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ORIGINAL PAPER

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

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Abstract Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* is a major disease of wheat (*Triticum aestivum* L.) that can be controlled by resistance breeding. The CIMMYT bread wheat line Saar is known for its good level of partial and race non-specific resistance, and the aim of this study was to map QTLs for resistance to powdery mildew in a population of 113 recombinant inbred lines from a cross between Saar and the susceptible line Avocet. The population was tested over 2 years in field trials at two locations in southeastern Norway and once in Beijing, China. SSR markers were screened for association with powdery mildew resistance in a bulked segregant analysis, and linkage maps were created based on selected SSR markers and supplemented with DArT genotyping. The most important QTLs for powdery mildew resistance derived from Saar were located on chromosomes 7DS and 1BL and corresponded to the adult plant rust resistance loci *Lr34/Yr18* and *Lr46/Yr29*. A major QTL was also located on 4BL with resistance contributed by Avocet. Additional QTLs were detected at 3AS and 5AL in the Norwegian testing environments and at 5BS in Beijing. The population was also tested for leaf rust (caused by *Puccinia triticina*) and stripe rust (caused by *P. striiformis* f. sp. *tritici*) resistance and leaf tip necrosis in Mexico. QTLs for these traits were detected on 7DS and 1BL at the same positions as the QTLs for powdery mildew resistance, and confirmed the presence of *Lr34/Yr18* and *Lr46/Yr29* in Saar. The powdery mildew resistance gene at the *Lr34/Yr18* locus has recently been named *Pm38*. The powdery mildew resistance gene at the *Lr46/Yr29* locus is designated as *Pm39*.

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Introduction

Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* is an important disease of wheat (*Triticum aestivum* L.) grown in regions with temperate and maritime climates, like Europe, North and South America, Africa and parts of China (Bennett 1984; Hsam and Zeller 2002). The disease is favoured by intensive cultivation methods associated with modern agriculture such as the use of semi-dwarf and high-yielding cultivars in combination with high levels of nitrogen fertilization, and yield losses have been reported in the range from 5 to 34% (Conner et al. 2003; Griffey et al. 1993; Lipps and Madden 1988).

Host resistance is considered to be a cost effective and an environmentally friendly way of controlling the disease (Bennett 1984; Hsam and Zeller 2002). Resistance breeding is often based on the incorporation of race-specific resistance genes that give complete protection of the crop, but such genes are usually associated with a very short durability since they can be overcome by simple genetic changes in the pathogen (McDonald and Linde 2002; Skinnes 2002). As a consequence, there is increased interest in the development of germplasm with partial and race non-specific resistance, which allows the plants to be infected with the pathogen, but significantly retards the development of disease in adult plants (Hautea et al. 1987; Shaner 1973). Such resistance has also been termed slow mildewing (Roberts and Caldwell 1970) and adult plant resistance (Gustafson and Shaner 1982). Partial resistance exhibits quantitative variation, and has been shown to be durable. Examples include the winter wheat cultivar Knox (Shaner 1973) and the derived cultivar Massey (Liu et al. 2001), which have provided effective resistance against powdery mildew in the southeastern United States for half a century. Also many European wheat cultivars are well known for their good levels of partial resistance.

The quantitative nature of partial resistance to powdery mildew makes it more complicated to handle in a breeding programme compared to race-specific resistance, especially if the symptoms in the field are confounded with the effects of race-specific resistance genes with a low frequency of matching virulence genes in the pathogen population (Yu et al. 2001). The selection for partial resistance in a breeding programme could therefore be more efficient with the aid of molecular markers. Molecular markers have recently been used to map quantitative trait loci (QTL) for partial resistance to powdery mildew in several wheat cultivars, including the Swiss winter wheat Forno (Keller et al. 1999), the French winter wheats RE714 (Chantret et al. 2000, 2001; Mingeot et al. 2002) and RE9001 (Bougot et al. 2006), the North American winter wheats Massey (Liu et al. 2001) and USG3209 (Tucker et al. 2007) and the Japanese cultivar Fukuho-komugi (Liang et al. 2006).

In addition to complementing phenotypic selection for resistance in the field, selection based on molecular markers linked to genes for resistance can also facilitate resistance breeding in environments where the disease does not occur. One example is Mexico, where the main breeding operations of CIMMYT (The International Maize and Wheat Improvement Center) are located, and the absence of natural epidemics of powdery mildew makes resistance breeding difficult although powdery mildew is considered to be an important disease in many areas of the world where CIMMYT germplasm is grown. International testing has recently identified several high-yielding and widely adapted spring wheat lines with good partial resistance to powdery mildew. One of them is the spring wheat line Saar, which also has good partial resistance to leaf rust (caused by *Puccinia triticina*) and stripe rust (caused by *P. striiformis* f.sp. *tritici*) (Navabi et al. 2003, 2004). Quantitative genetic analysis based on a segregating population from a cross with the susceptible cultivar Avocet showed that the powdery mildew resistance in Saar is governed by at least three genes with additive effects (Lillemo et al. 2006). High correlations among the disease scores of powdery mildew, leaf rust and stripe rust further indicated that the resistance to these biotrophic fungal pathogens could be under some common genetic control (Lillemo et al. 2007).

The objectives of the present study were to identify and map the main genetic factors behind the powdery mildew resistance in Saar with molecular markers and to test whether some of the detected genes also are involved in the resistance to leaf rust and stripe rust.

Materials and methods

Plant material

The present study was based on a segregating population of 113 recombinant inbred F_6 lines from the cross between Avocet-*YrA* and Saar. The pedigree of Saar is Sonoita F81/Trap#1//Baviacora M92 and the population was based on a pure inbred line with selection history CG25-099Y-099M-4Y-2M-3Y-0B. Avocet-*YrA* is a line selected from the heterogeneous Australian cultivar Avocet for lacking yellow rust resistance gene *YrA*, and will throughout the rest of this paper simply be referred to as Avocet. The population was developed from randomly selected F_5 lines that were kindly provided by A. Navabi, the University of Alberta, Edmonton, Canada (Navabi et al. 2003, 2004), and grown in a net house at El Batan, Mexico in 2003/2004. Bulk harvest of the seed from each plot was used for the quantitative genetic analysis previously reported (Lillemo et al. 2006), while one single head was selected from each plot and gave rise to the F_6 lines used in the present study.

