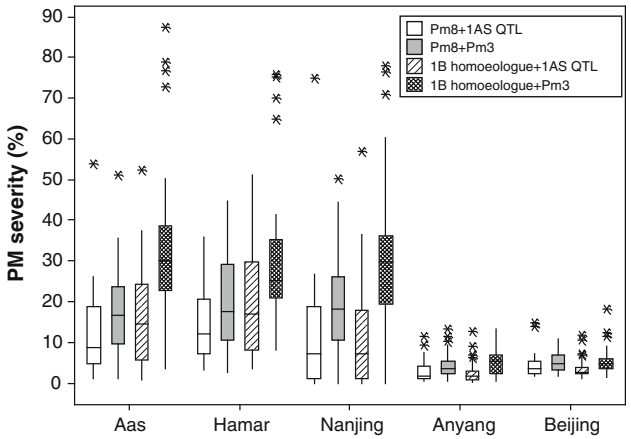

Fig. 5 *Boxplot* of *Pm3* haplotypes in the RIL population. *Pm3* allele determination was based on the functional marker UP3B/UP1A. Additionally, 1RS specific marker SCM 9 was used to differentiate the 1B homoeologue of *Pm3* from 1RS. *Pm3* homoeologue on 1B and 1AS QTL are from Naxos, while *Pm3* and *Pm8* are from SHA3/CBRD

Pm3 haplotype of SHA3/CBRD increased the susceptibility of RILs by different degrees ranging from 20 to 114% compared to the 1AS QTL from Naxos.

Discussion

Naxos has a long record of highly effective adult plant resistance to powdery mildew, and the lack of any race-specific resistance at the seedling stage (Lillemo et al. 2010a, b) suggested that the resistance should be polygenic. This was confirmed by both the phenotypic distributions and QTL mapping in the present study.

Comparison with other reports

Previous studies have already detected resistance QTL to powdery mildew at the *Pm3* locus on 1AS (Bougot et al.

2006; Liang et al. 2006; Mohler et al. 2011). At this locus, Liang et al. (2006) detected a QTL with resistance from Fukuho-komugi which was derived from the *Pm3a* carrier Norin 29 and suggested that the adult plant resistance might be a residual effect of *Pm3a*. Bougot et al. (2006) mapped a QTL for powdery mildew resistance at the vernalized seedling stage with resistance conferred by the *Pm3g* carrier RE9001. Recently, *Pm3e* conferred resistance at both seedling and adult plant stages in the German winter wheat cultivar Cortez (Mohler et al. 2011). Interestingly, in contrast to the previous studies where the favourable alleles were derived from parents carrying race-specific *Pm3* alleles, Naxos carries no race-specific resistance (Lillemo et al. 2010b) and apparently lacks the *Pm3* gene based on the UP3B/UP1A and Pm3MF/Pm3ER1 markers. The QTL with resistance from Naxos is therefore most likely due to a gene for race non-specific resistance located in the same area as the *Pm3* gene on 1AS.

Keller et al. (1999) reported a QTL for PM resistance from the winter wheat cultivar Forno on 2DL in the marker interval *Xpsr932–Xpsr331a*, explaining 8–12% of the phenotypic variation. Börner et al. (2002) detected a minor QTL on 2DL in the ITMI population with resistance from the synthetic wheat W7984. In addition, a partial QTL near marker *Xgwm301* was also detected in the Swedish winter wheat cultivar Folke (Lillemo et al. 2012). In the present study, this locus explained up to 22% of the phenotypic variation, much higher than that in other genetic and environmental backgrounds.

Börner et al. (2002) reported a 7DS QTL in W7984 at the adult plant stage. Subsequently, partial resistance QTL to PM were detected in the same region in Fukuho-komugi and Saar (Liang et al. 2006; Lillemo et al. 2008) and identified as *Lr34/Yr18/Pm38*. The 7DS QTL in the present study mapped to the same region based on the consensus map, but the functional marker *cssfr5* (Lagudah et al. 2009) amplified the 523 bp fragment specific for the non-*Lr34* allele in both Naxos and SHA3/CBRD (data not shown). The LOD curves for this QTL (Fig. 2) did, however, not peak at the *Xgwm1220* and *swm10* loci that are known to delineate the *Lr34* locus (Lillemo et al. 2008; Bossolini et al. 2006), but closer to *Xwmc438* and *Xwmc463* located 18 cM more distally on 7DS. Both parents showed expression of leaf tip necrosis (LTN), which is often used as a phenotypic marker for *Lr34* (Singh 1992), but this trait is also associated with *Lr46* (Rosewarne et al. 2006) and *Lr67* (Herrera-Foessel et al. 2011). We could, therefore, not use LTN to sort out this anomaly. In addition, *Lr34/Yr18/Pm38* usually shows stronger and more stable resistance across environments than the 7DS QTL detected in the present study (Spielmeyer et al. 2005; Lillemo et al. 2008). We therefore conclude that the resistance from Naxos at this locus is likely different from *Lr34/Yr18/Pm38*, but this warrants further study.

