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# 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

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**Abstract** Powdery mildew (*Blumeria graminis* f. sp. *tritici*) is one of the major diseases of wheat (*Triticum aestivum*). Adult plant resistance (APR) to powdery mildew is considered more durable than resistance conferred by major race-specific resistance genes. The objective of the present study was a better understanding of the genetic basis of APR in RE714 by means of QTL analysis of several resistance scores along the growing season. A population of 160 recombinant inbred lines obtained from the cross between RE714 and Hardi (susceptible) was assessed for APR under natural infection conditions during 3 years and a genetic map with whole genome coverage was developed with microsatellite and AFLP markers in this population. Two major QTL on chromosomes 5D and 6A were detected each year, and 6 minor QTL were detected only in 1 or 2 years. The QTL on chromosome 5D was detected during all the growing season each year and its $R^2$ value varied between 8.5 and 56.3%, whereas the QTL on chromosome 6A was detected at 1–4 scoring dates in the 3 years, and its $R^2$ value varied between 6.1 and 20.5%. The two QTL explained between 24.4 and 52.1% of the phenotypic variance for AUDPC, depending on the year. The models including QTL and cofactors in the composite interval mapping explained between 29 and 72% of the variance. The molecular markers linked to the two major QTL could be used in marker-assisted selection for adult plant resistance to powdery mildew.

**Keywords** QTL mapping · *Triticum aestivum* · *Blumeria graminis* f. sp. *tritici* · Adult plant resistance

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## Introduction

Powdery mildew, caused by the obligate parasitic fungus *Blumeria graminis* f. sp. *tritici*, is one of the major diseases of wheat in areas with maritime or semi-continental climates. Agricultural systems with high rates of nitrogen fertilizer and high cropping densities are particularly affected. The use of resistant cultivars is the most economical and environmentally safe means of controlling powdery mildew (Bennet

1984). To date, genes for powdery mildew resistance (*Pm* genes) have been described at 39 officially named and several temporarily designated gene loci in common wheat (Hsam et al. 2003; Lillemo et al. 2008; McIntosh et al. 2003; Miranda et al. 2007, 2006; Zhu et al. 2005). Usually, these genes are detected by powdery mildew resistance tests on seedlings or on detached leaves of seedlings (Hsam and Zeller 2002) but are thought to confer resistance which is expressed throughout the life cycle of the host. However, these resistances are race-specific and can be overcome when the pathogen population gains the matching virulence gene. Breeders therefore try to shift their efforts to breeding for more durable resistance. Incomplete resistance retarding growth and reproduction of powdery mildew in adult plants is considered to be non-race specific. It has been termed 'slow mildewing', adult-plant resistance (APR) or 'partial resistance'. Genetics studies on APR in winter wheat cultivars are more difficult to conduct than on race-specific resistance because resistance has to be assessed in field experiments that have to be replicated in several environments. Therefore, the identification of markers linked to quantitative trait loci (QTL) of APR would provide an opportunity for implementing marker-assisted selection (MAS) of resistant plants in breeding populations.

During the past 10 years, several studies on the genetic control of APR to powdery mildew were carried out in several wheat lines, including the Swiss cultivar Forno (Keller et al. 1999), the US' cultivar Massey and its derivatives (Liu et al. 2001; Tucker et al. 2006), the spring variety Opata 85 and the synthetic wheat W7984 (Börner et al. 2002), the French breeder lines RE714 (Chantret et al. 2001; Mingeot et al. 2002) and RE9001 (Bougot et al. 2006), the Japanese cultivar Fukuho-komugi (Liang et al. 2006), a *T. militinae* introgression line (Jakobson et al. 2006) and the CYMMIT cultivar Saar (Lillemo et al. 2008). In these studies, 3–18 QTL were identified, among which between one and three were consistent across environments.

Successful application of molecular markers for MAS in plant breeding depends on the availability of easy to use markers which are associated with validated QTL. Microsatellites (or SSRs) represent such markers that are amenable to automation. In wheat, the high number of microsatellite primer pairs that have been developed provide an efficient means to saturate QTL regions with markers. Their high polymorphism and genome specificity make them a marker system of choice to detect QTL. However, their use in practical wheat breeding programs has been limited so far because QTL-marker association has not been sufficiently validated in different genetic backgrounds and in various environments.

The winter wheat line RE714 results from a breeding program for disease resistance at INRA Rennes (France) and has been demonstrated to express powdery mildew resistance at the seedling, vernalized and adult stage. Moreover, RE714 adult plant resistance was expressed even when the field mildew population was able to attack RE714 seedlings (Robe et al. 1996) and this adult plant resistance was reported to be partially explained by the residual effect of *MlRE*, a resistance gene defined by resistance scoring at the seedling stage with specific isolates, in RE714 × Hardi segregating populations (Chantret et al. 1999). However, *MlRE* was latter broken up into two QTL, located on chromosomes 5D and 6A (Chantret et al. 2000). Chantret et al. (2001) and Mingeot et al. (2002) studied APR to powdery mildew in RE714 in two small doubled haploid line populations, namely a 41 DH line population from the cross RE714 × 'Festin' and a 44 DH line population from the cross RE714 × 'Hardi', and in a $F_{2:3}$ population with a partial map (4A, 5D, 6A, 7A, 7B). Only two QTL, mapped on chromosomes 5D and 6A, were consistent across the three populations and the eight environments. The objectives of the present study were : (1) to improve the power of QTL detection by the use of a population of 160 recombinant inbred lines from the cross RE714 × Hardi to analyse RE714 powdery mildew resistance at the adult stage, (2) to study the expression of the QTL along the growing season, by analysing both the AUDPC and the individual scores obtained at several scoring dates, and (3) to enrich with markers the main QTL regions and assess the potential of marker-assisted breeding for APR to powdery mildew. This study was conducted with a map representing good genome coverage and careful data analysis (composite interval mapping (CIM), estimation of appropriate threshold by permutation and sequential search).

## Materials and methods

### Plant material

A population of 160 recombinant inbred $F_6$ lines (RIL) was produced from the cross between RE714 and Hardi (a susceptible cultivar that does not possess any specific-resistance gene). The pedigree of RE714 is {[(*Aegilops squarrosa* n°33 × *Triticum dicoccum* n°119) × (VPM × Moisson)] × Beauchamp}. Several genotypes from the genealogy of RE714 were used as controls in the disease resistance assessments: the wheat lines VPM and Beauchamp, and the lines *Aegilops squarrosa* n°33 and *Triticum dicoccum* n°119, from related species of wheat. The cultivar Barbee (highly susceptible) was used as a contaminator.

### Disease assessments at the adult plant stage

Adult plant resistance was assessed under naturally infested conditions in Le Rheu (France) during three growing seasons. In 1999–2000, the test was done in the field, whereas in 2000–2001 and 2001–2002, the tests were done in market gardening tunnels. The tunnels were opened at each end so they did not restrict inoculation by the wind-dispersed powdery mildew spores. The sowing dates were October 14, 1999, October 20, 2000 and February 07, 2002. The 2002 test was sown late because there was not enough room for this test in the market gardening tunnels in October 2001. The RIL were sown as three replicate hill plots (25 seeds/hill plot). The very susceptible cultivar Barbee was sown as a line of hill plots for every two lines of tested hill plots in the field, and only around each replicate (7 × 25 hill plots) in the tunnel. The powdery mildew populations have several asexual multiplication cycles during the growing season, depending on weather conditions. The Barbee plants around the tested plants were intended to act as powdery mildew spreader plants. The level of disease was assessed on a 1–9 scale based on observation inside each hill plot of sporulation intensity, leaf area covered by sporulating colonies and distribution of symptoms along the plant (Robe et al. 1996). This assessment was done 4–6 times during the growing season, depending on the progression of the disease on the susceptible check Barbee. Plants were at the stem elongation stage in the field in 2000 and in the middle of the tillering stage in the market gardening tunnels in 2001 and 2002 for the first scoring and at the anthesis stage for the last scoring in the 3 years. The area under disease progress curve (AUDPC) was calculated for each hill plot using the several disease assessments.