Field evaluation

Powdery mildew (PM) was evaluated over 2 years at two locations in southeastern Norway: Vollebekk research farm at Ås, about 30 km south of Oslo (59°N, 90 m above sea level) and Staur research farm close to Hamar (60°N, 153 m above sea level). Both locations experience severe natural epidemics of PM every year, but are characterized by a different virulence composition (Skinnes 2002). Field trials were conducted with a randomized complete block design with two replicates at each location in 2005 and 2006. The lines were planted in hillplots to provide favour-

able conditions for mildew development, and the planting was delayed 3–4 weeks compared to the normal planting time for spring wheat to ensure an ample source of natural inoculum. The percentage of leaf area covered with PM was recorded on penultimate leaves at weekly intervals based on a modified Cobb scale (Peterson et al. 1948) commencing at the time of heading (GS 50–59) and ending when Avocet had reached maximum severity (around GS 69–71). The area under the disease progress curve was calculated according to Bjarko and Line (1988). Symptom severity of PM was also assessed in Beijng, China, in 2005, based on a 0–9 scale (Saari and Prescott 1975). The trial was planted on 2-m rows with 30-cm row spacing and followed a randomized complete block design with three replications.

The population was evaluated for leaf rust (LR) resistance at CIANO research station, near Ciudad Obregon in northwestern Mexico during the 2004–2005 cropping season and stripe rust (YR) at CIMMYT research station near Toluca in the Mexican highlands during the 2005 cropping season. The plot size at both locations was 2 rows of 1 m length with two replications. Artificial disease epidemics were created by inoculating spreader rows with selected races of the respective pathogens to which seedlings of the two parents were susceptible. Disease severity on flag leaves was scored according to the modified Cobb scale (Peterson et al. 1948) at the time when Avocet had just reached maximum severity. In addition, leaf tip necrosis (LTN) was scored at CIANO as absent (0) or present (1).

Molecular marker genotyping

Genomic DNA was extracted from young leaves of the parents and recombinant inbred lines using the DNeasy Plant DNA extraction Kit (QIAGEN). Simple sequence repeat (SSR) markers were screened for polymorphism between Saar and Avocet. SSR analysis was performed with fluorescently labelled primers and PCR products were either separated by polyacrylamide gel electrophoresis on an ABI PRISM 377 DNA Sequencer or subjected to capillary electrophoresis on an ABI 3730 Gene Analyzer. PCR was conducted as described by Semagn et al. (2006). Resistant and susceptible bulks were created by mixing equal amounts of DNA from the five most susceptible and the five most resistant lines, respectively, based on the PM field data from the two locations in Norway in 2005. SSR markers that showed a similar pattern of polymorphism between the bulks as between the parents were used to genotype individual lines of the population. Additional markers in the vicinity of those showing association with resistance were selected based on published linkage maps of wheat, and used to genotype the population. In addition, 111 recombinant inbred lines of the population were genotyped with DArT (Diversity Array Technologies) markers by Triticarte Pty. Ltd (Canberra, Australia).

Statistical analysis

Analysis of variance was performed with the PROC GLM procedure in SAS (SAS Institute Inc., v 9.1.). The information in the ANOVA table was used to calculate the heritability (h^2) of phenotypic traits based on the formula $h^2 = \sigma_g^2/\sigma_p^2$, where $\sigma_g^2 = (\sigma_L^2 - \sigma_E^2)/r$, and $\sigma_p^2 = \sigma_g^2 + \sigma_E^2$; in this formula, σ_p^2 = phenotypic variance, σ_g^2 = genetic variance, σ_L^2 = variance of the F_6 lines, σ_E^2 = error variance, and r = number of replications (Singh et al. 1995). Pearson correlation coefficients among traits were calculated by the PROC CORR procedure in SAS. PROC CORR was also used initially to determine statistically significant associations between single markers and powdery mildew resistance after the bulked segregant analysis (BSA).

