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Identification of quantitative trait loci for resistance to powdery mildew in a Spanish barley landrace

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Abstract The Spanish landrace-derived inbred line SBCC97, together with other lines from the Spanish Barley Core Collection, displays high resistance to powdery mildew, caused by the fungus *Blumeria graminis* f. sp. *hordei*. The objective of this study was to map quantitative trait loci (QTLs) for resistance to powdery mildew in a recombinant inbred line population derived from a cross between SBCC97 and the susceptible cultivar 'Plaisant'. Phenotypic analysis was performed using four *B. graminis* isolates, and genetic maps were constructed with mainly simple sequence repeat (SSR) markers, following a sequential genotyping strategy. Two major QTLs with large effects were identified on chromosome 7H, and they accounted for up to 45% of the total phenotypic variance. The alleles for resistance at each QTL were contributed by the Spanish parent SBCC97. One locus was mapped to the short arm of chromosome 7HS, and was flanked by the resistance gene analogue (RGA) marker S9202 and the SSR GBM1060. This corresponded to the same chromosomal region in which a major race-specific resistance gene from *Hordeum vulgare* ssp. *spontaneum*, designated as *mlt*, had been identified previously. The second QTL was linked tightly to marker EBmac0755, and it shared its chromosomal location with the qualitative resistance gene *Mlf*, which has only been described previously in the wild ancestor *H. spontaneum.* This is the first report of these two QTLs occurring together in cultivated barley, and it paves the way for their use in barley breeding programs that are designed to transfer resistance alleles into elite cultivars.

Keywords Barley · Powdery mildew · Disease resistance · Quantitative trait locus

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Introduction

Barley is an important cereal crop worldwide, being ranked fourth in terms of quantity produced and area of cultivation. More than 50% of global barley production occurs in Europe, where Spain is the third largest European producer of barley, after Germany and France (FAO 2006). Powdery mildew, caused by the biotrophic fungus *Blumeria graminis* f. sp. *hordei*, is one of the most destructive and widespread diseases of barley. It is of great economic importance

because it not only reduces yield but also lowers the quality of harvested grain (Zhang et al. 2005).

The control of the disease normally involves the use of fungicides. However, the risks that are associated with the widespread use of fungicides (development of resistance in pathogen populations and damage to the environment), together with their high cost, have led to the gradual limitation of their use (Gullino and Kuijpers 1994). The development of resistant barley cultivars is a relatively cheaper and more environmentally friendly method of disease control, compared to the use of fungicides.

The interaction between barley and powdery mildew is one of the best-characterised pathosystems in plants. Many genes and quantitative trait loci (QTLs) associated with resistance to powdery mildew have been identified and mapped to all seven barley chromosomes in different genetic backgrounds (Friedt and Ordon 2007), and many of them have already been used in barley breeding in Europe (Brown and Jørgensen 1991). The use of resistance genes, however, usually gives rise to the rapid development of novel virulence in the pathogen, which results in the 'breakdown' of resistance. Only the *mlo* resistance gene has remained highly effective against powdery mildew over the last 30 years. During this period, it has been introgressed widely into two-rowed spring barley grown throughout Europe. However, *mlo* has been scarcely used in winter barley breeding in Europe (Panstruga et al. 2005; Dreiseitl 2007). Therefore, new and effective sources of resistance are still needed, especially for six-rowed winter barleys, which are grown widely in Southern Europe.

Landraces represent valuable reservoirs of genetic variability that can be surveyed for new variation in traits such as disease resistance. Indeed, most of the genes for resistance to powdery mildew carried by current cultivars were found in barley landraces (Fischbeck and Jahoor 1991; Jørgensen 1994; Jørgensen and Jensen 1997). Identification of new resistances from these sources normally requires the evaluation of progenies from crosses among appropriate genotypes. The development of mapping populations is an effective tool for studying genetically complex traits, because it enables determination of not only the loci that are involved in resistance, but also the parent that contributes the allele and the effectiveness of each locus. The effort involved in the genotyping is usually substantial and expensive if a large number of individuals is studied. Researchers have resorted to several techniques to limit the genotyping burden of QTL studies. Selective genotyping and bulked segregant analysis have been proposed for this purpose. However, these strategies may not be very efficient when multiple QTLs are responsible for the variation in the trait (Sen et al. 2005). The method of sequential genotyping proposed here, slightly modified from Satagopan et al. (2007), represents an alternative mapping approach that reduces the number of individuals and markers that must be genotyped to detect QTL.

The Spanish Barley Core Collection (SBCC) (Igartua et al. 1998) comprises a representative sample of the landraces that were cultivated in Spain before the advent of modern breeding, and it represents a unique, distinct, and diverse genetic resource (Lasa et al. 2001; Yahiaoui et al. 2008). Previous studies have revealed the presence of significant levels of apparently uncharacterised resistance to powdery mildew in some landrace-derived inbred lines from the SBCC (Silvar et al. 2009). The main goal of the work described here was to investigate the genetic basis that underlies this resistance, using a mapping population that was obtained by crossing one of the most resistant lines from the SBCC with a susceptible cultivar.