As the tests were conducted under natural epidemics, the population of powdery mildew was analysed in the three environments in the middle of the growing season for the presence of virulence genes matching known resistance genes using a set of differential hosts as described by Chantret et al. (1999). Seedlings contaminated in the tests were scored after 10 days on the 0–9 scale (from no visible symptoms to heavily sporulating symptoms) usually used for seedling resistance assessment (Robe and Doussinault 1995). In 2000, when the test was done in the field, a more quantitative analysis was used: seedlings of 'Renan' (*Pm4b*), *T. dicoccum* 119 (*MlRE*), RE714, 'Hardi', *Ae. squarrosa* 33, 'Beauchamp' and 'Barbee' were maintained in the field for 4 h for contamination and the number of powdery mildew colonies on two leaves was counted 10 days later. The frequencies of powdery mildew genotypes with virulence matching the resistance of *T. dicoccum* 119, 'Renan' or RE714 were estimated on the basis of the number of powdery mildew colonies relative to the number of powdery mildew colonies on the susceptible check Barbee.

### Molecular marker analysis

Genomic DNA from the RIL and the parents was extracted from fresh leaves of $F_6$ plants following a modified CTAB method (Doyle and Doyle 1990). The DNA concentration was adjusted to 10 ng/µl. PCR reactions for microsatellite markers were performed as described by Tixier et al. (1997). PCR products were separated on 6% polyamide denaturing gels and visualized by silver nitrate staining or using a Li-Cor DNA analyzer (Li-Cor Inc., Lincoln, NE, USA). For the latter system, each right primer was 5′-tailed with the M13 forward consensus sequence. The M13-tailed right primers were then used in combination with a standard M13 primer dye-labelled at its 5′-end (Boutin-Ganache et al. 2001). A total of 766 wheat microsatellite primer pairs were screened for polymorphism. 272 primer pairs were used to genotype the population.

AFLP analysis was carried out according to the methods of Vos et al. (1995) with some modifications. 250 ng of genomic DNA was initially cut with *Mse*I and *Pst*I as the frequent- and rare-cutter

enzymes, respectively. The selective amplification step was performed with primers having three selective nucleotides and the PstI primers were labelled either with IRD700 or with IRD800 dye. Selective amplification was performed in 5 µl reaction volumes. After pooling of PCR products labelled with IRD700 and IRD800, AFLP fragments were separated on a Li-Cor DNA analyzer using polyacrylamide gels. A size marker was placed every 20 lanes to facilitate the semiautomatic analysis of the gel and the sizing of the fragments. Semiautomated analysis was performed with AFLP-Quantar® Pro (Keygene).

A total of 115 MseI-PstI primer combinations were screened for polymorphism and 16 informative primer pairs were used to genotype the population. The standard list for AFLP primer nomenclature was employed (http://wheat.pw.usda.gov/ggpages/keygeneAFLPs.html).

### Map construction

For each segregating marker, a $\chi^2$ analysis was performed to test for deviation from the expected 1:1 segregation ratio. Linkage analysis was performed using MAPMAKER/EXP ver. 3.0b (Lander et al. 1987). Linkage groups were established using a minimum LOD score of 3.0 and a maximum distance of 50 cM (no restriction) after preliminary analysis using LOD scores ranging from 3 to 7. Linkage groups were assigned to chromosomes by comparison with previously published maps. Recombination fractions were converted into map distances in centiMorgans (cM) using the Haldane mapping function (Haldane 1919).

### Statistical analyses

Statistical analyses of the trait data were performed with Splus and R (R-Development-Core-Team 2006). Replicate and genotype effects were estimated by analysis of variance (ANOVA). For each trait, heritability of the means was estimated from ANOVA using the formula

$$\hat{h}^2 = 1 - \frac{\mathrm{MS_e}}{\mathrm{MS_{gt}}},$$

where $MS_{gt}$ and $MS_e$ are mean square for genotype and residual error, respectively (Knapp et al. 1985). Adjusted means were used for QTL analysis.

### QTL analysis

QTL analysis was performed by CIM (Zeng 1993, 1994) using the QTL CARTOGRAPHER package (Basten et al. 2003). A forward–backward regression analysis was performed to choose cofactors before QTL detection by CIM for each trait. Five cofactors with the highest *F*-value were taken into account. A window of 10 cM size was blocked out on either side of the markers flanking the test position when picking background markers. Permutation tests were performed to estimate appropriate 5% significance thresholds for each trait (1,000 permutations). To detect QTL with minor effects, we applied a sequential search: new traits were derived from the initial ones as the residuals from a model fitted for major QTL effects and permutations and QTL search were performed on these new traits (Doerge and Churchill 1996). This was repeated until no more QTL were detected. QTL × year interactions for AUDPC were tested using R (R-Development-Core-Team 2006). To identify digenic epistatic interactions, we tested all possible marker × marker interactions and QTL × QTL interactions using R (R-Development-Core-Team 2006).

## Results

### Environmental conditions

During the 1999–2000 growing season in Le Rheu, December was particularly wet (182.5 mm) and April and May were slightly wetter than usual with around 36 mm more rain. Temperatures were higher than usual in December (+1.4°C), February (+2.7°C), May (+2.1°C) and June (+1.8°C), whereas the others months were near usual. During the 2000–2001 growing season, December (+2.7°C) and March (+1.9°C) were warmer than usual and in 2002, February was particularly warmer than usual (+3.0°C).

### Phenotypic variation in the mapping population

In 2002, two replicates planted in a different market-gardening tunnel from the one used for the third replicate did not grew normally and had to be dismissed.

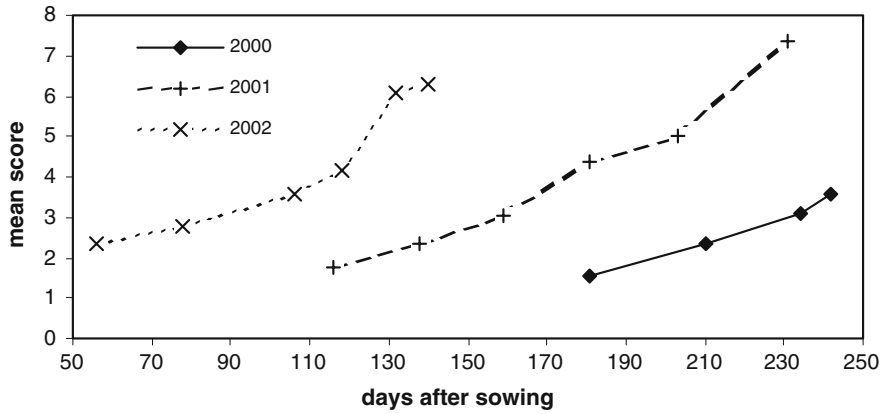



**Fig. 1** Mean powdery mildew disease progression in 3 years of test in the field (2000) or in market-gardening tunnels (2001 and 2002)

The susceptible check Barbee was heavily covered with powdery mildew colonies when the RIL were scored throughout the growing season in the 3 years. Phenotypic means of the population progressed from 1.54 to 3.59 between the first and the last scoring dates in 2000 (four scoring dates), when the test was done in the field, and from 1.79 to 7.36 in 2001 (six scoring dates) and from 2.34 to 6.28 in 2002 (six scoring dates), when the tests were done in market-gardening tunnels (Fig. 1). The most susceptible RIL and the susceptible parental line 'Hardi' reached the highest score of nine at the end of the scorings in market-gardening tunnels in 2001 and 2002 but not in the field in 2000 (Supplementary Table 1). The market-gardening tunnels provided an environment more favourable to powdery mildew because they were warmer and more humid (the plants were regularly watered) than the field. In 2002, scoring began much sooner after sowing than the other years because the experiment was late sown and encountered warm conditions. The resulting mean AUDPC were 149 in 2000, 463 in 2001 and 312 in 2002 (Table 1).

There was significant genotypic variation among RIL for powdery mildew resistance at adult plant stage (genotypic effect significant with $P < 10^{-3}$ in ANOVA), and a few transgressive lines with increased resistance or susceptibility were observed. The mean-based heritabilities observed for AUDPC and the individual scorings varied between 0.38 and 0.89 (Tables 1, 2). An ANOVA for AUDPC data from the 3 years showed that all the effects (year, replication within year, genotype and genotype × year interaction) were highly significant ($P < 0.0001$). However, between year correlations for AUDPC were positive and highly significant, ranging from 0.60 between 2000 and 2002 to 0.71 between 2001 and 2002.