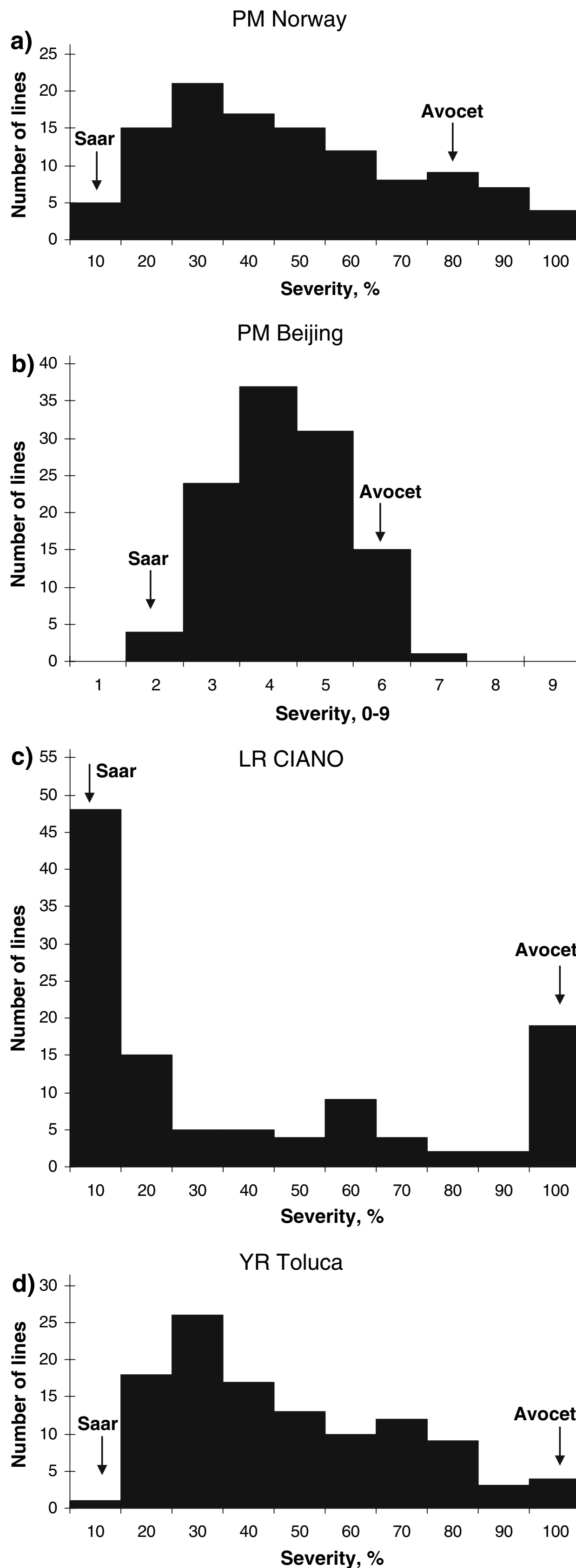
Map construction and QTL analysis

The genotypic data of SSR and DArT markers was used to construct genetic linkage maps with the software JoinMap v. 3.0 (van Ooijen and Voorrips 2001). Markers were assigned to linkage groups with a minimum logarithm of the odds (LOD) threshold of 4.0, and map distances were calculated based on the Kosambi mapping function (Kosambi 1944). QTL analysis was done with PLABQTL v. 1.2 (Utz and Melchinger 2003). Simple interval mapping (SIM) was conducted first to detect the major QTLs for powdery mildew resistance. The most closely linked markers to each of the QTLs that consistently showed effects in all environments were then selected as cofactors for composite interval mapping (CIM). The LOD threshold for declaring a significant QTL at a type 1 error rate of $\alpha = 0.05$ in one environment was set to 3.2 based on 1,000 permutations. QTLs reaching this level in one environment were also reported for other environments if their LOD scores reached an arbitrary LOD level of 2.0 and showed significant effects in multiple regression. Genetic maps and LOD curves were drawn using the programme MapChart, v. 2.1 (Voorrips 2002).

Results

Phenotypic evaluation

The disease development was good under all testing environments. Histograms of the average of the last PM score in the four testing environments in Norway, PM in Beijing and YR in Toluca revealed continuous distributions close to normality (Fig. 1), which is typical of traits with polygenic



◀ **Fig. 1** Frequency distributions of disease severities for 113 RILs of the cross between Avocet and Saar. **a** The average of the last severity score for PM across four testing environments in Norway (Vollebekk and Staur, 2005 and 2006), **b** PM in Beijing, China in 2005, **c** LR at CIANO, Mexico 2005, **d** YR in Toluca, Mexico 2005

inheritance. The LR data from CIANO showed a more bimodal distribution, which indicated that this trait is likely under control of a major genetic factor. For PM, transgressive segregation was apparent, with a substantial number of lines exhibiting higher susceptibility than Avocet, indicating that the susceptible parent may carry a gene that contributes to resistance. The same was also observed for F_5 recombinant inbred lines of the same population (Lillemo et al. 2006).

Phenotypic correlations among traits and heritabilities are presented in Table 1. The PM scores were highly correlated across the Norwegian environments. The heritability estimates were also very high in these environments, except at Hamar in 2006 trial which was affected by poor germination of some plots resulting in less favourable conditions for disease development. The lower heritability for the PM data in Beijing and weaker correlation with the Norwegian test environments is likely reflecting the use of a different scale for disease rating (0–9), which gives less discrimination among the lines. The PM data were significantly correlated with the disease scores for LR and YR, indicating that the resistance to these three biotrophic pathogens might be under some common genetic control in this population. Moreover, LTN, the phenotypic marker for the adult plant rust resistance genes *Lr34/Yr18*, was significantly correlated not only with LR and YR but also with PM (Table 1).

Construction of linkage maps

A total of 572 SSR markers were selected from the wheat consensus map (Somers et al. 2004), based on coverage of all chromosome arms and approximately even distribution along the genome. Of these, 334 markers (58%) were polymorphic between the parents, and 56 marker loci showed differentiation when tested on the resistant and susceptible bulks for PM. These markers were genotyped on individual RILs from the population and used to develop linkage maps for QTL analysis. In addition, the population was genotyped with DArT markers, and 209 polymorphic DArT loci were included in the linkage maps. A total of 34 linkage groups were created, representing chromosomal areas from 16 chromosomes.

Detection of QTLs for PM resistance

Simple interval mapping (SIM) based on the preliminary linkage maps detected QTLs on 1BL (close to *Xwmc44*),

Table 1 Pearson correlation coefficients among phenotypic traits and broad sense heritability estimates

	Correlation coefficients											Heritability h^2
	PM Ås 2005, AUDPC	PM Ås 2006, Severity	PM Ås 2006, AUDPC	PM Hamar 2005, Severity	PM Hamar 2005, AUDPC	PM Hamar 2006, Severity	PM Hamar 2006, AUDPC	PM Beijing 2005, Severity	LR CIANO 2005, Severity	YR Toluca 2005, Severity	Leaf Tip Necrosis	
PM Ås 2005, Severity	0.989	0.867	0.848	0.903	0.905	0.864	0.810	0.672	0.797	0.768	−0.713	0.95
PM Ås 2005, AUDPC		0.875	0.860	0.910	0.917	0.866	0.814	0.661	0.785	0.779	−0.688	0.97
PM Ås 2006, Severity			0.983	0.860	0.872	0.896	0.876	0.630	0.577	0.692	−0.438	0.92
PM Ås 2006, AUDPC				0.840	0.854	0.882	0.888	0.612	0.552	0.678	−0.432	0.91
PM Hamar 2005, Severity					0.989	0.878	0.821	0.695	0.764	0.718	−0.674	0.93
PM Hamar 2005, AUDPC						0.877	0.820	0.688	0.752	0.721	−0.663	0.93
PM Hamar 2006, Severity							0.960	0.663	0.645	0.702	−0.523	0.79
PM Hamar 2006, AUDPC								0.584	0.554	0.660	−0.481	0.76
PM Beijing 2005, Severity									0.547	0.552	−0.575	0.51
LR CIANO 2005, Severity										0.762	−0.849	0.98
YR Toluca 2005, Severity											−0.640	0.96