Materials and methods

Plant material

Two hundred and sixty-two recombinant inbred lines (RILs) from a cross between the Spanish landrace SBCC97 and the French cultivar 'Plaisant' were used. The mapping population was developed by single-seed descent and consisted of 262 RILs, 75 in F_6 and 187 in F_5 at the start of the experiment. These two sets constitute independent separate tranches of the population. The pedigree of each line traces back to a different F_2 plant (for a total of 262 F_2 different plants). SBCC97 is a six-rowed barley landrace belonging to the Spanish Barley Core Collection, which possesses high resistance to powdery mildew. 'Plaisant' is a six-rowed winter commercial cultivar that is susceptible to powdery mildew and shows good adaptation to Spanish conditions. It carries the *Mlra* resistance gene, on chromosome 1H, which is ineffective against all isolates of *B. graminis* used in this study.

The RILs were multiplied in the 2006–2007 season, using a single plant per line (F_5 and F_6). Five leaves per plant (line) were collected for DNA extraction in March 2007. Two heads of each plant were bagged, and their seed (actually, F_6 and F_7 seed) was used for phenotyping in the fall of 2007.

Pathogen isolates and disease assessment

Four isolates of *B. graminis* f. sp. *hordei* (R79, R126, R178, and R180) were chosen according to their virulence/avirulence pattern on 19 near-isogenic lines from the Pallas differential set (Kølster et al. 1986) and 10 cultivars with previously-identified resistance genes (Table 1).

Five barley seedlings from each RIL were inoculated artificially in the greenhouse. Eight days after inoculation, the infection on the primary leaves of the seedlings was scored on a scale of 0–4 (including intermediate points), according to the protocol of Torp et al. (1978) and Jensen et al. (1992). Plants that showed infection types lower than 2 were classified as resistant, and plants with infection types of 2 or higher were included in the susceptible group.

Genotyping

Five leaves per RIL were harvested and ground in liquid nitrogen in 2007. Total genomic DNA was isolated using the NucleoSpin Plant XL kit (Macherey–Nagel), following the manufacturer's instructions. One hundred and eleven markers, which covered the entire genome, were used sequentially for genetic analysis (see next section). The majority of the markers were simple sequence repeats (SSRs). The sequences of the primer pairs and amplification protocols were obtained from Pillen et al. (2000), Ramsay et al. (2000), Thiel et al. (2003), Rostoks et al. (2005), and Varshney et al. (2007). PCR markers were developed for resistance gene analogues (RGAs) PIC25-1 (Collins et al. 2001) and S9202 (Madsen et al. 2003), and resistance gene *Mla* (Table S1). PCR amplification was carried out in a final volume of 15 µl, which contained 50 ng of genomic DNA, 1× PCR Buffer (Biotools), 2 mM $MgCl_2$, 15 pmol each of the forward and reverse primers, dNTPs (Biotools) at 0.2 mM each, and 0.4 U of Tth DNA Polymerase (Biotools). Amplified products were separated on either 5% (w/v) denaturing polyacrylamide gels or 2–3% agarose gels and visualised by staining with silver or ethidium bromide, respectively.

Mapping and QTL analysis

A two-stage mapping strategy that was based on sequential genotyping was used (Satagopan et al. 2007). In the first step, a subset of 75 RILs (which corresponded to the F_6 lines) was genotyped using 101 SSR markers in order to identify promising chromosomal regions. Secondly, 10 additional markers located in these promising regions were analysed, six of them for the whole population.

A linkage map was constructed with JoinMap 3.0 (van Ooijen and Voorrips 2001), using Kosambi's map function (Kosambi 1944) and a minimum logarithm of the odds ratio (LOD score) of 3. Linkage groups were aligned and assigned to chromosomes using markers that were in common with previously published maps (Varshney et al. 2007).

Table 1 Virulence/avirulence patterns for the four *B. graminis* isolates used and infection scores for the parents and RIL population

Isolate	Virulence/avirulence[a]	Infection scores		
		SBCC97	Plaisant	Mean ± SD[b]
R79	Mla6, a7, a9, a10, a12, a13, ra, k, nn, p, at, g, La, h, Bw, St, Kr, Ab/a1, a3, a22, a23, o5, o9, Tu2, f, t, j, a28	0	2.5	1.56 ± 0.66
R180	Mla3, a6, a7, a12, a13, a22, ra, nn, p, at, g, h, Bw, Tu2, Kr, Ab, a28/a1, a9, a10, a23, k, o5, La, o9, St, f, t, j	0	2	1.66 ± 0.69
R126	Mla3, a6, a7, a22, ra, nn, p, g, La, h, Tu2, St, Kr/a1, a9, a10, a12, a13, a23, k, at, o5, o9, Bw, Ab, f, t, j, a28	0	2.5	1.11 ± 0.86
R178	Mla6, a7, a10, a12, a13, ra, k, nn, g, h, Bw, Kr, Ab, a28, f, t, j/a1, a3, a9, a22, a23, p, at, o5, La, o9, Tu2, St	0	3.5	1.44 ± 0.85