The 2BL QTL from Naxos was located in the same region as PM resistance QTL from Massey (Liu et al. 2001), the progeny line USG3209 (Tucker et al. 2007), Chinese cv. Lumai 21 (Lan et al. 2010) and Japanese cv. Fukuho-komugi (Liang et al. 2006). This QTL was not detected in China, which might result from the insufficient symptom development and lower heritabilities in the Chinese disease nurseries. This QTL was relatively stable and detectable across environments in Norway, but showed consistently less effect than the 1AS and 2DL QTL.

The resistance QTL on 1B was located to the 1B/1R translocation of SHA3/CBRD based on the rye-specific markers SCM9 (Saal and Wricke 1999) and NOR (Koebner 1995). Since *Pm8* is known to be located on this translocation, we conclude that this QTL was caused by *Pm8*.

The 6BL QTL near *Xwmc539* in this study is located more than 40 cM away from the previously reported PM resistance QTL on this chromosome in Bainong 64 (Lan et al. 2009) and Folke (Lillemo et al. 2012) based on the consensus map, and must therefore be a different locus. Interestingly, based on another marker *Xbarc354*, the QTL in Naxos is located close to the high-temperature adult plant yellow rust resistance gene *Yr36* originating from wild emmer wheat (Uauy et al. 2005), and recently cloned and found to be absent from modern wheat varieties (Fu et al. 2009). Hence, this 6BL QTL is likely caused by a novel and previously uncharacterized gene for partial and race non-specific resistance to powdery mildew.

The 2DLc QTL with resistance from SHA3/CBRD mapped in the same region as *Pm43* originating from *Thinopyrum intermedium* (He et al. 2009) and an adult plant resistance (APR) QTL from Lumai 21 (Lan et al. 2010). It is more likely to share the same resistance basis with the latter rather than *Pm43*, which was derived from a wheat relative. The 7AL QTL was located close to *Xbarc108* near the centromere similar to the QTL detected in RE714 (Chantret et al. 2001). Besides, a putative QTL on 7AL close to *Xgwm334* in this study (Fig. 2) mapped to the same position as similar QTL reported by Chantret et al. (2001) and Lillemo et al. (2012).

Effect of race-specific genes

Pm8 was more effective in Norway, while negligible in China. This could be attributed to the differences in the composition of PM pathogen populations in Norway and China. Besides, the trials in Beijing were inoculated with strain E20, which is virulent to *Pm8* (Wang et al. 2005). The 1B/1R translocation with *Pm8* derived from 'Petkus' rye has been widely used in Chinese breeding programs since the 1970s (He et al. 2001). Almost half of the Chinese varieties carry the 1B/1R translocation (Yu 2000; Wang et al. 2005), and the frequency of matching virulence in the

pathogen populations had already reached over 90% in the late 1990s (Duan and Sheng 1998). Nevertheless, *Pm8* still had a weak effect in Nanjing which might be due to the lower frequency of 1B/1R cultivars in its belonging wheat production region (20%) compared to the area around Anyang (42%) (Zhou et al. 2004).

In Norway, however, *Pm8* is rare and not present in any of the current spring and winter wheat cultivars (Lillemo et al. 2010b). In addition, only 1% of Nordic wheat accessions carry *Pm8* (Hysing et al. 2007). This indicates a much weaker selection pressure for *Pm8* virulence in the local pathogen populations in Norway and neighbouring countries, and accordingly *Pm8* was more effective.