**Table 1** Mean AUDPC for powdery mildew of the parental lines RE714 and 'Hardi', and the RIL derived from their cross, minima and maxima for each year of test, and mean-based heritabilities

| Year | RE714 | Hardi | RIL | Minimum | Maximum | $h^2$ |
|---|---|---|---|---|---|---|
| 2000 | 84.5 | 238.5 | 149 | 61 | 332.8 | 0.82 |
| 2001 | 325 | 668 | 463 | 259.7 | 680.2 | 0.89 |
| 2002 | 170 | 543 | 312 | 132 | 549 | – |

The analysis of the virulence genes present in the natural populations infecting the plants in the phenotypic tests showed that the virulence genes present did not vary greatly between years. In particular, the virulence genes matching the resistance genes of 'Renan' (*Pm4b*), *T. dicoccum* 119 (*MlRE*) and RE714 (*Pm4b* + *MlRE*) were present in the population in the three tests. However, in 2000, the estimation of genotype frequencies on the basis of colony numbers showed that powdery mildew genotypes with virulence genes matching the resistance genes of RE714 seemed to be less frequent than expected, if virulence genes matching its two resistance genes were randomly assorted.

## Map construction

A total of 534 marker loci were obtained, including 315 obtained with microsatellites and 219 with AFLP. Seventy-eight marker loci (14.6%) deviated significantly from the expected ratio for RIL (1:1) at $P < 0.01$ (30 from microsatellites (9.6%) and 48 from AFLP (21.9%)). Among the framework marker loci

**Table 2** Mean-based heritabilities of the individual disease scorings

| Year | Scoring dates | | | | | |
|---|---|---|---|---|---|---|
| 2000 | 13-apr | 12-May | 05-June | 13-June | | |
| | 0.60 | 0.73 | 0.80 | 0.77 | | |
| 2001 | 13-feb | 07-Mar | 28-Mar | 19-Apr | 11-May | 08-June |
| | 0.39 | 0.38 | 0.82 | 0.88 | 0.79 | 0.60 |

belonging to linkage groups with three or more loci, the number of distorted loci was significantly greater at the ends of the groups than in the middle ($P < 0.01$). 43 linkage groups were obtained; between one and three linkage groups could be assigned to each chromosome, whereas four groups could not be assigned to any chromosome. 20 marker loci remained unlinked and nine marker loci gave conflicting results. The number of loci per chromosome ranged from six on chromosome 1D to 49 on chromosome 2A. The A-genome chromosomes had the highest number of loci, with 181 marker loci consisting in 116 loci from microsatellites and 65 loci from AFLP, while the D genome had 160 loci (109 from microsatellites, 51 from AFLP) and the B-genome 140 loci (65 from microsatellites, 75 from AFLP).

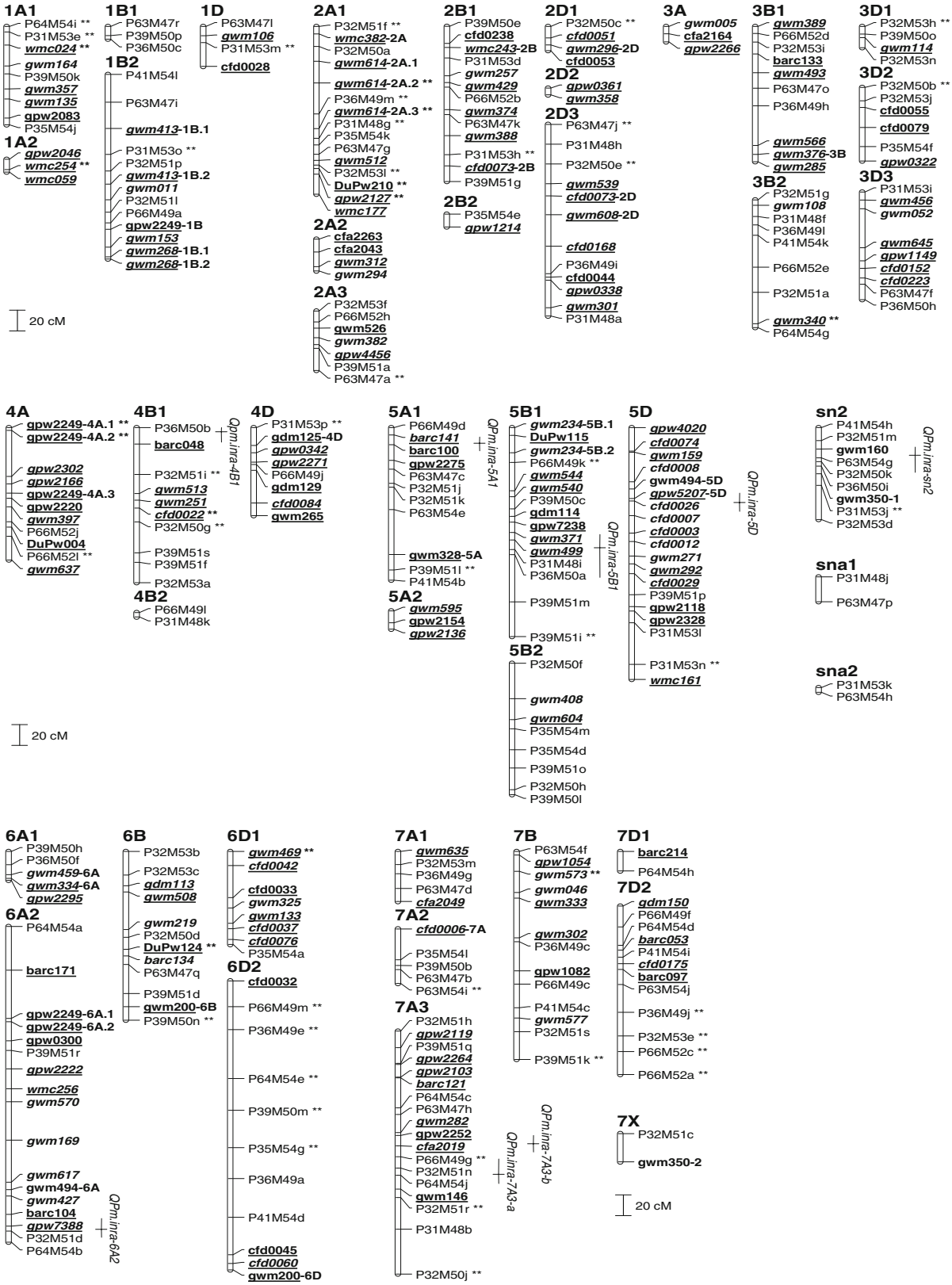
A total of 342 marker loci were used to construct a framework map (Fig. 2). The total length of the framework map was 4,263.9 cM, with an average inter-loci distance of 14.26 cM. The A-genome was the densest, containing 116 loci on the framework map, which accounted for 1,334.1 cM (average inter-loci distance of 13.1 cM). The B-genome chromosome framework maps spanned 1,456.5 cM with 110 loci (average inter-loci distance of 14.9 cM). The D-genome was the least dense, with 101 loci accounting for 1,328.7 cM (average inter-loci distance of 15.1 cM). The lengths of individual chromosomes ranged from 14.9 cM for chromosome 3A to 369.3 cM for chromosome 6D. Twenty-eight large "gaps" of more than 30 cM (between 30.2 and 47.3 cM) were obtained, among which seven were assigned to chromosome 6D. Breaking the maps in these large gaps would have added fourteen linkage groups and reduced the total length of the framework map to 3,226.3 cM, with an average inter-loci distance of 11.9 cM. The large gaps of more than 30 cM accounted for 328.5 cM in the A-genome, 287.6 cM in the B-genome and 388.5 cM in the D-genome. After switching 'error detection' to on in MAPMAKER, the total map length with the same linkage groups and the same marker orders was reduced to 3,790.8 cM. In this analysis, 2.1% of the data points of the framework map had a LOD of error >1.0. The map lengths used for QTL analysis were those obtained with 'error detection' off.

## QTL analysis

Likelihood ratio thresholds for QTL detection obtained by permutations tests for individual disease assessments and AUDPC varied between 20.7 and 23.0 (which corresponds to 4.5–5.0 when expressed in LOD score). With these thresholds, zero to four QTL were detected, depending on the resistance trait (Table 3).