[a] Designation of pathotypes was performed according to Limpert and Müller (1994)

[b] Mean of disease score for 262 RI lines

After both steps, QTL analysis was performed using the composite interval mapping (CIM) procedure (Zeng 1994) implemented in Windows QTL Cartographer 2.5 (Wang et al. 2007). Three to six cofactors were chosen using a forward-selection stepwise regression procedure. The walk speed was set to 2 cM and the scan window to 10 cM. The permutation test was conducted 1000 times at a significance level of 0.05, to determine QTL threshold levels. QTLs that were above the significance threshold were considered significant and the Likelihood Ratio Test statistic (LR) was expressed as a LOD score (LOD = 0.2171 LR).

Possible interactions between the QTLs that were identified were analysed using the SAS GLM procedure (SAS 1988). The markers that were closest to the QTL were included as fixed factors and their interaction was tested against the residual variance (due to the variation within each allelic class). Protection against spurious significant positives that arose from multiple testing was performed using a Bonferroni correction.

Results

Trait analysis

The Spanish landrace SBCC97 displayed high levels of resistance to all four *B. graminis* isolates (score 0), whereas 'Plaisant' ranged from moderately susceptible (score 2 for isolate R180) to highly susceptible (score 3.5 for isolate R178). The RILs differed in their resistance to powdery mildew, ranging from 0 (resistant) to 3.5 (susceptible), depending on the isolate. Three lines were as resistant as SBCC97 (score 0 for all isolates) and 69% showed resistance (scores between 0 and 2) to all isolates. For all isolates, the frequency distribution of infection types showed a continuous response (Fig. 1). A two-peak distribution was observed after infection with isolates R126 and R178, whereas inoculation with isolates R79 and R180 gave a bell-shaped distribution that was more similar to a Gaussian distribution. However, the Kolmogorov–Smirnov test showed that none of the distributions fitted a normal curve. For R126 and R178, the distribution was skewed towards resistance, and the population mean was lower than the mid-parent mean, especially for R178 (Table 1). For isolates R79 and R180, however, a deviation towards susceptibility was observed, with a population mean higher than the mid-parent value, especially for R180, for which a marked transgressive segregation was noted (Fig. 1; Table 1). Pearson correlation coefficients between the isolates were high and significant in all cases ($P < 0.0001$), although the highest coefficients, and hence the greatest similarity, were detected between isolates R126 and R178 ($r = 0.82$) and isolates R79 and R180 ($r = 0.86$).

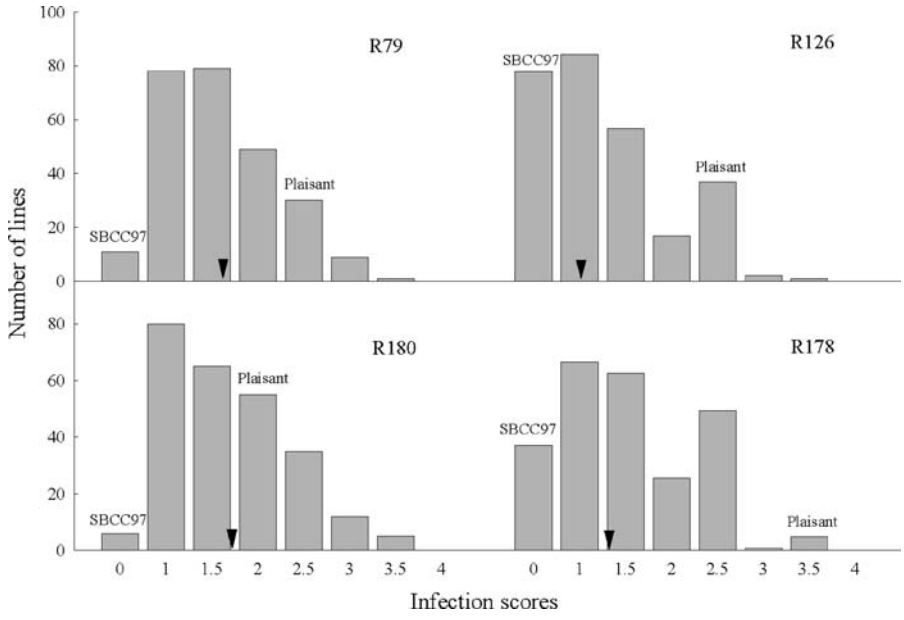

Fig. 1 Frequency distribution of resistance/susceptibility to the different isolates of powdery mildew in the 262 RI lines. *Vertical arrows* indicate the population means