Although PCR amplification with both UP3B/UP1A and Pm3MF/Pm3ER1 primers indicated the presence of a *Pm3* gene in SHA3/CBRD, the identity is unknown as the allele-specific markers did not detect any of the previously characterized *Pm3a-g* alleles. Moreover, no conclusions can be made as to whether SHA3/CBRD carries a functional allele of this gene since the resistance of the line has not been tested with differential isolates. In the QTL analysis, the effect of the *Pm3* allele can only be compared with the corresponding 1AS QTL for partial resistance located at the same position in Naxos. It indicates that the Naxos QTL provided much stronger effect on PM resistance than the *Pm3* allele from SHA3/CBRD.

Pm8 was suppressed at the seedling stage in certain 1B/1R translocation lines (Ren et al. 1997; Hanusova et al. 1996). The suppressor of *Pm8* has been mapped to the short arm of chromosome 1A, and McIntosh et al. (2011) recently demonstrated that *Pm3* could act as a suppressor of the *Pm8* resistance. Both SHA3/CBRD and its parent Catbird carry the *Pm3* haplotype and *Pm8* based on diagnostic markers, but Catbird did not express the *Pm8* resistance at the seedling stage (Lillemo et al. 2010b; Wang et al. 2005). If the *Pm3* allele of SHA3/CBRD functioned as a suppressor of *Pm8* in our mapping population, we would not have expected to see any effect of *Pm8* in the background of *Pm3*. However, the opposite was observed, with *Pm8* providing additional resistance in the backgrounds of both *Pm3* and the 1AS QTL from Naxos. As McIntosh et al. (2011) suggested that translation of *Pm3* is necessary for *Pm8* suppression, we may conclude that the *Pm3* allele of SHA3/CBRD is likely not functional. But this requires further investigations that are beyond the scope of this paper.

Implications for breeding

In this study, genetic analysis showed that the high level of partial resistance to powdery mildew in Naxos was mainly governed by two major QTL on 1AS and 2DL, and minor QTL on 2BL and 7DS. Markers closely linked to these loci have potential to improve the selection for PM resistance in breeding populations generated from this highly valuable source of resistance.

Several other promising sources of partial resistance have also been identified and demonstrated their effectiveness under highly conducive environments for powdery mildew epidemics (Lillemo et al. 2010a, b). Markers are already available for some of them such as Saar (Lillemo et al. 2008), Folke (Lillemo et al. 2012), Massey and USG 3209 (Liu et al. 2001; Tucker et al. 2007) and RE714 (Muranty et al. 2009). The QTL reported here for Naxos and their corresponding closely linked molecular markers might serve to diversify the genetic basis of partial and potentially durable resistance to powdery mildew and accelerate the breeding process.

Acknowledgments The PhD scholarship of the first author was funded by the Norwegian University of Life Sciences, and the research was supported by grants from the Research Council of Norway (projects 178273 and 185046), and the National Science Foundation of China (projects 30821140351 and 30671294). Additionally, we gratefully acknowledge the technical contributions from Anne Guri Marøy in the lab and Yalew Tarkegne in the field.

References

Bjarko ME, Line RF (1988) Heritability and number of genes controlling leaf rust resistance in four cultivars of wheat. Phytopathology 78(4):457–461

Blanco A, Gadaleta A, Cenci A, Carluccio AV, Abdelbacki AMM, Simeone R (2008) Molecular mapping of the novel powdery mildew resistance gene *Pm36* introgressed from *Triticum turgidum* var. *dicoccoides* in durum wheat. Theor Appl Genet 117(1):135–142

Börner Schumann, Fürste Cöster, Leithold Röder, Weber (2002) Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (*Triticum aestivum* L.). Theor Appl Genet 105(6):921–936. doi:10.1007/s00122-002-0994-1

Bossolini E, Krattinger S, Keller B (2006) Development of simple sequence repeat markers specific for the *Lr34* resistance region of wheat using sequence information from rice and *Aegilops tauschii*. Theor Appl Genet 113(6):1049–1062. doi:10.1007/s00122-006-0364-5

Bougot Y, Lemoine J, Pavoine MT, Guyomar'ch H, Gautier V, Muranty H, Barloy D (2006) A major QTL effect controlling resistance to powdery mildew in winter wheat at the adult plant stage. Plant Breeding 125(6):550–556

Chantret N, Sourdille P, Roder M, Tavaud M, Bernard M, Doussinault G (2000) Location and mapping of the powdery mildew resistance gene *MIRE* and detection of a resistance QTL by bulked segregant analysis (BSA) with microsatellites in wheat. Theor Appl Genet 100(8):1217–1224