All correlations were highly significant at the level $\alpha = 0.0001$

4BL (close to *XwPt-6209*) and 7DS (close to *Xgwm1220*) in all testing environments for PM, with the resistance on 1BL and 7DS contributed by the resistant parent Saar and the resistance on 4BL contributed by Avocet. In addition, putative QTLs were detected in some environments on 2DS (close to *Xgwm296a*), 3AS (close to *Xbarc310*) and 5AL (close to *Xgwm617b*) with the resistance contributed by Saar (data not shown). The linkage maps around these loci were refined with more SSR markers based on other published maps (Bougot et al. 2006; Hayden et al. 2006; Parida et al. 2006; Somers et al. 2004; Torada et al. 2006) and two markers tightly linked to *Lr34* on 7DS; *swm10* (Bossolini et al. 2006) and *csLV34* (Lagudah et al. 2006).

Results of the final composite interval mapping (CIM) with the consistent QTL factors on 1BL, 4BL and 7DS as cofactors are shown in Table 2 and Fig. 2. The most important QTL for PM resistance was located on chromosome 7DS, with the LOD curves peaking at the *Lr34* locus between the closely linked markers *Xgwm1220* and *Xswm10* (Fig. 2). At this QTL, which explained from 19.0 to 56.5% of the phenotypic variance of the trait, the resistance was contributed by Saar. The second largest QTL was located on 4BL, around the SSR markers *Xgwm251* and *Xgwm375* and the DArT marker *XwPt-1505*. The resistance at this locus was derived from the susceptible parent Avocet, and explained from 21.0 to 40.2% of the phenotypic variance. A third QTL with significant effects in all environments was located on 1BL, between the SSR markers *Xwmc719* and *Xhbe248*, corresponding to the location of *Lr46*. The resistance at this locus was contributed by Saar and explained from 7.3 to 35.9% of the phenotypic variance. Two additional QTLs for PM resistance were detected in the Norwegian environments, on 3AS and 5AL with resistance contributed by Saar. The QTL on 3AS was located between *Xstm844tcac* and *Xbarc310* and explained from 8.1 to 20.7% of the phenotypic variance. The QTL on 5AL was located close to *Xgwm617b* and was detected above the LOD threshold of 3.2 only in Hamar 2005 and for the mean data from Norway. The contribution of this QTL in multiple regression was, however, significant in all Norwegian environments and ranged from 4.2 to 15.2%. The QTLs on 3AS and 5AL were not detected in Beijing, but the data from this location revealed a QTL on 5BS explaining 9.7% of the phenotypic variance. This QTL also had marginal effects in the Norwegian environments. The LOD curve for PM in Beijing also revealed a peak at the presumed location for *Pm5a*, close to *Xwmc581* on 7BL (Nematollahi et al. 2007), but the contribution of this QTL in multiple regression was only 4.9% and barely significant at the $\alpha = 0.05$ level. The putative QTL on 2DS, although showing a significant, but small effect on PM resistance when tested individually, was not detected in the CIM analysis and showed no significant contribution in multiple

Table 2 Results of composite interval mapping (CIM) of PM resistance with the marker loci *Xwmc719* (1BL), *Xgwm149* (4BL) and *Xswm10* (7DS) as cofactors

Chromosome arm	Position (cM)	Marker interval	Source of resistance	Ås 2005 severity	Ås 2005 AUDPC	Ås 2006 severity	Ås 2006 AUDPC	Hamar 2005 severity	Hamar 2005 AUDPC	Hamar 2006 severity	Hamar 2006 AUDPC	Mean severity Norway	Mean AUDPC Norway	Beijing 2005 severity
1BL	23	*Xwmc719-Xhbe248*	S	**32.3**	**32.1**	**18.4**	9.1	**35.4**	**35.9**	**17.9**	7.3	**31.1**	**23.9**	9.5
3AS	1	*Xstm844tcac-Xbarc310*	S	**18.8**	**20.0**	**17.7**	**19.3**	8.1	**11.4**	**18.3**	**18.0**	**19.5**	**20.7**	
4BL	28	*XwPt-1505-Xgwm149*	A	**30.6**	**29.9**	**33.6**	**30.8**	**33.4**	**33.9**	**36.9**	**28.5**	**40.2**	**35.3**	**21.0**
5AL	54	*Xgwm617b- Xwmc327*	S	4.3	4.2	8.0	9.7	**13.0**	**15.2**	5.9	7.0	**10.2**	**10.1**	
5BS	22	*Xbarc4-Xgwm274b*	S	8.2	7.8	8.1		9.1	8.8	4.5		9.0	5.5	**9.7**
7BL	70	*Xwmc581- XwPt-8007*												**4.9**
7DS	31	*Xgwm1220-Xswm10*	S	**56.5**	**53.6**	**20.5**	**19.0**	**49.7**	**49.1**	**32.0**	**24.5**	**46.3**	**40.3**	**27.9**
Percentage of the phenotypic variance explained				72.2	71.0	59.7	54.1	71.6	72.3	61.8	51.8	72.0	67.3	48.8
Percentage of the genotypic variance explained				75.6	73.5	64.7	59.1	77.1	77.4	78.0	68.2	80.4	77.7	96.0

R^2 values obtained from multiple regression in PlabQTL. QTLs that were detected with a LOD score above the threshold of 3.2 determined by permutation tests are highlighted in bold. Other putative QTL are also listed if they showed significant association with the trait in the multiple regression

S Saar, *A* Avocet

regression when tested together with the other detected QTLs. No significant epistatic interactions were found among the QTLs for PM resistance.