Linkage map

A linkage map was constructed with 111 polymorphic markers (Fig. 2a). In the first stage of the sequential mapping approach, 101 SSR markers were examined in 75 F_6 lines. In the second step, 10 additional SSR and RGA-based markers were included in promising candidate regions on chromosomes 2H, 3H and 7H. The dotted lines represent regions on the chromosomes where no polymorphic markers were identified (106 markers that were distributed throughout the major gaps in the map were monomorphic). Deviation from the expected 1:1 segregation ratio in favour of SBCC97 alleles was observed for eight markers on chromosomes 1H and 5H, whereas distortion of the segregation ratio in favour of 'Plaisant' alleles was significant ($P < 0.05$) in regions of chromosomes 3H and 4H (one marker each). The first map consisted of 25 linkage groups (LOD score >3.0) with a combined total genetic distance of 1090.5 cM and an average density of 9.8 cM per marker. Average LOD score for linkage group merger was 6.4, with 4 groups merged at LOD 3, 8 at LODs of 4–5, 13 at LODs of 6 and above. The linkage groups were assigned to chromosomes according to previously published data (Varshney et al. 2007). Consensus maps were also used to place isolated markers on chromosomes. The inclusion of additional markers did not change the linkage map significantly, and only the regions on chromosome 7H were considered sufficiently interesting to warrant further analysis. Therefore, chromosome 7H was genotyped in the whole population. The resulting map for chromosome 7H comprised three linkage groups, rather than the initial four (Fig. 2b).

QTL analysis

Preliminary QTL analysis was performed on the subset of 75 F_6 RILs. CIM identified only two regions on chromosome 7H that were associated with resistance to powdery mildew (Fig. 3). Additional analyses using multiple linear regression and non-parametric (Wilcoxon rank-sum) approaches, better suited to non-normal data, gave essentially the same results. One and three additional QTL were detected, respectively. In all cases, these additional QTL affected only one

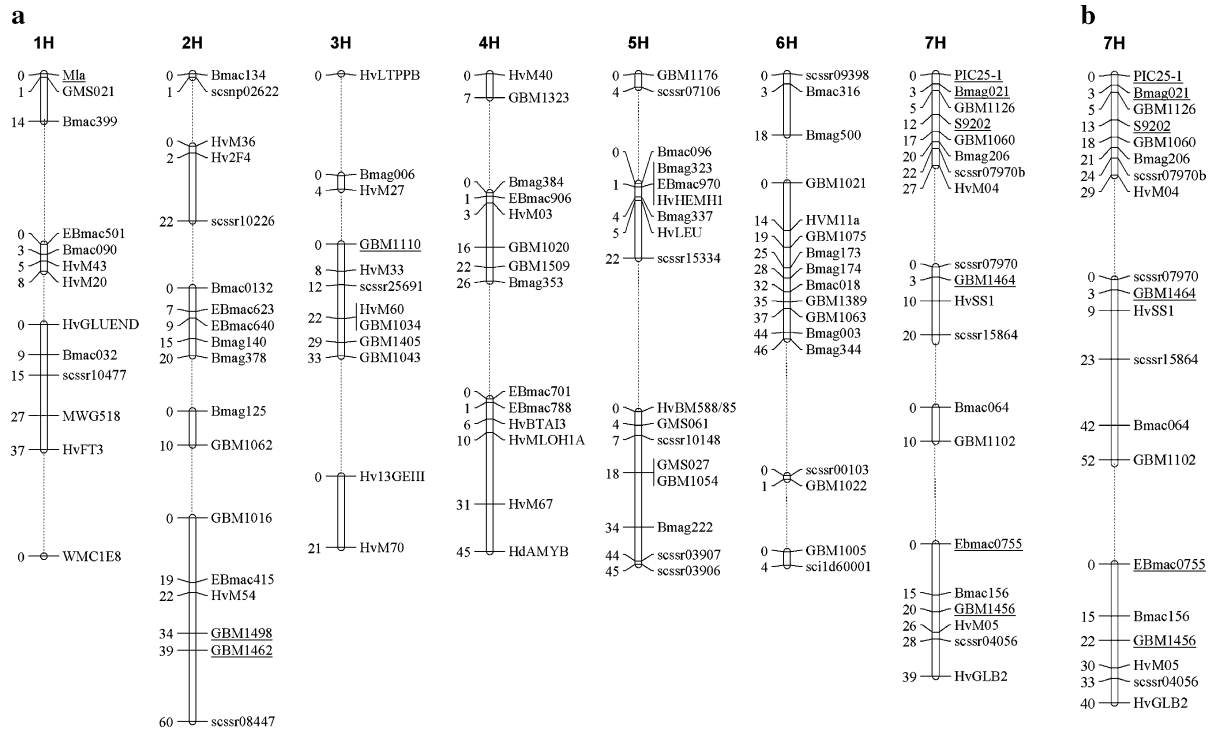

Fig. 2 Linkage map of SBCC97 × 'Plaisant' constructed with 75 F_6 lines (**a**) or 262 F_5 and F_6 lines (**b**). Markers that were incorporated in the second stage of sequential genotyping are *underlined*