Chantret N, Mingeot D, Sourdille P, Bernard M, Jacquemin JM, Doussinault G (2001) A major QTL for powdery mildew resistance is stable over time and at two development stages in winter wheat. Theor Appl Genet 103(6–7):962–971

Conner RL, Kuzyk AD, Su H (2003) Impact of powdery mildew on the yield of soft white spring wheat cultivars. Can J Plant Sci 83(4):725–728

Duan XY, Sheng BQ (1998) Identification of isolates of *Blumeria graminis* f. sp. *tritici* and the monitoring of their virulence frequencies. Acta Phytopathol Sinica 25:31–36

Fu DL, Uauy C, Distelfeld A, Blechl A, Epstein L, Chen XM, Sela HA, Fahima T, Dubcovsky J (2009) A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. Science 323(5919):1357–1360. doi:10.1126/science.1166289

Griffey CA, Das MK, Stromberg EL (1993) Effectiveness of adult-plant resistance in reducing grain yield loss to powdery mildew in winter wheat. Plant Dis 77(6):618–622

Hanusova R, Hsam SLK, Bartos P, Zeller FJ (1996) Suppression of powdery mildew resistance gene *Pm8* in *Triticum aestivum* L. (common wheat) cultivars carrying wheat-rye translocation T1BL.1RS. Heredity 77:383–387

He ZH, Rajaram S, Xin ZY, Huang GZ (2001) A history of wheat breeding in China. CIMMYT, Mexico

He RL, Chang ZJ, Yang ZJ, Yuan ZY, Zhan HX, Zhang XJ, Liu JX (2009) Inheritance and mapping of powdery mildew resistance gene *Pm43* introgressed from *Thinopyrum intermedium* into wheat. Theor Appl Genet 118(6):1173–1180

Herrera-Foessel S, Lagudah E, Huerta-Espino J, Hayden M, Bariana H, Singh D, Singh R (2011) New slow-rusting leaf rust and stripe rust resistance genes *Lr67* and *Yr46* in wheat are pleiotropic or closely linked. Theor Appl Genet 122(1):239–249. doi:10.1007/s00122-010-1439-x

Hua W, Liu ZJ, Zhu J, Xie CJ, Yang TM, Zhou YL, Duan XY, Sun QX, Liu ZY (2009) Identification and genetic mapping of *pm42*, a new recessive wheat powdery mildew resistance gene derived from wild emmer (*Triticum turgidum* var. *dicoccoides*). Theor Appl Genet 119(2):223–230

Huang XQ, Röder MS (2004) Molecular mapping of powdery mildew resistance genes in wheat: a review. Euphytica 137(2):203–223

Hysing SC, Merker A, Liljeroth E, Koebner RMD, Zeller FJ, Hsam SLK (2007) Powdery mildew resistance in 155 Nordic bread wheat cultivars and landraces. Hereditas 144(3):102–119

Keller M, Keller B, Schachermayr G, Winzeler M, Schmid JE, Stamp P, Messmer MM (1999) Quantitative trait loci for resistance against powdery mildew in a segregating wheat × spelt population. Theor Appl Genet 98(6–7):903–912

Koebner RMD (1995) Generation of PCR-based markers for the detection of rye chromatin in a wheat background. Theor Appl Genet 90(5):740–745

Kolmer JA (1996) Genetics of resistance to wheat leaf rust. Annu Rev Phytopathol 34:435–455

Lagudah ES, Krattinger SG, Herrera-Foessel S, Singh RP, Huerta-Espino J, Spielmeyer W, Brown-Guedira G, Selter LL, Keller B (2009) Gene-specific markers for the wheat gene *Lr34/Yr18/Pm38* which confers resistance to multiple fungal pathogens. Theor Appl Genet 119(5):889–898. doi:10.1007/s00122-009-1097-z

Lan CX, Liang SS, Wang ZL, Yan J, Zhang Y, Xia XC, He ZH (2009) Quantitative trait loci mapping for adult-plant resistance to powdery mildew in Chinese wheat cultivar Bainong 64. Phytopathology 99(10):1121–1126. doi:10.1094/phyto-99-10-1121

Lan CX, Ni XW, Yan J, Zhang Y, Xia XC, Chen XM, He ZH (2010) Quantitative trait loci mapping of adult-plant resistance to powdery mildew in Chinese wheat cultivar Lumai 21. Mol Breeding 25(4):615–622