Four QTL were detected for AUDPC in 2001, on chromosomes 4B, 5D, 6A and 7A. These QTL individually explained 5.9–48.7% of the phenotypic variance. The models including QTL and cofactors in the CIM explained 32.1–66.9% of the variance. Among the five cofactors taken into account in the full model, one was located near *QPm.inra-5B1*, a QTL detected at two scoring dates in 2001 (see Table 3). Only one QTL was detected for AUDPC in 2000 and 2002, namely *QPm.inra-5D*, explaining 24.3 and 27.2% of the phenotypic variation, respectively. However, the full model including QTL and cofactors in the CIM explained 47.2 and 44.3% of the variance, respectively. Among the five cofactors taken into account in the full model, two were located near QTL detected for AUDPC in 2001. These were *QPm.inra-6A2* and *QPm.inra-7A3-b* for AUDPC in 2000, and *QPm.inra-6A2* and *QPm.inra-4B1* for AUDPC in 2002.

**Fig. 2** Framework genetic map of the RE714 × Hardi RIL ▶ population with 185 microsatellite markers, written in *bold*, and 157 AFLP markers. The "new" microsatellite markers, that were not previously described in studies of RE714 powdery mildew resistance by Chantret et al. (2000), (2001) or Mingeot et al. (2002), are *underlined*. The microsatellite loci that can be used to compare marker order between this map and the microsatellite consensus map (Somers et al. 2004) or the Genoplante microsatellite map of the ITMI population (Sourdille et al. 2003) are in *italics*. The position of the QTL detected for powdery mildew resistance at the adult stage are indicated at *right*, with a *horizontal line* representing the peak of each QTL and a *vertical line* representing the confidence interval of each QTL. Loci showing a segregation distortion at $P = 0.01$ significance level are denoted by *asterisks* (**)


1A1
P64M54i **
P31M53e **
wmc024 **
gwm164
P39M50k
gwm357
gwm135
gpw2083
P35M54j
1A2
gpw2046
wmc254 **
wmc059
1B1
P63M47r
P39M50p
P36M50c
1B2
P41M54l
P63M47i
gwm413-1B.1
P31M53o **
P32M51p
gwm413-1B.2
gwm011
P32M51l
P66M49a
gpw2249-1B
gwm153
gwm268-1B.1
gwm268-1B.2
1D
P63M47l
gwm106
P31M53m **
cfd0028
20 cM
2A1
P32M51f **
wmc382-2A
P32M50a
gwm614-2A.1
gwm614-2A.2 **
P36M49m **
gwm614-2A.3 **
P31M48g **
P35M54k
P63M47g
gwm512
P32M53l **
DuPw210 **
gpw2127 **
wmc177
2A2
cfa2263
cfa2043
gwm312
gwm294
2A3
P32M53f
P66M52h
gwm526
gwm382
gpw4456
P39M51a
P63M47a **
2B1
P39M50e
cfd0238
wmc243-2B
P31M53d
gwm257
gwm429
P66M52b
gwm374
P63M47k
gwm388
P31M53h **
cfd0073-2B
P39M51g
2B2
P35M54e
gpw1214
2D1
P32M50c **
cfd0051
gwm296-2D
cfd0053
2D2
gpw0361
gwm358
2D3
P63M47j **
P31M48h
P32M50e **
gwm539
cfd0073-2D
gwm608-2D
cfd0168
P36M49i
cfd0044
gpw0338
gwm301
P31M48a
3A
gwm005
cfa2164
gpw2266
3B1
gwm389
P66M52d
P32M53i
barc133
gwm493
P63M47o
P36M49h
gwm566
gwm376-3B
gwm285
3B2
P32M51g
gwm108
P31M48f
P36M49l
P41M54k
P66M52e
P32M51a
gwm340 **
P64M54g
3D1
P32M53h **
P39M50o
gwm114
P32M53n
3D2
P32M50b **
P32M53j
cfd0055
cfd0079
P35M54f
gpw0322
3D3
P31M53i
gwm456
gwm052
gwm645
gpw1149
cfd0152
cfd0223
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P36M50h
4A
gpw2249-4A.1 **
gpw2249-4A.2 **
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gpw2166
gpw2249-4A.3
gpw2220
gwm397
P66M52j
DuPw004
P66M52l **
gwm637
4B1
P36M50b
barc048
P32M51i **
gwm513
gwm251
cfd0022 **
P32M50g **
P39M51s
P39M51f
P32M53a
QPm.inra-4B1
4B2
P66M49l
P31M48k
4D
P31M53p **
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gpw0342
gpw2271
P66M49j
gdm129
cfd0084
gwm265
5A1
P66M49d
barc141
barc100
gpw2275
P63M47c
P32M51j
P32M51k
P63M54e
gwm328-5A
P39M51l **
P41M54b
QPm.inra-5A1
5A2
gwm595
gpw2154
gpw2136
5B1
gwm234-5B.1
DuPw115
gwm234-5B.2
P66M49k **
gwm544
gwm540
P39M50c
gdm114
gpw7238
gwm371
gwm499
P31M48i
P36M50a
P39M51m
P39M51i **
QPm.inra-5B1
5B2
P32M50f
gwm408
gwm604
P35M54m
P35M54d
P39M51o
P32M50h
P39M50l
5D
gpw4020
cfd0074
gwm159
cfd0008
gwm494-5D
gpw5207-5D
cfd0026
cfd0007
cfd0003
cfd0012
gwm271
gwm292
cfd0029
P39M51p
gpw2118
gpw2328
P31M53l
P31M53n **
wmc161
QPm.inra-5D
sn2
P41M54h
P32M51m
gwm160
P63M54g
P32M50k
P36M50i
gwm350-1
P31M53j **
P32M53d
QPm.inra-sn2
sna1
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P63M47p
sna2
P31M53k
P63M54h
20 cM
6A1
P39M50h
P36M50f
gwm459-6A
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gpw2295
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gpw2222
wmc256
gwm570
gwm169
gwm617
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gpw7388
P32M51d
P64M54b
QPm.inra-6A2
6B
P32M53b
P32M53c
gdm113
gwm508
gwm219
P32M50d
DuPw124 **
barc134
P63M47q
P39M51d
gwm200-6B
P39M50n **
6D1
gwm469 **
cfd0042
cfd0033
gwm325
gwm133
cfd0037
cfd0076
P35M54a
6D2
cfd0032
P66M49m **
P36M49e **
P64M54e **
P39M50m **
P35M54g **
P36M49a
P41M54d
cfd0045
cfd0060
gwm200-6D
7A1
gwm635
P32M53m
P36M49g
P63M47d
cfa2049
7A2
cfd0006-7A
P35M54l
P39M50b
P63M47b
P63M54i **
7A3
P32M51h
gpw2119
P39M51q
gpw2264
gpw2103
barc121
P64M54c
P63M47h
gwm282
gpw2252
cfa2019
P66M49g **
P32M51n
P64M54j
gwm146
P32M51r **
P31M48b
P32M50j **
QPm.inra-7A3-a
QPm.inra-7A3-b
7B
P63M54f
gpw1054
gwm573 **
gwm046
gwm333
gwm302
P36M49c
gpw1082
P66M49c
P41M54c
gwm577
P32M51s
P39M51k **
7D1
barc214
P64M54h
7D2
gdm150
P66M49f
P64M54d
barc053
P41M54i
cfd0175
barc097
P63M54j
P36M49j **
P32M53e **
P66M52c **
P66M52a **
7X
P32M51c
gwm350-2
20 cM

**Table 3** QTL detected for powdery mildew resistance at the adult plant stage in a recombinant inbred line population derived from the cross RE714 × Hardi