Effects of detected QTLs on LR, YR and LTN

SIM with the LR and YR data from Mexico detected two consistent QTLs for both diseases, corresponding to the *Lr46/Yr29* and *Lr34/Yr18* loci on 1BL and 7DS with resistance contributed by Saar. CIM with these two QTLs as cofactors did not detect any other significant QTL for LR (Table 3, Fig. 2). The effects of 1BL and 7DS on LR showed a significant and negative deviation from a simple additive model, and the final fitted model with the interaction term accounted for 82.5% of the genetic variance for LR, with the biggest contribution coming from the *Lr34* locus on 7DS (Table 3). The interaction between *Lr46* and *Lr34* is illustrated in Fig. 3, showing that the additive effect of *Lr46* on LR is much less in the presence of *Lr34*.

CIM detected two further QTLs for YR resistance with a LOD score above the significance threshold of 3.2. One was located on 3AS, at the same position as the QTL for PM resistance with resistance from Saar, while the other was mapped on 6AL between *Xbarc3* and *XwPt-7063*, and had resistance contributed by Avocet (Table 3, Fig. 2). In contrast to the situation for LR, no significant interactions were found among the detected QTLs for YR resistance. Altogether, the four QTLs explained about half of the genetic variance for the trait (Table 3).

The phenotypic marker LTN deviated slightly from the expected 1:1 ratio of a single gene with 61 lines with and 45 lines without LTN ($\chi^2 = 2.42$, $P = 0.12$). Instead of trying to place it on the linkage map as a marker, SIM was used to study the genetic basis for the trait. As expected, the LOD curve peaked at the *Lr34/Yr18* locus on 7DS (Fig. 2), which accounted for most of the variation, but there was also a small, but significant contribution from the *Lr46/Yr29* locus on 1BL (Table 3).

Discussion

In this study, we have conducted QTL mapping for resistance to three different biotrophic pathogens in the same population, which was feasible due to the high levels of partial and race non-specific resistance to PM, LR and YR in Saar and the corresponding susceptibility to all three diseases by Avocet. The high correlations among the disease data suggested that resistance to these three diseases might be under some common genetic control, and this was indeed confirmed by the QTL analysis. Although the bulked segregant analysis (BSA) and subsequent QTL mapping was only based on the PM data, two of the