Liang SS, Suenaga K, He ZH, Wang ZL, Liu HY, Wang DS, Singh RP, Sourdille P, Xia XC (2006) Quantitative trait loci mapping for adult-plant resistance to powdery mildew in bread wheat. Phytopathology 96(7):784–789

Lillemo M, Asalf B, Singh RP, Huerta-Espino J, Chen XM, He ZH, Bjornstad A (2008) The adult plant rust resistance loci *Lr34/Yr18* and *Lr46/Yr29* are important determinants of partial resistance to powdery mildew in bread wheat line Saar. Theor Appl Genet 116(8):1155–1166

Lillemo M, Singh RP, van Ginkel M (2010a) Identification of stable resistance to powdery mildew in wheat based on parametric and nonparametric methods. Crop Sci 50(2):478–485

Lillemo M, Skinnes H, Brown JKM (2010b) Race specific resistance to powdery mildew in Scandinavian wheat cultivars, breeding lines and introduced genotypes with partial resistance. Plant Breeding 129(3):297–303

Lillemo M, Bjørnstad Å, Skinnes H (2012) Molecular mapping of partial resistance to powdery mildew in winter wheat cultivar Folke. Euphytica doi:10.1007/s10681-011-0620-x (online)

Liu SX, Griffey CA, Maroof MAS (2001) Identification of molecular markers associated with adult plant resistance to powdery mildew in common wheat cultivar Massey. Crop Sci 41(4):1268–1275

Ma H, Kong Z, Fu B, Li N, Zhang L, Jia H, Ma Z (2011) Identification and mapping of a new powdery mildew resistance gene on chromosome 6D of common wheat. Theor Appl Genet 123(7):1099–1106

McDonald BA, Linde C (2002) The population genetics of plant pathogens and breeding strategies for durable resistance. Euphytica 124(2):163–180

McIntosh R, Zhang P, Cowger C, Parks R, Lagudah E, Hoxha S (2011) Rye-derived powdery mildew resistance gene *Pm8* in wheat is suppressed by the *Pm3* locus. Theor Appl Genet 123(3):359–367

Mingeot D, Chantret N, Baret PV, Dekeyser A, Boukhatem N, Sourdille P, Doussinault G, Jacquemin JM (2002) Mapping QTL involved in adult plant resistance to powdery mildew in the winter wheat line RE714 in two susceptible genetic backgrounds. Plant Breeding 121(2):133–140

Miranda LM, Murphy JP, Marshall D, Leath S (2006) *Pm34*: a new powdery mildew resistance gene transferred from *Aegilops tauschii* Coss. to common wheat (*Triticum aestivum* L.). Theor Appl Genet 113(8):1497–1504

Miranda LM, Murphy JP, Marshall D, Cowger C, Leath S (2007) Chromosomal location of *Pm35*, a novel *Aegilops tauschii* derived powdery mildew resistance gene introgressed into common wheat (*Triticum aestivum* L.). Theor Appl Genet 114(8):1451–1456

Mohler V, Bauer A, Bauer C, Flath K, Schweizer G, Hartl L (2011) Genetic analysis of powdery mildew resistance in German winter wheat cultivar Cortez. Plant Breeding 130(1):35–40. doi:10.1111/j.1439-0523.2010.01824.x

Muranty H, Pavoine MT, Jaudeau B, Radek W, Doussinault G, Barloy D (2009) Two stable QTL involved in adult plant resistance to powdery mildew in the winter wheat line RE714 are expressed at different times along the growing season. Mol Breeding 23(3):445–461

Ren SX, McIntosh RA, Lu ZJ (1997) Genetic suppression of the cereal rye-derived gene *Pm8* in wheat. Euphytica 93(3):353–360

Rosewarne G, Singh R, Huerta-Espino J, William H, Bouchet S, Cloutier S, McFadden H, Lagudah E (2006) Leaf tip necrosis, molecular markers and β1-proteasome subunits associated with the slow rusting resistance genes *Lr46/Yr29*. Theor Appl Genet 112(3):500–508. doi:10.1007/s00122-005-0153-6

Saal B, Wricke G (1999) Development of simple sequence repeat markers in rye (*Secale cereale* L.). Genome 42(5):964–972