isolate, and presented minor effects (data not shown). The same regions on the short and long arms of chromosome 7H were identified for all four isolates, although the significance of the putative QTLs differed depending on the isolate. QTL analysis on chromosome 7H with the whole population (262 RILs) confirmed the presence of two peaks on the short and long arms of the chromosome (Fig. 4). The same significant QTLs were detected for all four isolates and, in all cases, the resistant allele was contributed by the Spanish parent. The first QTL was located on the short arm of chromosome 7H (bins 1–2), in the interval between markers GBM1126 and HvM004. The closest marker to the peak was S9202 for isolates R79 and R180, whereas for isolates R126 and R178, it was GBM1060. This QTL exhibited a LOD score between 3.5 and 13.2, and accounted for 3.8–18.5% of the phenotypic variance. The second QTL had an even more significant effect and was located on the long arm of chromosome 7H (bins 11–12), with the peak at EBmac0755 for all isolates. The LOD score for this QTL ranged from 10.5 to 23.3, and it accounted for 15.6–30.7% of the phenotypic variance, depending on the isolate.

The analyses of variance, including the markers that were closest to the QTL peaks as sources of variation, showed no interaction between the QTLs above a significance threshold of 0.05, according to a Bonferroni correction that considered all possible pairwise tests among the chromosome 7H markers (data not shown).

Given that only two QTLs were apparently responsible for resistance to powdery mildew in this population, the RILs were split into four groups according to the allele that was present at the closest marker to each QTL, i.e. GBM1060 and EBmac0755 (Fig. 5). As expected, the QTLs behaved additively and clear differences were observed among the classes for all isolates. The group that carried both alleles from SBCC97 showed a greater degree of resistance to all isolates, whereas those plants with the same allelic composition as 'Plaisant' were susceptible. The group of genotypes that carried both QTL alleles from SBCC97 (approximately 25% of the population)

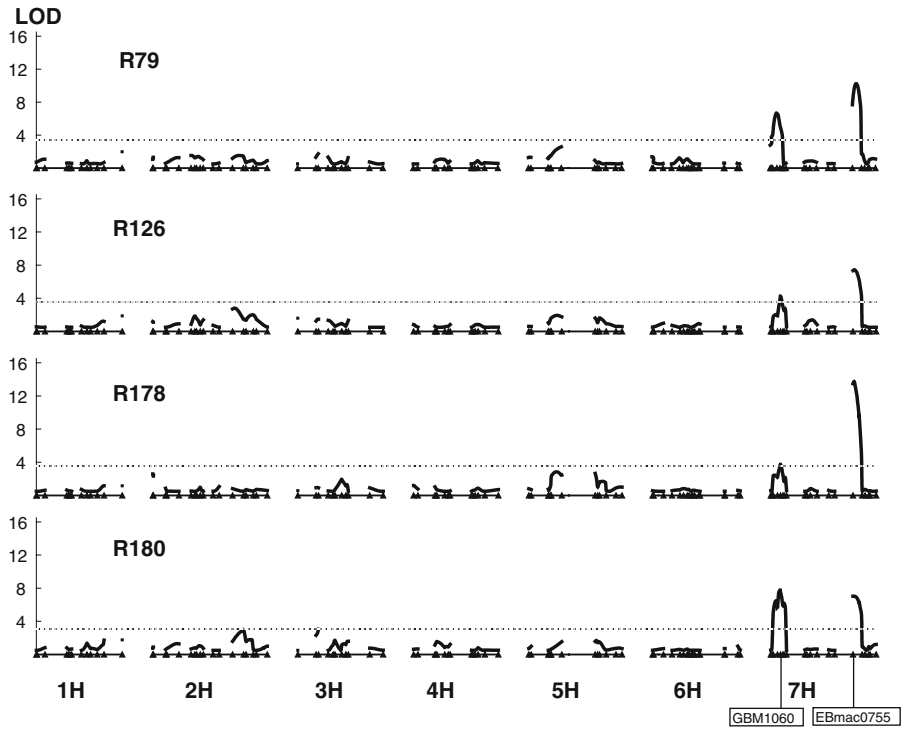

Fig. 3 Composite interval mapping LOD scans for resistance scores to different isolates of powdery mildew in the subsample of 75 F_6 RI lines. The *horizontal dotted lines* indicate the significance threshold for QTL detection based on an experiment-wise error rate of less than 5%, estimated with 1000 permutations