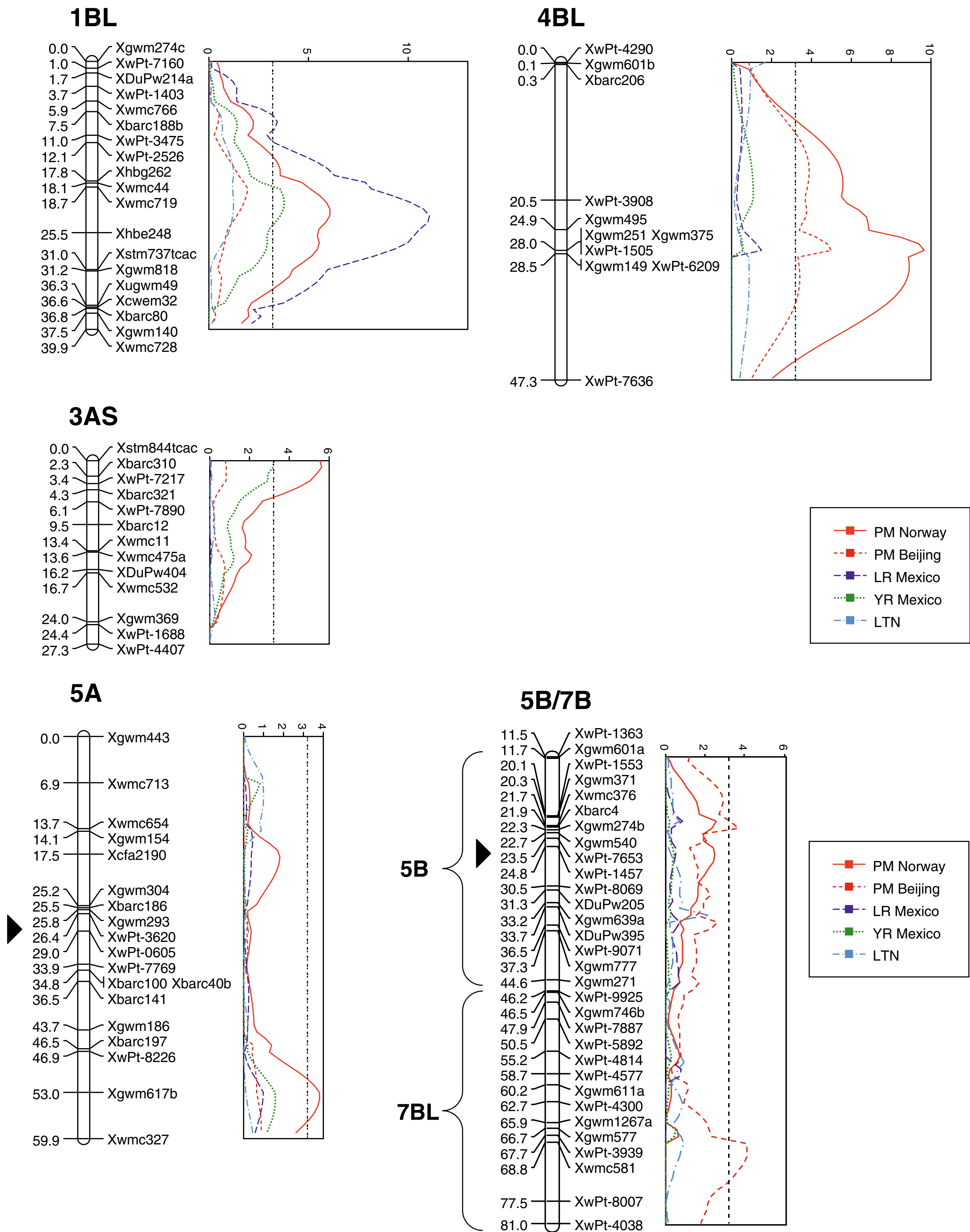


Fig. 2 Linkage groups showing significant association with PM, LR and YR resistance and LTN, with corresponding LOD curves obtained from either CIM or SIM as described in the text. Genetic distances are shown in centimorgans to the left of each linkage group. The LOD significance threshold of 3.2 is indicated by a *dashed line*. The approximate positions of centromeres are indicated by *closed arrowheads*

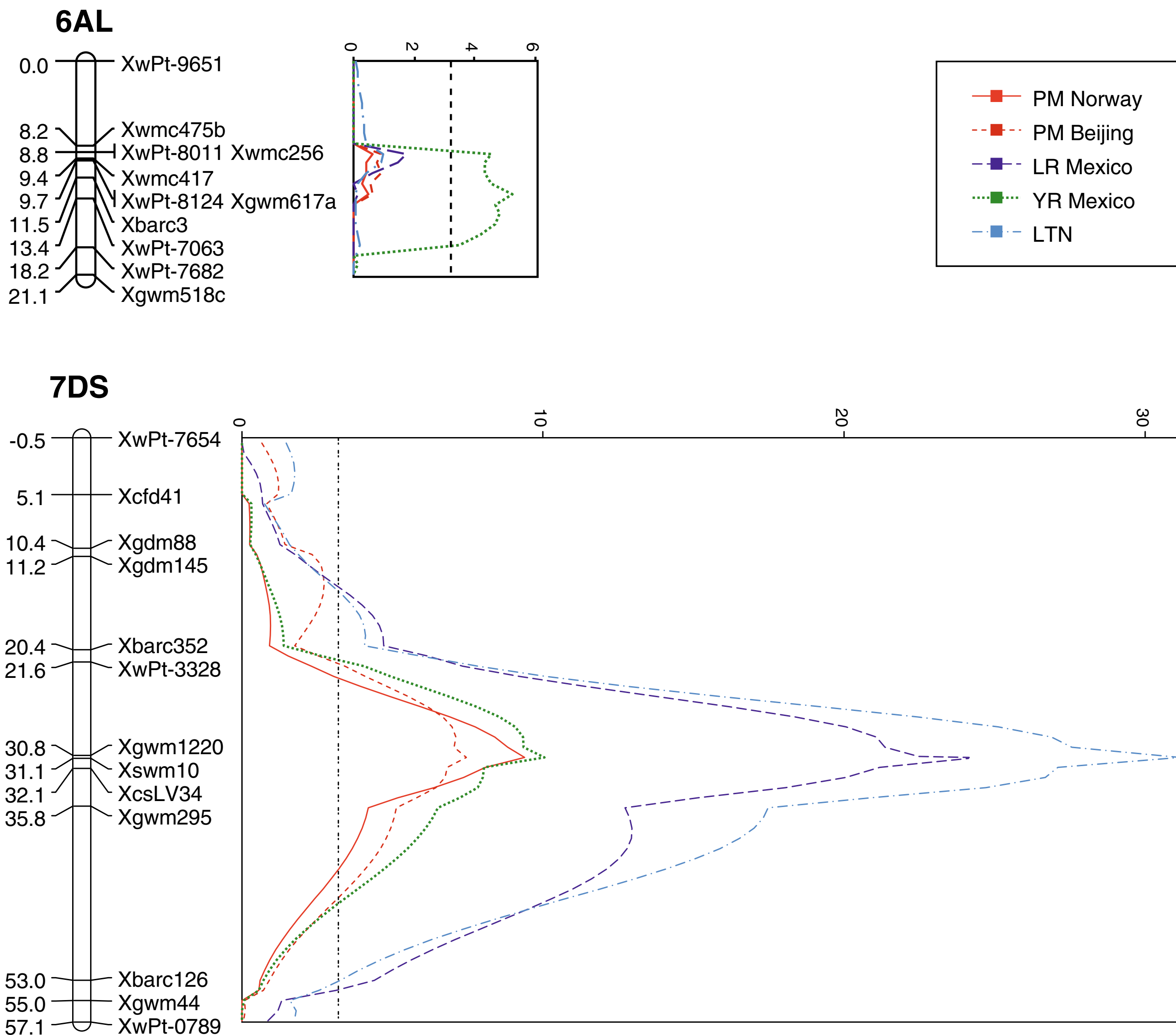


Fig. 2 continued

detected QTLs for PM resistance also had major effects on LR and YR. The genetic aspects and potential impact of this important finding will be discussed at the end of this section.

Genetic control of PM resistance in Saar

The PM resistance of Saar in the Norwegian testing environments was shown to be mainly controlled by three major QTLs on 7DS, 1BL and 3AS as well as a minor QTL on 5AL and a barely detectable locus on 5BS. This is largely in agreement with the quantitative genetic study of the same population (Lillemo et al. 2006) indicating at least three genes for partial resistance from Saar. The other indication of a resistance gene from Avocet was also confirmed by a major QTL mapped on 4BL. Altogether, these QTLs explained from 59 to 80% of the genetic variance for PM resistance in the Norwegian environments (Table 2), which indicates that the BSA strategy was successful in detecting the most important genetic factors behind the resistance.