| Chr. | QTL | Year | Trait | Closest marker | Peak position (cM) | Support interval (−1.5 LOD) | LOD score | $R^2$% | Additive effect |
|---|---|---|---|---|---|---|---|---|---|
| 4B1 | *QPm.inra-4B1* | 2001 | AUDPC (Res 1) | P36M50b | 4.0 | 0.0–14.0 | 5.0 | 14.4 | 24.767 |
| 5A1 | *QPm.inra-5A1* | 2001 | 13-feb (Res 2) | barc141 | 15.0 | 9.0–22.5 | 4.8 | 12.0 | −0.158 |
| 5B1 | *QPm.inra-5B1* | 2001 | 28-Mar (Res 3) | P31M48i | 117.7 | 110.5–144.8 | 4.8 | 11.1 | −0.224 |
| | | | 19-Apr | gwm499 | 114.6 | 103.3–140.8 | 5.1 | 5.9 | −0.336 |
| 5D | *QPm.inra-5D* | 2000 | 13-Apr | cfd0026 | 73.0 | 65.3–79.0 | 6.2 | 17.7 | −0.258 |
| | | | 12-May | cfd0026 | 73.0 | 63.3–79.0 | 5.8 | 17.9 | −0.313 |
| | | | 05-June (Res 4) | cfd0026 | 71.0 | 59.3–79.0 | 6.3 | 14.0 | −0.410 |
| | | | 13-June (Res 4) | cfd0026 | 71.0 | 67.3–79.0 | 7.0 | 14.7 | −0.483 |
| | | | AUDPC | cfd0026 | 73.0 | 70.5–77.0 | 9.1 | 24.3 | −22.133 |
| | | 2001 | 13-Feb | cfd0026 | 71.0 | 67.3–79.0 | 7.1 | 13.4 | −0.176 |
| | | | 07-Mar | gpw5207 h | 70.5 | 65.3–79.0 | 4.7 | 8.5 | −0.162 |
| | | | 28-Mar | cfd0026 | 73.0 | 70.5–75.0 | 29.4 | 48.8 | −0.683 |
| | | | 19-Apr | cfd0026 | 73.0 | 70.5–75.0 | 34.2 | 56.3 | −1.037 |
| | | | 11-May | cfd0026 | 71.0 | 70.5–73.0 | 21.2 | 39.4 | −0.822 |
| | | | AUDPC | cfd0026 | 73.0 | 70.5–75.0 | 27.9 | 48.7 | −68.592 |
| | | 2002 | 04-Apr | cfd0026 | 73.0 | 70.5–79.0 | 7.6 | 19.5 | −0.408 |
| | | | 26-Apr | cfd0026 | 73.0 | 65.3–81.0 | 5.3 | 13.6 | −0.341 |
| | | | 24-May | cfd0026 | 71.0 | 70.5–77.0 | 8.9 | 19.0 | −0.647 |
| | | | 05-June | cfd0026 | 73.0 | 65.3–79.0 | 6.8 | 16.0 | −0.649 |
| | | | AUDPC | cfd0026 | 73.0 | 70.5–77.0 | 10.7 | 27.2 | −45.984 |
| 6A2 | *QPm.inra-6A2* | 2000 | 05-June | gpw7388 | 293.6 | 284.2–300.4 | 5.7 | 12.2 | −0.419 |
| | | | 13-June | gpw7388 | 291.6 | 286.2–300.4 | 7.7 | 15.5 | −0.546 |
| | | 2001 | 13-Feb | gpw7388 | 293.6 | 291.6–300.4 | 10.0 | 20.5 | −0.218 |
| | | | 07-Mar | gpw7388 | 293.6 | 291.6–298.4 | 9.2 | 17.7 | −0.240 |
| | | | 28-Mar | gpw7388 | 291.6 | 284.2–294.4 | 9.6 | 11.5 | −0.334 |
| | | | 19-Apr | gpw7388 | 293.6 | 282.2–296.4 | 5.5 | 6.1 | −0.343 |
| | | | AUDPC | gpw7388 | 293.6 | 286.2–298.4 | 7.0 | 9.0 | −29.596 |
| | | 2002 | 24-May | gpw7388 | 291.6 | 282.2–294.4 | 5.9 | 13.0 | −0.543 |
| 7A3 | *QPm.inra-7A3-a* | 2000 | 05-June | P32M51n | 138.4 | 123.4–147.3 | 5.1 | 10.8 | −0.391 |
| | | | 13-June (Res 2) | P32M51n | 138.4 | 134.4–145.3 | 8.2 | 18.7 | −0.567 |
| | *QPm.inra-7A3-b* | 2001 | AUDPC | gpw2252 | 108.8 | 100.8–116.9 | 4.7 | 5.9 | −24.080 |
| SN2 | *QPm.inra-sn2* | 2000 | 13-June (Res 5) | gwm160 | 20.6 | 20.6–43.2 | 4.7 | 10.6 | −0.393 |
| | | 2001 | 28-Mar (Res 6) | P32M50 k | 32.8 | 18.6–31.3 | 4.9 | 9.5 | −0.223 |

Negative additive effects indicate that the RE714 allele improved resistance

Res 1: The trait analysed was the residual from a model of the initial AUDPC score fitted for the *QPm.inra-5D*, *QPm.inra-6A2* and *QPm.inra-7A3-b* effects

Res 2: The trait analysed was the residual from a model of the initial resistance score fitted for the *QPm.inra-6A2* effect

Res 3: The trait analysed was the residual from a model of the initial resistance score fitted for the *QPm.inra-5D* and *QPm.inra-6A2* effects

Res 4: The trait analysed was the residual from a model of the initial resistance score fitted for the *QPm.inra-6A2* and *QPm.inra-7A3-a* effects

Res 5: The trait analysed was the residual from a model of the initial resistance score fitted for the *QPm.inra-6A2*, *QPm.inra-7A3-a* and *QPm.inra-5D* effects

Res 6: The trait analysed was the residual from a model of the initial resistance score fitted for the *QPm.inra-5D* effect

QTL × year interaction for AUDPC was analysed at the four QTL positions significant for AUDPC in 2001. QTL × year interaction was not significant for the *QPm.inra-4B1*, *QPm.inra-6A2* and *QPm.inra-7A3-b* QTL ($P > 0.10$) while it was highly significant at the *QPm.inra-5D* QTL ($P < 0.0001$). This is due to the large difference in additive effects between years at this QTL (Table 3).

The evolution of the percentage of phenotypic variation explained by all detected QTL during the growing season is represented in Fig. 3. No QTL was detected for the last scoring in 2001 and the two last scorings in 2002. Between one and four QTL were detected for the other scoring dates. Two QTL were consistently detected over the 3 years of experimentation. *QPm.inra-5D* was detected over all the growing season each year (Fig. 3). Its $R^2$ value varied between 8.5 and 56.3% (Table 3). *QPm.inra-6A2* was detected at the two last scoring dates in 2000, at the four-first scoring dates in 2001 and only at the third scoring date in 2002 (Fig. 3). Its $R^2$ value varied between 6.1 and 20.5% (Table 3). The other QTL were detected only in 1 or 2 years and for one or two disease scorings. They were located in four regions, on chromosomes 5A, 5B, 7A and on an unassigned linkage group. Their $R^2$ value varied between 5.9 and 18.7% (Table 3). Altogether, the models including QTL and cofactors in the CIM explained 29.1–72.1% of the variance, and the lower values were obtained when the trait analysed was a residual from a model of the original trait fitted for the significant QTL.

At all QTL except *QPm.inra-4B1*, the favourable allele, i.e. the one improving resistance, came from the resistant parent RE714.

### Epistatic interactions

Only one QTL × QTL interaction was significant at the 5% level, namely the interaction between *QPm.inra-5D* and *QPm.inra-6A2* at the third scoring date (28 March) in 2001 ($P = 0.0288$). Three QTL × QTL interactions were significant at the 10% level, and concerned the third scoring date (5 June) in 2000 and the third (28 March) and the fourth (19 April) scoring dates in 2001.

In marker × marker interaction tests, between 4.88 and 5.82% of the tests were significant at the 5% level, depending on the traits, and between 0.7 and 1.7‰ of the tests were significant at the 1‰ level. This indicates that if there is some epistasis for APR for powdery mildew, it cannot be split into significant digenic interactions.

### Assessment of MAS efficiency for powdery mildew resistance

RE714 × 'Hardi' RI lines were classified into four classes according to their presumed genotype at the two main QTL *QPm.inra-5D* and *QPm.inra-6A2*, on the basis of their marker alleles at the loci of the framework map comprised in the 1.5 LOD unit fall confidence intervals of the QTL. Eleven lines had a missing data at one of the four markers, 19 lines had a recombination at one of the QTL. Thirty RIL were identified as carrying the RE714 allele at both QTL, 33 had the RE714 allele only at the *QPm.inra-5D* QTL, 32 had the RE714 allele only at the *QPm.inra-6A2* QTL and 33 had the 'Hardi' allele at both QTL.

The RIL that had the RE714 allele at the two QTL had the lowest mean scores at the end of the growing season in 2000, throughout the growing season in 2001 except at the last scoring date and throughout the growing season in 2002 (Fig. 4). These RIL were similar to those that had the RE714 allele only at *QPm.inra-5D* at the beginning of the growing season in 2000 and at the last scoring date in 2001. The RIL that had the 'Hardi' allele at the two QTL had the highest mean scores throughout the growing season in the 3 years (Fig. 4). Applying MAS and selecting for the RE714 allele at the *QPm.inra-5D* and *QPm.inra-6A2* QTL should therefore provide a substantial genetic gain in adult powdery mildew resistance.