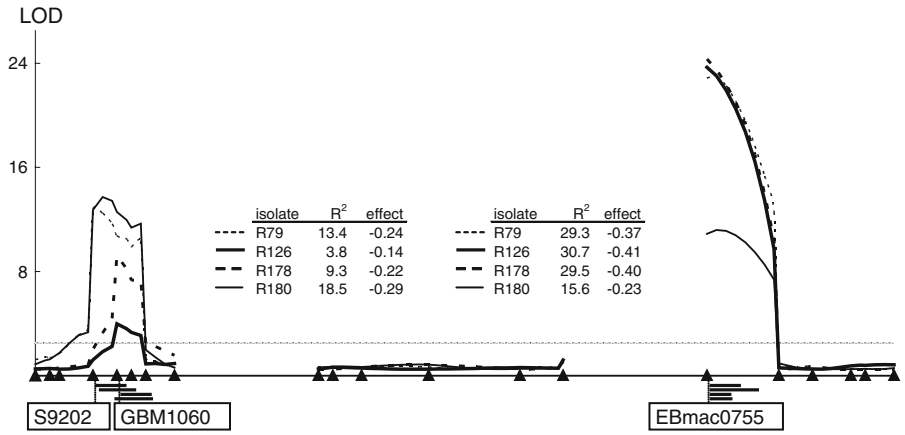

Fig. 4 Composite interval mapping LOD scans for resistance scores to different isolates of powdery mildew in 262 F_5 and F_6 RI lines. *Horizontal dotted lines* indicate the significance threshold for QTL detection based on an experiment-wise error rate of less than 5%, estimated with 1000 permutations. *Small horizontal bars* represent 2-LOD confidence intervals for the different isolates. R^2 is the percentage of phenotypic variance explained by the QTL. A negative value for the additive effect indicates that the allele from SBCC97 reduced the value of the trait

showed a much greater degree of resistance to all isolates. Actually 66% of plants with total resistance to any isolate (0 score) were identified within this group. Only 1.6% of susceptible plants (score of 2 or higher) were in this group. On the other hand, the group of lines with both QTL alleles from 'Plaisant' (also approximately 25% of the population) included no lines with a score of 0, and 71% of the lines with a susceptible score (2 or higher).

Discussion

In a previous study, high levels of resistance to powdery mildew were found in some landrace-derived lines from the SBCC (Silvar et al. 2009). Spanish barleys present adaptation to conditions in Southern Europe, where they are likely to have evolved under abiotic stresses and pathogen pressures (Lasa et al. 2001; Yahiaoui et al. 2008). From the results of previous studies (Silvar et al. 2009), we can speculate that these landraces are a potential source of unexploited genes or alleles for disease resistance.

The sequential mapping approach described here is similar to approaches used to identify loci in human linkage analyses and population-based association studies (Satagopan et al. 2002, 2007 Wang et al. 2006). In our case, this method proved to be a

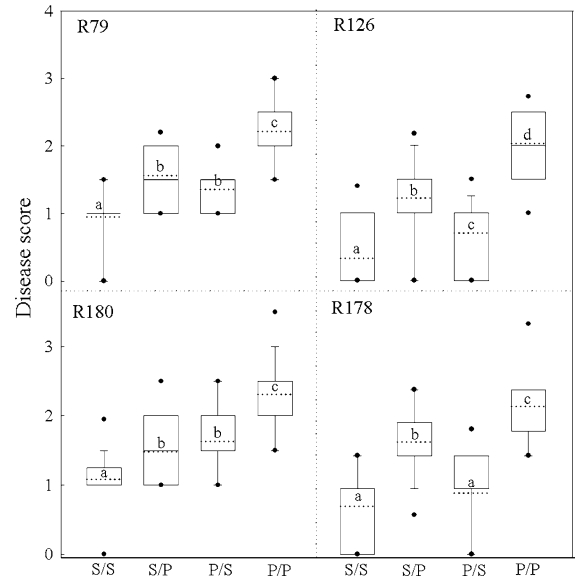

Fig. 5 *Box* and *whisker plots* showing the distribution of 262 F_5 and F_6 RI lines according to the alleles present at the closest marker to each QTL (GBM1060 and EBmac0755). '*S*' represents the SBCC97 allele and 'P' the 'Plaisant' allele. The ends of the whiskers represent the 5th and 95th percentiles and the *dots* indicate outliers. The *boxes* limit the two central quartiles, and the *dotted lines* within the *boxes* represent the mean disease score for each group. *Different letters* above the *dotted lines* indicate that the difference between the means is significant ($P < 0.05$), as shown by analysis of variance (ANOVA) (SAS 1988)

very effective strategy, because it reduced considerably the costs required by classical approaches. The linkage mapping and QTL analysis were performed successfully by generating only 12,065 genotypic data points, 8,325 in the first step and 3,740 in the second. If the whole set of markers had been analysed in the entire RIL population, 29,082 data points would have been generated. The main problem with this two-step analysis is that regions containing minor QTLs could be inadvertently discarded, due to the reduced sample size of the first step. However, in practical terms, this drawback was probably not a major issue, as the aim of this work was to identify major QTLs that conferred resistance to powdery mildew, and these were detected readily in the first step. The effect of the two large QTLs, calculated in the second step, however, is probably overestimated to some extent, as the QTLs on 7H may explain part of the variance actually caused by minor QTL on the other chromosomes. Other QTL that could be detected with alternative analyses (linear regression and non-parametric) showed only minor effects. If they were true QTL, it is questionable whether their effect would be large enough to be of practical value.