Other recent QTL mapping studies have also confirmed that partial resistance to PM is under control of a few loci with relatively large effects. Three genes explained about half of the phenotypic variance for resistance to PM in the winter wheat Massey (Liu et al. 2001) and in the derived cultivar USG3209 (Tucker et al. 2007). Liang et al. (2006) detected four QTLs for PM resistance in the Fukuho-komugi by Oligoculm population, each explaining from 6 to 27% of the phenotypic variance. Several QTLs were detected in the winter wheat line RE714, though only two major loci showed stable expression across environments and genetic backgrounds (Mingeot et al. 2002). Likewise,

Table 3 Results of composite interval mapping (CIM) of LR and YR resistance with the marker loci *Xwmc719* (1BL) and *Xswm10* (7DS) as cofactors, and simple interval mapping (SIM) for Leaf Tip Necrosis

Chromosome arm	Position (cM)	Marker interval	Source of resistance	LR Cd. Obregon, Mexico 2005	YR Toluca, Mexico 2005	Leaf Tip Necrosis
1BL	23	*Xwmc719-Xhbe248*	S	**49.1**	**17.4**	12.1
3AS	0	*Xstm844tcac-Xbarc310*	S		**8.7**	
6AL	13	*Xbarc3-XwPt-7063*	A		**14.4**	
7DS	31	*XwPt-3328 -XcsLV34*	S	**73.1**	**40.0**	**76.6**
1BL × 7DS				34.3		
Percentage of the phenotypic variance explained				81.2	52.8	76.9
Percentage of the genotypic variance explained				82.5	55.3	–

R^2 values obtained from multiple regression in PlabQTL. QTLs that were detected with a LOD score above the threshold of 3.2 determined by permutation tests are highlighted in bold

S Saar, *A* Avocet

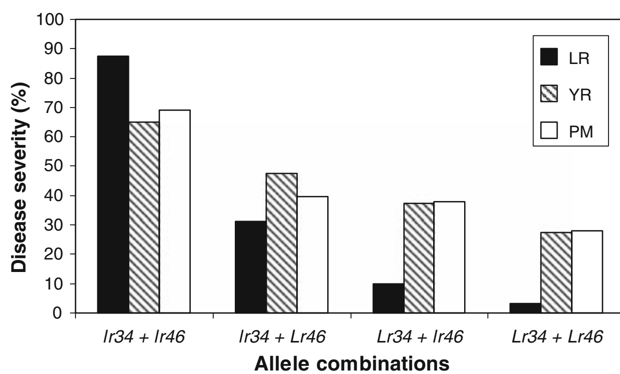


Fig. 3 Effects of the *Lr34/Yr18* and *Lr46/Yr29* loci on LR, YR and PM of 113 RILs derived from the cross between Avocet and Saar. The effects are calculated as the average disease severity of lines with different allelic combinations as determined by the flanking markers *Xgwm1220* and *Xswm10* for *Lr34/Yr18* and *Xwmc719* and *Xhbe248* for *Lr46/Yr29*. The figure is based on LR and YR data from Mexico and the mean PM severity score for the Norwegian testing environments

although Keller et al. (1999) reported 18 QTLs for PM resistance in a segregating wheat by spelt population, only two QTLs with major effects were consistent across environments.

As demonstrated in the present study, the two most important genetic factors behind the PM resistance in Saar were located at the *Lr34/Yr18* and *Lr46/Yr29* loci on chromosomes 7DS and 1BL, respectively. Previous studies have also detected important QTLs for PM resistance in chromosomal areas corresponding to these loci, although no direct genetic relationship with rust resistance was suggested in these studies. The QTL on 7DS corresponds to similar QTLs detected in the Synthetic × Opata (Börner et al. 2002) and Fukuho-komugi × Oligoculm (Liang et al. 2006) mapping populations. Likewise, the QTL on 1BL corresponds with a QTL detected at s similar position in the winter wheat Massey (Liu et al. 2001) and the derived cultivar USG3209 (Tucker et al. 2007).

To our best knowledge, this is the first report of a QTL for powdery mildew resistance on the short arm of chromosome 3A. Although this locus was significant only in the Norwegian testing environments, the LOD curves indicate that this QTL also had effects on PM in Beijing and on YR in Toluca (Fig. 2). Considering that no gene for race-specific resistance to PM is known at this location (Huang and Röder 2004), and that Saar exhibits race non-specific PM resistance, the QTL detected is likely a gene for partial resistance to PM.

The minor QTL for PM resistance 5AL close to the marker *Xgwm617b* could correspond to similar loci for partial resistance to PM detected in the Swiss winter wheat Forno (Keller et al. 1999) and *T. militinae* (Jakobson et al. 2006).

A major genetic factor for PM resistance was found to be inherited from the susceptible parent Avocet. This QTL was located on chromosome 4BL at the same position as similar QTLs for PM resistance detected in the Forno × Oberkulmer (Keller et al. 1999), Synthetic × Opata (Börner et al. 2002) and Fukuho-komugi × Oligoculm (Liang et al. 2006) populations. The same QTL from Avocet showed significant effects on LR and YR in the Avocet × Pavon population (William et al. 2006); but although the LOD curves for LR and YR also peaked at the same location in the present study (Fig. 2), the effects were far from being significant.