The effects of MAS were also examined in terms of distributions of the RIL mean AUDPC (Table 4). Selecting for the RE714 allele at the two main QTL *QPm.inra-5D* and *QPm.inra-6A2* or only at the *QPm.inra-5D* QTL should eliminate most of the more susceptible lines.

## Discussion

### Marker order and map coverage

The marker order in the map obtained in the RE714 × 'Hardi' RIL population was in general in

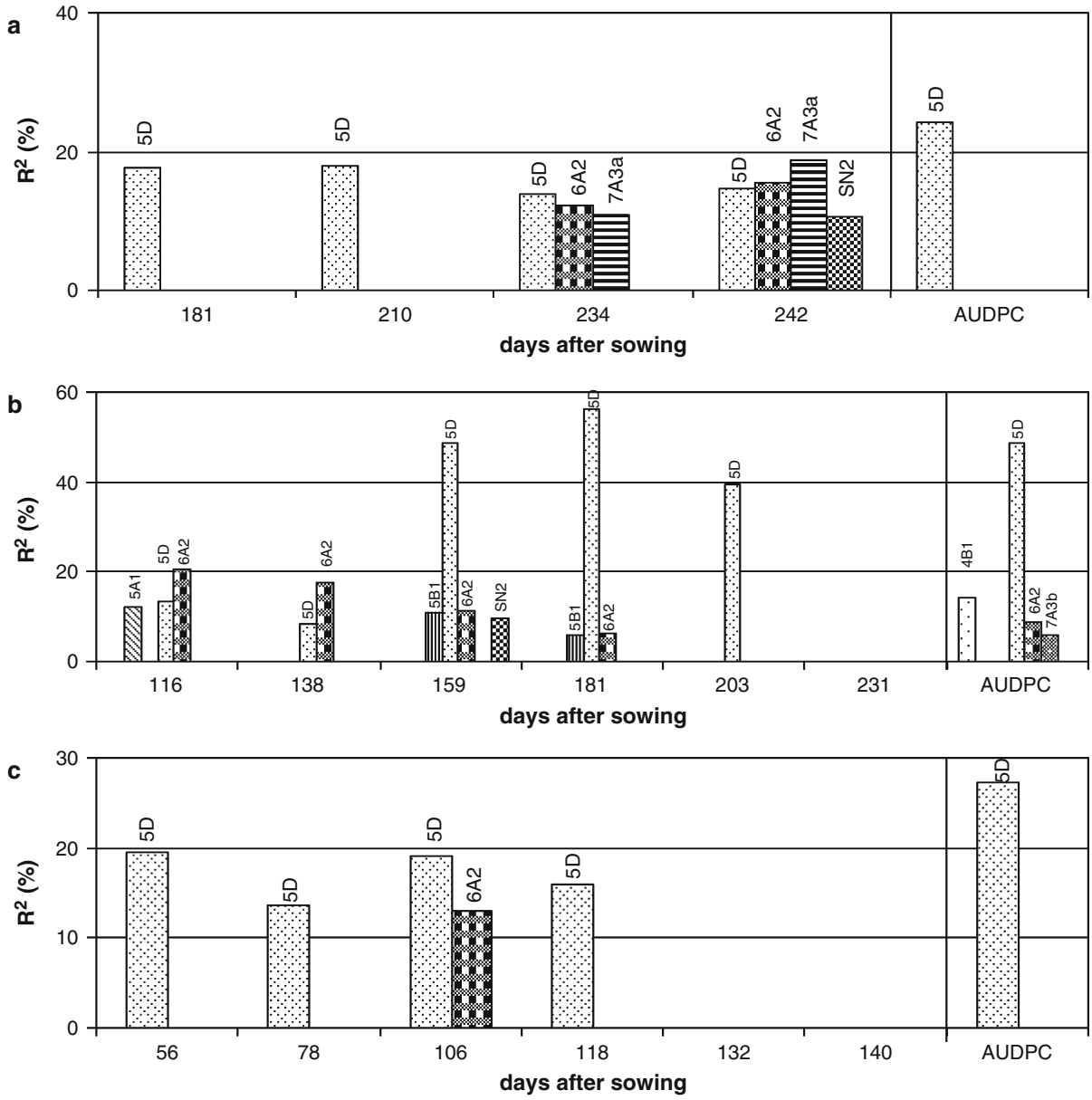


**Fig. 3** Percentage of phenotypic variation explained by QTL for powdery mildew resistance detected along the growing season **a** in 2000 in the field, **b** in 2001 in a market-gardening tunnel and **c** in 2002 in a market-gardening tunnel

agreement with the order in the microsatellite consensus map (Somers et al. 2004) and in the Genoplante microsatellite map of the ITMI population (Sourdille et al. 2004) although some differences in marker order were found, that mainly concerned close markers.

The total map length of the RE714 × 'Hardi' RIL population reported here was 3,790.8 cM with 'error detection' on in MAPMAKER. This length is in the range of map length reported for other populations used for the detection of QTL for adult plant resistance to powdery mildew when a complete map was obtained (ITMI 'Synthetic' × 'Opata' population, 'Fukuho-komugi' × Oligoculm population or RE9001 × 'Courtôt' population, see Table 5) and below the estimation by Sourdille et al. (2003) of 4,000 cM for an intervarietal bread wheat map. Besides, the map of the RE714 × 'Hardi' RIL

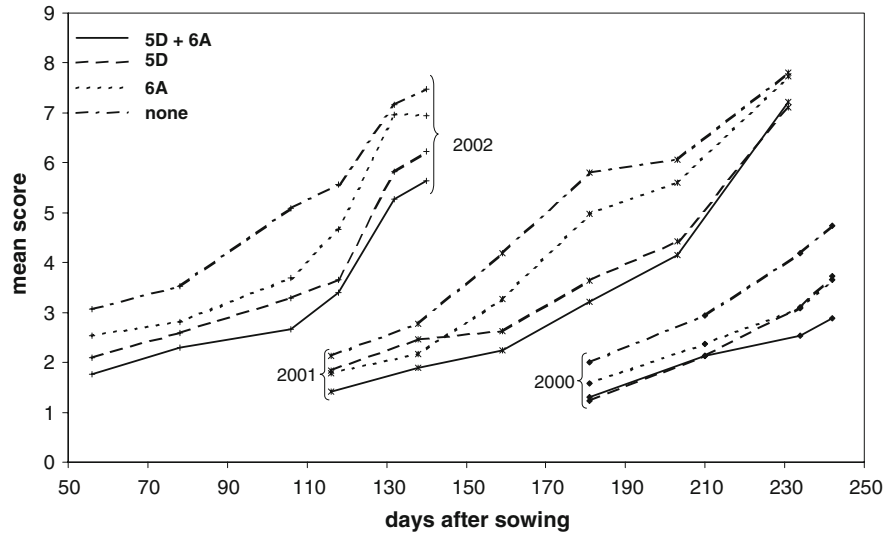


**Fig. 4** Progression of the mean of the RE714 × Hardi RI lines classified according to their genotype at the *QPm.inra-5D* and *QPm.inra-6A2* QTL along the growing season in the field (2000) or in market-gardening tunnels (2001 and 2002). *5D + 6A*: lines with the RE714 allele at the two QTL; *5D*: lines with the RE714 allele at *QPm.inra-5D* and the Hardi allele at *QPm.inra-6A2*; *6A*: lines with the RE714 allele at *QPm.inra-6A2* and the Hardi allele at *QPm.inra-5D*; *none*: lines with the Hardi allele at the two QTL

**Table 4** Approximate 90% range of mean AUDPC of RE714 × Hardi RI lines classified according to their genotype at the *QPm.inra-5D* and *QPm.inra-6A2* QTL in 2000 in the field, in 2001 and in 2002 in market-gardening tunnels

| QTL combinations | Line number | 2000 | 2001 | 2002 |
|---|---|---|---|---|
| *QPm.inra-5D + QPm.inra-6A2* | 30 | 97–158 | 318–493 | 193–326 |
| *QPm.inra-5D* | 33 | 103–183 | 328–513 | 206–411 |
| *QPm.inra-6A2* | 32 | 115–202 | 423–570 | 248–438 |
| None | 33 | 131–267 | 435–668 | 297–532 |

The approximate 90% range of mean AUDPC was obtained by picking the mean AUDPC value of the third lowest and the third highest lines after ranking the lines according to AUDPC within each group defined by genotype at the *QPm.inra-5D* and *QPm.inra-6A2*. In doing so, the two lowest and two highest lines, that could be outliers for any reason, were not considered. This represents an approximate 90% range because 1–4/line number is between 86.7 and 87.9%

population reported here had a far better genome coverage than the maps of the other populations involving RE714 as the resistant parent previously reported (Table 5); (Chantret et al. 2001; Mingeot et al. 2002).