Schneider A, Molnár-Láng M (2009) Detection of the 1RS chromosome arm in Martonvásár wheat genotypes containing 1BL.1RS or 1AL.1RS translocations using SSR and STS markers. Acta Agron Hung 57(4):409–416

Singh RP (1992) Association between gene *Lr34* for leaf rust resistance and leaf tip necrosis in wheat. Crop Sci 32(4):874–878

Singh RP, Mujeeb-Kazi A, Huerta-Espino J (1998) *Lr46*: a gene conferring slow-rusting resistance to leaf rust in wheat. Phytopathology 88(9):890–894

Singh RP, Nelson JC, Sorrells ME (2000) Mapping *Yr28* and other genes for resistance to stripe rust in wheat. Crop Sci 40(4):1148–1155. doi:10.2135/cropsci2000.4041148x

Skinnes H (2002) Breakdown of race specific resistance to powdery mildew in Norwegian wheat. Cereal Rusts and Powdery Mildews Bulletin 30. Available at http://www.crpmb.org/2002/1201skinnes/

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109(6):1105–1114

Spielmeyer W, McIntosh RA, Kolmer J, Lagudah ES (2005) Powdery mildew resistance and *Lr34/Yr18* genes for durable resistance to leaf and stripe rust cosegregate at a locus on the short arm of chromosome 7D of wheat. Theor Appl Genet 111(4):731–735

Tommasini L, Yahiaoui N, Srichumpa P, Keller B (2006) Development of functional markers specific for seven seven *Pm3* resistance alleles and their validation in the bread wheat gene pool. Theor Appl Genet 114(1):165–175. doi:10.1007/s00122-006-0420-1

Tucker DM, Griffey CA, Liu S, Brown-Guedira G, Marshall DS, Maroof MAS (2007) Confirmation of three quantitative trait loci conferring adult plant resistance to powdery mildew in two winter wheat populations. Euphytica 155(1–2):1–13

Uauy C, Brevis JC, Chen XM, Khan I, Jackson L, Chicaiza O, Distelfeld A, Fahima T, Dubcovsky J (2005) High-temperature adult-plant (HTAP) stripe rust resistance gene *Yr36* from *Triticum turgidum* ssp. *dicoccoides* is closely linked to the grain protein content locus *Gpc-B1*. Theor Appl Genet 112(1):97–105. doi:10.1007/s00122-005-0109-x

Utz HF, Melchinger AE (1996) PLABQTL: a computer program to map QTL. Institute of plant breeding, seed science and population genetics, University of Hohenheim, Stuttgart

Van Ooijen J, Voorrips R (2001) Joinmap 3.0 software for the calculation of genetic linkage maps. Plant Research International, Wageningen

Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93(1):77–78

Wang ZL, Li LH, He ZH, Duan XY, Zhou YL, Chen XM, Lillemo M, Singh RP, Wang H, Xia XC (2005) Seedling and adult plant resistance to powdery mildew in Chinese bread wheat cultivars and lines. Plant Dis 89(5):457–463

William M, Singh RP, Huerta-Espino J, Islas SO, Hoisington D (2003) Molecular marker mapping of leaf rust resistance gene *Lr46* and its association with stripe rust resistance gene *Yr29* in wheat. Phytopathology 93(2):153–159

Xu WG, Li CX, Hu L, Zhang L, Zhang JZ, Dong HB, Wang GS (2010) Molecular mapping of powdery mildew resistance gene *PmHNK* in winter wheat (*Triticum aestivum* L.) cultivar Zhoumai 22. Mol Breeding 26(1):31–38

Yahiaoui N, Brunner S, Keller B (2006) Rapid generation of new powdery mildew resistance genes after wheat domestication. Plant J 47(1):85–98

Yu DZ (2000) Wheat powdery mildew in Central China: pathogen population structure and host resistance. PhD thesis, Wageningen University and Research Centre, Wageningen

Zhou Y, He ZH, Zhang GS, Xia LQ, M. CX, C. GY, B. JZ, J. YG (2004) Utilization of 1BL/1RS translocation in wheat breeding in China. Acta Agronomica Sinica 30(6):531–535

Zhu ZD, Zhou RH, Kong XY, Dong YC, Jia JZ (2005) Microsatellite markers linked to 2 powdery mildew resistance genes introgressed from *Triticum carthlicum* accession PS5 into common wheat. Genome 48(4):585–590