Although the three most important QTLs on 1BL, 4BL and 7DS in the present study showed significant and relatively similar effects across all the testing environments for PM, there were some discrepancies among the other QTLs detected in Norway and China. The lack of significance of the 3AS and 5AL QTLs in Beijing and a higher contribution

by the 5BS QTL in that environment could be due to the different scales used for scoring the disease. While PM severity in Norway was scored as the percentage of leaf area of penultimate leaves covered with the disease, the whole canopy was assessed on a 0–9 scale in Beijing. Moreover, the Beijing trial used single rows while hillplots were used in Norway. These methods are likely to emphasize different aspects of the PM resistance, and the slightly different results obtained in the QTL mapping were therefore not unexpected. Environmental differences could also account for some of the variation in the QTL effects between Norway and China, while the different virulence composition of *Blumeria graminis* f. sp. *tritici* in the two countries was not expected to influence the results due to the partial and race non-specific nature of the PM resistance in Saar (Lillemo et al. 2006). Saar has been tested against a collection of 18 differential isolates at the seedling stage and only found to have a reaction pattern corresponding to *Pm5a* (M. Lillemo, unpublished). The presence of *Pm5a* was also confirmed by the QTL analysis, but this gene did not affect the PM scores in Norway and had only negligible effects on the PM severity in Beijing, which is in agreement with the widespread virulence against this defeated resistance gene in both Norway (Skinnes 2002) and China (Yu 2000). Since *Pm5a* was the only race-specific resistance detected at the seedling stage, and all the other QTLs showed relatively consistent effects across all environments, one might conclude that all the detected QTLs for PM resistance in Saar are likely to represent genes for partial and race non-specific resistance.

An interesting finding of the map construction was the detection of an interchromosomal translocation between the centromeric part of 5B and the distal part of the long arm of 7B in Saar (Fig. 2). This translocation is known from Capelle Desprez and many other European wheat cultivars (Riley et al. 1967), and has been associated with increased adult plant resistance to YR (Law and Worland 1997). The YR resistance was attributed to the 5BS arm of the translocation (Law and Worland 1997), and in the present study we found a significant QTL for PM resistance on 5BS, close to the SSR marker *Xbarc4*. However, we did not find any significant QTL for YR resistance associated with the 5B/7B translocation, which may indicate that the translocation in Saar is different from the one in Capelle Desprez or that the YR resistance was not effective in the Toluca testing environment.

Genetic control of LR and YR resistance in Saar

Since the primary objective of the present QTL study was to detect the main genetic factors behind the PM resistance in Saar, the linkage maps obtained from the BSA approach have on purpose excluded most of the wheat genome that did not represent chromosomal areas involved in the PM resistance. Thus, the QTL mapping of LR and YR resistance presented in this study does not constitute any complete molecular genetic analysis of these traits, and was included just for the purpose of testing whether some of the detected QTLs for PM resistance could also be involved in resistance to LR and YR.

This analysis confirmed the presence of *Lr34*/*Yr18* in Saar, while the presence of *Lr46/Yr29* had not been inferred by previous studies (Navabi et al. 2003, 2004). While the analysis of the LR data indicated that these two loci account for most of the genetic variation for this trait at Cd. Obregon, their magnitude of effect on YR is more similar to the effect on PM (Tables 2, 3). The QTL on 6AL with YR resistance contributed by Avocet was also detected in the Avocet × Pavon population and is likely due to a translocation from *Agropyron elongatum* in Avocet that is known to carry the stem rust resistance gene *Sr26* (William et al. 2006). The Avocet × Saar population is possibly segregating for other important, but yet undetected genetic factors for YR resistance, as the four QTLs on 1BL, 3AS, 6AL and 7DS did not explain much more than half of the genetic variance for the trait (Table 3).

The non-additive interaction between *Lr34* and *Lr46* for LR (Table 3, Fig. 3) can probably be explained by the similar resistance mechanisms of these genes. They both confer partial resistance with increased latent period and decreased infection frequency and uredinium size, and both are more clearly expressed in adult plants than in seedlings (Martinez et al. 2001; Rubiales and Niks 1995).

General disease resistance loci on 7DS and 1BL

The co-location of genes for partial and race non-specific resistance to three biotrophic pathogens at the *Lr34/Yr18* and *Lr46/Yr29* loci on 7DS and 1BL is the most important finding of this study. The tight linkage or potential pleiotropy of genes for LR and YR resistance at these two loci has been widely known and utilized in wheat breeding (Singh 1992b; William et al. 2003), but their involvement in resistance to PM has not been suggested until recently. The first direct evidence for the association of *Lr34/Yr18* with resistance to PM came from a field experiment in Australia, where a population of recombinant inbred lines that segregated for *Lr34/Yr18* in the near-isogenic background of the susceptible cultivar Thatcher became infected with PM (Spielmeyer et al. 2005). The uniform genetic background of the population allowed for the unambiguous scoring of lines as either homozygous resistant or homozygous susceptible to all three diseases, and no recombination was detected among the resistance genes to these three diseases in the 107 lines tested (Spielmeyer et al. 2005). Independently from this study, Lillemo et al. (2007) tested

near-isogenic lines of *Lr34/Yr18* and *Lr46/Yr29* in the genetic background of Avocet and found significant effects of both loci on PM. Another common feature of these two disease resistance loci is the expression of LTN, a premature senescence of the leaf tips about 1–2 weeks after flowering, which is widely used as a phenotypic marker for the rust resistance at these loci (Rosewarne et al. 2006; Singh 1992a). Another indication that the resistances to LR, YR and PM at these two loci are not just caused by genetic linkage is that the resistance effects of the *Lr34/Yr18* locus are stronger than the effects of the *Lr46/Yr29* locus for all three diseases (Lillemo et al. 2007; Martinez et al. 2001). This was also confirmed by the present study. The partial resistance to PM at the *Lr34/Yr18* locus has been given the gene designation *Pm38* (R. McIntosh, personal communication), and based on the analogous effect of the *Lr46/Yr29* locus on PM as shown in this study and previously on the Avocet NILs (Lillemo et al. 2007) we designate the corresponding PM resistance gene as *Pm39*.

Interestingly, Saar was never exposed to PM and selected for resistance to this disease during its breeding in Mexico, but nevertheless obtained a good partial resistance to PM through the intense selection for race non-specific resistance to LR and YR based on *Lr34/Yr18* and *Lr46/Yr29*. It is a major advantage to resistance breeding that race non-specific and potentially durable resistance to three of the most important biotrophic pathogens in wheat can be selected for simultaneously by these two loci by field testing for only one of the diseases. Moreover, the resistance of these loci can also be selected based on the expression of LTN or facilitated by the closely linked molecular markers presented in this study.

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