The scores for resistance to isolates R79 and R180 gave a bell-shaped curve, which was close to a normal distribution, and was coherent with the presence of several QTLs. In contrast, the bimodal distribution that was obtained for R126 and R178 suggested the presence of at least two QTLs that had effects of different size. This was also supported by the large number of resistant lines in the population. A clear transgressive segregation was observed for the response to isolate R180. A remarkable proportion of the lines were more susceptible to the pathogen than either parent. This may be due to the contribution of resistance and susceptibility factors by both parents, as has been reported in many other QTL studies of disease resistance (Hayes et al. 2003).

The estimated positions of the markers in the linkage groups were in agreement with previously published consensus maps, which enabled the linkage groups to be assigned to chromosomes (Varshney et al. 2007). Segregation distortion was found at only 9% of markers located on chromosomes 1H, 3H, 4H and 5H. The distortion at 1H and 5H (4 markers in each) corresponds to regions of major photoperiod and vernalization genes (*PpdH2* and *VrnH1*, respectively). This distortion seems to be common under our conditions, as we have noticed it in other crosses as well. The plants were vernalized at each generation, to avoid selection for vernalization requirement but, still, it seems that the population has been under some sort of selection pressure. In any case, the regions affected are far from the powdery mildew QTL detected in this study and should not affect the results presented here. The distortion in favour of the 'Plaisant' allele at just another two markers was not as strong, and seems the result of chance events.

Two RGA-based markers (PIC25-1 and S9202) were included in the genotypic analysis, because they may be close to one of the QTLs located. Many of the cloned genes for resistance to plant diseases encode proteins with nucleotide binding site (NBS) and leucine rich repeat (LRR) domains, and they often belong to complex loci that comprise arrays of related genes (Martin et al. 2003). These genes, called resistance gene analogues (RGAs), are abundant in plant genomes, and may represent undiscovered resistance genes and therefore may be potential candidates for QTLs (Bulgarelli et al. 2004). RGA PIC25-1 was isolated by Collins et al. (2001) and was mapped in a Steptoe × Morex population to the short arm of chromosome 7H, in the vicinity of the *Rpg1* gene. RGA probe S9202 was also localised in a Steptoe × Morex cross at the distal region of chromosome 7HS, but it was located closer to the *mlt* and *Rph2* genes (Madsen et al. 2003).

Our QTL analysis identified two regions for resistance to powdery mildew on chromosome 7H, which together accounted for up to 42.7% of the total phenotypic variance. Both alleles for resistance were contributed by the Spanish barley landrace SBCC97. One of these QTLs localised to the first linkage group of this chromosome, which corresponded approximately to the distal portion of 7HS. It was more effective against isolates R79 and R180, with LOD scores of 12.3 and 13.2, respectively, than against isolates R126 and R178. For R79 and R180, the closest marker to the peak, RGA S9202, was derived from a gene, whereas for R126 and R178, the closest marker to the peak, GBM1060, was derived from an EST. This region of chromosome 7HS is known to contain families of NBS RGAs, such as pic20 (Ayliffe et al. 2000), Hvb9 (Leister et al. 1998), ssCH4 (Seah et al. 1998), and ABG1019 (Brueggeman et al. 2002), which are different from S9202 and unrelated to one another. Other studies have also located major genes

and QTLs for other diseases, such as *Rpg1* (Brueggeman et al. 2002), *Rdg2a* (Bulgarelli et al. 2004) and *Rrs2* (Schweizer et al. 1995), in this region. With regard to powdery mildew resistance in barley, a QTL on chromosome 7HS was first reported by Backes et al. (1995, 1996), who identified a QTL in the vicinity of the restriction fragment length polymorphism (RFLP) marker MWG530. Subsequently, Schönfeld et al. (1996) identified *mlt*, a recessive gene that provided race-specific resistance to powdery mildew, in the line RS42-6*, which was derived from a *Hordeum vulgare* ssp. *spontaneum* accession. This gene was localised to bin 7H-01, at 2.6 cM from MWG555a, and may correspond to the QTL on 7HS that we have identified. According to Madsen et al. (2003), this position probably excludes RGA S9202 as a candidate for the *mlt* gene. In a more recent study, Řepková et al. (2006) identified a QTL for resistance to powdery mildew, in a cross between cultivar 'Tiffany' and the *H. spontaneum* accession PI466461, which is linked tightly to the marker Bmag0021. We did not find any association with Bmag0021, even though the position of this marker in our map agreed with that in the consensus map (Varshney et al. 2007). On the other hand, Řepková et al. (2006) found that this is a highly recombinogenic region and, therefore, the association of a specific marker with a QTL may be lost in other germplasm groups. The resolution of our map meant that we could not draw definite conclusions about the relationship between this QTL and genes that were identified in previous studies. This region seems rich in resistance genes and it is well known that many resistance genes are located in clusters that contain several closely related genes, although the latter are not always involved directly in resistance (Williams 2003). Ongoing work to increase marker density should shed light on the genetic basis that governs resistance to powdery mildew in this region.