We compared the map length of the chromosomes obtained in the RE714 × 'Hardi' RIL population reported here with the map length of the chromosomes in the maps reported in other studies of QTL for APR to powdery mildew (Table 5). Two chromosomes (1D and 3A) were much shorter in the RE714 × 'Hardi' RIL population than in the ITMI 'Synthetic' × 'Opata' RIL population or in the 'Fukuho-komugi' × Oligoculm DH population or in the RE9001 × 'Courtôt' RIL population, whereas the 4B chromosome was much larger. These differences in map length could reflect a true difference in genome coverage, e.g. due to a lack of polymorphism (see the small number of markers on chromosomes 1D and 3A, Fig. 2) or could reflect differences in recombination frequencies owing to genetic background.

## Complexity of the genetic control of powdery mildew adult plant resistance

Altogether, eight regions controlling powdery mildew adult plant resistance were detected in the

**Table 5** Wheat (*Triticum aestivum*) populations used for the detection of QTL for APR to powdery mildew and QTL detected

| Cross | Population type | Population size | Mapping function | Number of loci | Map length (cM) | Number of QTL | LOD score for QTL detection | QTL location | Reference |
|---|---|---|---|---|---|---|---|---|---|
| RE714 × 'Festin' | DH | 41 | K | 227 | 1,654 | 5 | 3.1 | 1A, 2A, 2B, 5D, 6A | a |
| RE714 × 'Hardi' | DH | 44 | K | 161 | 993 | 3 | 2.4 | 4A, 5D, 6A | a, b |
| RE714 × 'Hardi' | $F_{2:3}$ | 140 | K | 68 | 510 | 4 | 3.0 | 5D, 6A, 7A, 7B | b |
| 'Becker' × 'Massey' | $F_{2:3}$ | 180 | H | 40 | 296 | 3 | 3.0 | 1BL, 2AL, 2BL | c |
| USG3209 × 'Jaypee' | $F_{6:7}$ RIL | 293 | H | 20 | 357 | 3 | 3.0 | 1BL, 2AL, 2BL | c |
| ITMI map ('Synthetic' × 'Opata') | RIL | 114 | H | 510 | 3,826 | 3 (+3) | 3.0 (2.0) | 2DL, 4Bc, 7DS (3BL, 5DL, 6AL) | d |
| ('Tähti' × T militinae) 8/1 × 'Tähti' | $F_{2:3}$ | 130 | K | 38 | 251 | 1 (+4) | 3.5 (1.2) | 4A (1B, 2A, 5A, 5B) | e |
| 'Fukuho-komugi' × Oligoculm | DH | 107 | H | 432 | 3,948 | 4 | 2.5 | 1AS, 2BL, 4BL, 7DS | f, g |
| RE9001 × 'Courtôt' | $F_8$ RIL | 104 | K | 404 | 3,825 | 11 | 3.2 | 1D, 2A, 2B, 2D, 3B, 3D, 4A, 5B, 5D, 7D | h |
| 'Forno' × 'Oberkulmer' | $F_{5:6}$ RIL | 226 | H | 182 | 2,469 | 18 | 3.0 | 1A, 1B, 1D, 2A, 2D, 3A, 3D, 4A (2), 4B, 4D, 5A (3), 5B, 6B, 7B (2) | i, j |
| Avocet × Saar | $F_6$ RIL | 113 | K | 265 | 334 | 7 | 3.2 | 1BL, 3AS, 4BL, 5AL, 5BS, 7BL, 7DS | k |

*Map function*: K, Kosambi (Kosambi 1944); H, Haldane (Haldane 1919); population type: DH, doubled haploid lines; RIL, recombinant inbred lines

References: *a* (Mingeot et al. 2002), *b* (Chantret et al. 2001), *c* (Tucker et al. 2007), *d* (Börner et al. 2002), *e* (Jakobson et al. 2006), *f* (Suenaga et al. 2005), *g* (Liang et al. 2006), *h* (Bougot et al. 2006), *i* (Messmer et al. 1999), *j* (Keller et al. 1999), *k* (Lillemo et al. 2008)

RE714 × 'Hardi' RIL population, four in 2000, seven in 2001 and two in 2002 (Table 3; Fig. 3). The total variation explained by the full model of CIM, including QTL and cofactors, was 29.1–72.1% over the 3 years, and was 35.8–48.9% in 2000, 29.1–72.1% in 2001 and 31.5–44.3% in 2002. The lower complexity and lower part of variance explained in 2002 is probably related to the fact that the phenotypic data were obtained from only one replicate because two had to be discarded. With these data, the power of QTL detection was probably lower. The lower complexity and slightly lower part of variance explained in 2000 relative to 2001 could be explained by the higher level of disease obtained in the market-gardening tunnel in 2001 than in the field in 2000 because of more favourable conditions for powdery mildew in the tunnel (Fig. 1). Powdery mildew could be scored two months earlier in the market-gardening tunnel in 2001 than in the field in 2000. Also, even if the powdery mildew populations did not seem to be very different between the 3 years on the basis of data obtained with differential hosts at the seedling stage, they could be different in terms of frequencies of virulence genes, which are difficult to measure, and in terms of aggressiveness. If some of the QTL we try to reveal are race-specific, their effects on disease symptoms and consequently on disease scorings depend on the frequencies of the matching virulence genes in the population (Parlevliet 1983). In particular, the QTL *QPm.inra-6A2* is detected at only two and one scoring dates and not with AUDPC in 2000 and 2002, respectively, whereas it is detected at four scoring dates and with AUDPC in 2001. As a QTL for powdery mildew resistance at the seedling stage was detected at the same location as *QPm.inra-6A2* with some specific isolates (Muranty et al. 2008), we can suppose that *QPm.inra-6A2* is race-specific and that the frequency of the virulence gene matching this resistance varied in the powdery mildew populations that occurred in our trials.

Among the eight regions controlling powdery mildew adult plant resistance detected in the RE714 × 'Hardi' RIL population, four were detected only with one or two disease scorings whereas two were detected only with AUDPC. This indicates that analyzing not only AUDPC but also individual disease scorings has enriched our knowledge of genetic control of powdery mildew resistance in this population. Some QTL have an effect only at a few stages during wheat growing season and are masked in the AUDPC analysis, whereas some QTL that are expressed during all the growing season but have low effects, too low to be detected with individual disease scorings, are only detected via AUDPC. No QTL was detected for the last scoring in 2001 and the two last scorings in 2002, and this corresponded to a reduction in the 90% range of the RIL mean values: the discrimination of the RILs diminished at the end of the growing season.

The number of powdery mildew resistance QTL detected in the RE714 × 'Hardi' RIL population is lower than what was obtained by Keller et al. (1999) (18 QTL with a LOD score higher than 3.0) or by Bougot et al. (2006) (11 QTL, LOD > 3.2), but is higher than what was obtained by Liang et al. (2006) (4 QTL, LOD > 2.5) or by Börner et al. (2002) (3 QTL, LOD > 3.0) (see Table 5). These differences can be explained by differences in the genetic background of the wheat population analysed leading to differences in polymorphism at powdery mildew resistance QTL and in composition of the powdery mildew population.

## Comparison of detected QTL with QTL mapped in other populations involving RE714 as the resistant parent

The detection of two QTL on chromosomes 5D and 6A over the 3 years of experimentation confirmed the results of Chantret et al. (2001) and Mingeot et al. (2002). None of the other QTL detected by Mingeot et al. (2002) were detected again and only one of the QTL detected on chromosome 7A in the $F_{2:3}$ mapping population by Chantret et al. (2001), near the marker Xgwm344, could correspond to one of the QTL detected in the RIL population on chromosome 7A. Many of the QTL detected by Mingeot et al. (2002) and not detected in the present study had quite low LOD scores between 1.9 and 4.0. Only two QTL detected by Mingeot et al. (2002) and not detected in the present study had LOD scores higher than 5.0. For one of these, the favourable allele came from the susceptible parent Festin, so it was not unexpected that it could not be detected in a RE714 × Hardi population. The other one is a residual effect of *Pm4b* (present in RE714) that was specifically detected in the RE714 × Festin population and not in the RE714 × Hardi population (Mingeot et al. 2002).

Similarly, among the QTL detected by Chantret et al. (2001) in the $F_{2:3}$ mapping population, only the QTL on chromosome 5D and chromosome 7A near the marker Xgwm344 have LOD scores higher than 5.0. All the non-reproducible QTL could either be false positives or environment specific or genetic background specific QTL.

The two main QTL *QPm.inra-5D* and *QPm.inra-6A2* were first detected on the basis of a bulk segregant analysis of powdery mildew resistance scores at the seedling stage in the $F_{2:3}$ population (Chantret et al. 2000), followed by a complete genotyping only in the regions pointed out by BSA. The analysis reported in this study in the RE714 × 'Hardi' RIL population involved genotyping of the whole population with full genome coverage and did not reveal other stable regions. This can be due to the limited population size (160 RIL) and the quite stringent significance level (5%) chosen by permutation analysis (between 4.5 and 5.0 on the LOD scale) or to the absence of other stable regions.

When expressed in terms of resolving power, following the method of Darvasi and Soller (1997), the lengths of the confidence intervals of the QTL in this study were between 5.9 and 39.1 cM for *QPm.inra-5D* and between 16.1 and 54.3 cM for *QPm.inra-6A2*, depending on the trait considered, which is lower than that obtained in DH populations by Chantret et al. (2001) and Mingeot et al. (2002) and similar to that obtained in an $F_{2:3}$ population by Chantret et al. (2001) (Table 6). This is mainly due to the bigger size of the RE714 × 'Hardi' RIL population as compared to the sizes of the DH populations studied by Chantret et al. (2001) and Mingeot et al. (2002). When expressed in terms of 1.5 LOD unit fall, the lengths of the confidence intervals of *QPm.inra-5D* and *QPm.inra-6A2* were between 2.5 and 15.7 cM and between 6.8 and 16.2 cM, respectively, depending on the quantitative trait considered, which is lower than the resolving power. This could reflect the fact that when using CIM, the power of QTL detection is higher, the precision of the map position is better or simply the fact that the peak of the test statistic is sharper.

## Comparison of detected QTL with major powdery mildew resistance genes and QTL derived from other sources

The QTL *QPm.inra-5D* and *QPm.inra-6A2* detected here at the adult plant stage correspond to QTL detected in the same regions at the seedling stage (Muranty et al. 2008). No major gene has been described in the region of *QPm.inra-6A2* in wheat for the moment. However, a minor QTL for powdery mildew resistance was detected in one environment, with a LOD score between two and three, in the ITMI population by Börner et al. (2002) in a genomic localization similar to the QTL *QPm.inra-6A2* (Table 5). On chromosome 5D, the QTL detected in this study and at the seedling stage (Muranty et al. 2008.) lies in a similar region as *Pm35*, a major gene derived from *Aegilops tauschii* and introgressed in NC96BGTD3 (Miranda et al. 2007), and as the major genes described by Sun et al. (2006) in *Aegilops*

**Table 6** Range of the length of confidence intervals of the two major QTL *QPm.inra-5D* and *QPm.inra-6A2* in this study and in previous studies involving RE714 as the resistant parent

| QTL | RE714 × Hardi 160 RIL | | | | RE714 × Hardi 140 $F_{2:3}$ families | | RE714 × Hardi 44 DH | | RE714 × Festin 41 DH | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Support interval[a] (−1.5 LOD) | | Resolving power[b] | | Resolving power | | Resolving power | | Resolving power | |
| | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max |
| QPm-5D | 2.5 | 15.7 | 5.9 | 39.1 | 10.1 | 13.5 | 31.8 | 45.8 | 23.8 | 58.2 |
| QPm-6A | 6.8 | 16.2 | 16.1 | 54.3 | 28.3 | 43.0 | 50.2 | 98.7 | 24.0 | 51.9 |

[a] The support interval was estimated on the basis of a 1.5 LOD unit fall

[b] Resolving power is an estimate of the length of the 95% confidence interval of a QTL location following a method developed by Darvasi and Soller (1997). It was calculated as $530/(N \times R^2)$, where the constant 530 is derived from simulations of backcross and $F_2$ populations (Darvasi and Soller 1997), $N$ is the population size and $R^2$ the proportion of variance explained by the QTL

*tauschii*, namely *PmY201* and *PmY212*. Moreover, it lies in a similar region as *QPmVPn.inra-5D* detected by Bougot et al. (2006) at the vernalized seedling stage in the RE9001 × 'Courtôt' population (Table 5).

We tried to compare the positions of minor QTL on chromosome 4B, 5A, 5B and 7A to the positions of the QTL for powdery mildew resistance described in other papers by projecting these QTL on the composite map by Appels (2003). On chromosome 4B, a QTL was detected at the top of our linkage group and could correspond to one of the QTL detected on chromosome 4B by Börner et al. (2002). On chromosome 5A, a QTL was detected in the second interval of the first linkage group, and could correspond to one of the QTL detected in *Triticum militinae* by Jakobson et al. (2006). On chromosome 5B, a QTL was detected near marker Xgwm499 and could be similar to the QTL detected in *Triticum militinae* by Jakobson et al. (2006). On chromosome 7A, as discussed previously, one of the QTL detected in the RIL population could correspond to the QTL detected near the marker Xgwm344 in the $F_{2:3}$ mapping population by Chantret et al. (2001). Two alleles of *Pm1*, namely *Pm1c* and *Pm1e*, have been mapped near this marker by Singrün et al. (2003), while two powdery mildew resistance genes from einkorn (*Triticum monococcum*) have also been mapped in this region (Yao et al. 2007).

## Perspectives for marker-assisted selection for adult plant resistance to powdery mildew

The two QTL on chromosomes 5D and 6A are good candidates for marker-assisted selection because they were stable over several environments and detected in different populations derived from RE714 (this study, Chantret et al. (2001) and Mingeot et al. (2002)). In our study, these QTL together explained 24.4, 52.1 and 31.1% of the phenotypic variance for AUDPC in 2000, 2001 and 2002 respectively. In particular, selecting lines with the RE714 allele at *QPm.inra-5D* should eliminate the greatest number of the most susceptible lines (Table 4) and should result in a decrease of AUDPC of about 14.8% (additive effect divided by mean, Table 3). The difference between genotypes with RE714 allele at both QTL and genotypes with RE714 allele at *QPm.inra-5D* and anything at the other QTL was quite low, between 4.5 and 6.8% in AUDPC means (see Fig. 4; Table 4). Therefore breeders should carefully consider the additional genotyping costs and the selection scheme to apply, before deciding to select also for RE714 allele at *QPm.inra-6A2*. Six microsatellite markers are located in the confidence interval of *QPm.inra-5D*, namely Xgpw5207-5D, Xgwm639-5D, Xcfd0057, Xgwm174, Xgpw5098 and Xcfd0026. Two of these microsatellite markers (Xgwm174 and Xcfd0026) were in the one LOD support interval defined by Chantret et al. (2001) on the $F_{2:3}$ population and two were mapped in this population but outside the 1 LOD support interval (Xgwm639-5D and Xcfd0057). This difference is associated with a marker order difference between the $F_{2:3}$ and the RIL population. Moreover, we considered a 1.5 LOD support interval because it is more likely to contain the true position of the QTL than a 1 LOD support interval (van Ooijen 1992). With this study, two microsatellites, namely Xgpw5207 and Xgpw5098 were added to the *QPm.inra-5D* region. Two microsatellite markers are located in the confidence interval of *QPm.inra-6A2*, namely Xgpw7388 and XDuPw167, and one AFLP marker, namely XP32M51d. These three markers were not mapped in previous studies of RE714 resistance. All these markers could be used for marker-assisted selection. No QTL was detected for the last scoring in 2001 and the two last scorings in 2002, and the last scoring was much nearer to the first scoring in 2000 (61 days) than in 2001 (115 days) and 2002 (84 days; Figs. 1 or 4): consequently we can suppose that the two QTL *QPm.inra-5D* and *QPm.inra-6A2* from RE714 do not provide an efficient powdery mildew resistance at the end of the wheat growing season. The markers linked to these QTL could be used to cumulate RE714 alleles at these QTL with RE9001 allele at the *QPm.inra-2B* QTL (Bougot et al. 2006) which is efficient at the adult plant stage.

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