A second QTL, which had a larger effect than the first, was identified on the third linkage group of chromosome 7H (long arm). It showed high LOD scores (10.5–23.3) and accounted for approximately 30% of the phenotypic variance for three isolates. For all four isolates, the closest marker was EBmac0755. In spite of the small confidence interval, the fact that EBmac0755 is the first marker in the linkage group adds uncertainty to the position of the QTL. Other SSRs in this region (GBM1419, Bmag0120, GBM5225, GBM1362, GBM1017 and GBM1065) were tested but none was polymorphic. Despite this uncertainty, it seems likely that this QTL is located close and proximal to EBmac0755, as indicated by the figures presented in Fig. 5 and by the profile of the genome scan (Fig. 4). The low number of false positives when using only EBmac0755 to tag the QTL means that it cannot lie far from the marker. This region has been repeatedly identified in the literature as carrying QTL for powdery mildew resistance, in crosses involving different *H. spontaneum* accessions. Schönfeld et al. (1996) found a semi-dominant resistance gene, ultimately contributed by a *H. spontaneum* accession, which they named *Mlf*. Its position, based on common markers (according to the consensus map of Marcel et al. 2007), is very close to EBmac0755, on bin 7H-11. Backes et al. (2003) identified a QTL for resistance to powdery mildew in this region, derived from a different *H. spontaneum*, in the cross 1B-87 × 'Vada'. Recently, Řepková et al. (2006) found a tight linkage between marker EBmac0755 and a resistance QTL against powdery mildew in the cross 'Tiffany' × PI466297, with the resistance allele contributed by yet another *H. spontaneum* parent. von Korff et al. (2005) also reported a QTL on the long arm of chromosome 7H, at the position of marker HVCHI26A, in an advanced backcross population derived from the cross 'Scarlett' × ISR42-8. Again, the resistant allele was contributed by the *H. spontaneum* accession. Recently, Schmalenbach et al. (2008) developed a set of introgression lines from the same population, and identified a QTL that strongly reduced susceptibility to powdery mildew in three lines. These authors postulated that this QTL was in fact *Mlf*. These introgressed regions overlap with our QTL on 7HL, according to consensus maps. Other quantitative resistance reported on 7HL (Rossi et al. 2006), seems different from the one found in the present study, according to the distances of associated markers on the consensus map of Marcel et al. (2007). The question of whether the QTL we have found on 7HL is (or is not) *Mlf* remains open. Since this is a relevant locus for barley breeding, it would be interesting to investigate if the QTL found in different studies are the same.

The QTLs presented a slightly different response profile to the four isolates. It is worth noting that two of these isolates (R79 and R126) were selected based on the different responses caused by them on the

genotypes of the Spanish Barley Core Collection. Thus, we expected that the use of these two isolates would allow the possible identification of QTL with different specificity. The QTL on 7HS was most effective against isolates R79 and R180, and the QTL on 7HL showed a larger effect for all isolates except for R180. On the other hand, both QTL presented significant effects against all four isolates. We can conclude that we have found two QTLs with a broad effect and slightly different reaction spectra.

In the field of plant disease resistance, it is not surprising that QTLs are detected in the genomic regions that contain major resistance genes (Pflieger et al. 1999; Wagner et al. 2008). This appears to be our case, as our QTLs were in the chromosomal regions of resistance genes *mlt* and *Mlf*. Interestingly, both *mlt* and *Mlf* have been found in lines that are derived from the wild species *H. vulgare* ssp. *spontaneum*. Only once, a QTL close to the *mlt* gene was reported in cultivated barley, in a cross between cultivars 'Igri' and 'Danilo', with the resistance allele contributed by 'Danilo' (Backes et al. 1995, 1996).

With this study, we have found the approximate location of two QTL, and determined their large effect on resistance to powdery mildew, to the degree of detail needed to advocate their use in plant breeding. Future work with high resolution mapping populations for each of these two QTL in this population is envisaged for the future. One outstanding observation from our results was that both QTLs arose in a single inbred line of cultivated barley, SBCC97. This fact will facilitate their use in breeding, as the sources of resistance identified in previous works contributed only one QTL that, in most cases, came from a wild source (Schönfeld et al. 1996; von Korff et al. 2005; Řepková et al. 2006). Therefore, we expect that the transfer of these two QTLs from SBCC97 into elite material will require a reduced number of crosses compared to pyramiding of the two QTL from two different sources. Also, although SBCC97 is quite different from current elite cultivars, and linkage drag will still be an issue when used for introgression, the transfer of their QTLs will possibly entail less linkage drag than caused by *H. spontaneum* sources. Therefore, although further work is needed to localise the QTLs more precisely and to validate the resistance in other backgrounds, we recommend the use of SBCC97 as a donor for resistance to powdery mildew in barley breeding programmes.

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