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Genetic basis of control of *Rhynchosporium secalis* infection and symptom expression in barley

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Abstract The genetic basis of several different components of resistance to *Rhynchosporium secalis* in barley was investigated in a mapping population derived from a cross between winter and spring barley types. Both the severity of visual disease symptoms and amount of *R. secalis* DNA in leaf tissues were assessed in field trials in Scotland in the 2007/2008 and 2008/2009 growing seasons. Relative expression of symptoms was defined as the residual values from a linear regression of amount of *R. secalis* DNA against visual plot disease score at GS 50. Amount of *R. secalis* DNA and visual disease score were highly correlated traits and identified nearly identical QTL. The genetic control of relative expression of symptoms was less clear. However, a QTL on chromosome 7H was identified as having a significant effect on the expression of visual disease symptoms relative to overall amount of *R. secalis* colonisation.

Keywords Asymptomatic colonisation · Disease resistance · Leaf scald · Mapping population · QTL

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Introduction

Rhynchosporum secalis (Oudem) J.J. Davis, the pathogen that causes 'rhynchosporium', 'barley leaf blotch' or 'scald', in *Hordeum vulgare* L. (barley). is one of the most economically important barley pathogens worldwide, particularly in cool humid environments, causing reductions in both yield and grain quality (Zhan et al. 2008). Average yield losses (from Canada) have been estimated at 5–10% (Turkington et al. 1998), though losses of up to 40% have been reported under conditions favourable for the disease (Xi et al. 2000). Mapping studies have located a number of major resistance (*R*) genes and quantitative trait loci (QTL) affecting expression of resistance to *R. secalis;* these are predominantly located on barley chromosomes 2H, 3H and 7H (Zhan et al. 2008). Whilst current control strategies in the UK frequently include a fungicide treatment, commercial cultivars with good levels of resistance, probably due

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to major gene factors on chromosomes 3H and 7H, are available. However, breakdown of such sources of host resistance is generally rapid, if they are used in widespread commercial deployment of resistant cultivars, as *R. secalis* populations are able to evolve rapidly (Abang et al. 2006; Newton et al. 2001). Therefore, novel sources of resistance to *R. secalis* represent a valuable resource for plant breeders. In particular, the identification of quantitative resistance loci, which have previously been shown to be more durable than major resistance loci in other host-pathogen systems (Brun et al. 2010), is of considerable importance. To ensure food security, particularly for subsistence farmers who cannot afford to use fungicides, it is essential to breed for resistance that is not rapidly rendered ineffective by changes in pathogen populations.

In the UK, ratings for 'field resistance' to *R. secalis*, based on visual assessment of disease symptoms on leaves of barley crops/field plots, are generally and consistently greater in winter (autumn sown) barley than in spring barley (http://www.hgca.com). This difference is greatest when spring types are autumn-sown and scored for disease symptoms alongside winter types (Newton et al. 2004) but it is maintained even when each type is grown in the appropriate season. Whilst the origin of this difference remains a subject for speculation, such observations suggest that winter barley germplasm represents a potential source of resistance genes that could be incorporated into spring lines. Thus, populations derived from crosses between spring and winter parents are of considerable interest to the study of the genetic basis of resistance to *R. secalis*.

A problem associated with the use of populations segregating for major developmental genes to address such questions is the extent to which field resistance QTLs are simply pleiotropic expressions of broader morphological differences. For example, in a cross between the spring barley genotypes B83-12/21/5 and Derkado, two known semi-dwarfing genes (*sdw1* and *ari*-eGP) were consistently associated with QTL for resistance to *R. secalis* (Thomas et al. 2010), with semi-dwarf types showing more disease symptoms. Given that secondary infection is mediated by splash dispersal of *R. secalis* spores (Fitt et al. 1988), this finding almost certainly reflects a pleiotropic effect of height rather than an interaction between host and pathogen and therefore needs to be accounted for in selection for resistance.

R. secalis is known to have a long asymptomatic phase in crop leaves between infection and development of visual symptoms (Davis and Fitt 1990; Walters et al. 2008) and recent work has shown that the pathogen may complete its life cycle and produce asexual spores on apparently healthy leaf tissue (Atkins et al. 2010; Fountaine et al. 2010). Understanding the mechanisms that cause the switch between asymptomatic and symptomatic *R. secalis* colonisation and its genetic basis could be important for devising breeding strategies for producing cultivars with durable resistance. It is therefore necessary to determine whether suppression of disease symptoms (or more generally, the level of disease symptom expression relative to the amount of pathogen colonisation) represents a separate mechanism of resistance from that which prevents the infection that precedes colonisation (Hahn et al. 1993; Lehnackers and Knogge 1990). Viewed from an evolutionary perspective, such a mechanism would imply that the expression of disease symptoms represented a yield cost to the plant greater than that caused by pathogen colonisation alone. Resistance that differentially restricts colonisation and symptom development will also affect disease risk in relation to other epidemiological factors (e.g. through differential effects on amounts of inoculum within a field and thus, potentially, a differential response to environmental factors that may cause a switch between asymptomatic and symptomatic colonisation). Therefore a better understanding of the genetic basis of resistance in UK barley crops will also facilitate more appropriate targeting of fungicides.

Severity of disease symptoms and amount of pathogen colonisation can be measured using visual assessment and quantitative real-time PCR (qPCR), respectively, as described by Fountaine et al. (2007). The current study utilised these two approaches to investigate the genetic basis of resistance to rhynchosporium in a mapping population from a cross between winter and spring barley types. An additional aim was to define relative disease expression based on these measurements, and use this to investigate whether the suppression of rhynchosporium symptom expression (following successful infection by *R. secalis*) has a distinct genetic basis in barley.

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Materials and methods

Plant material, mapping population and markers

A doubled-haploid mapping population was produced by microspore culture from the F_1 progeny of a cross between the spring barley cultivar Cocktail and the winter barley inbred line WB05-13, derived from a cross between the winter cultivars Leonie and Pearl. Cv Leonie was bred by Nordsaat in Germany and was the most resistant cultivar on the UK recommended list during its period of special recommendation from 2001 to 2003. As it also had resistance to barley yellow mosaic virus strains BaMVV and BaYMV-1 and good malting quality, it had a special recommendation for the UK (http://www.hgca.com/varieties/2003/common/200212/recommendedlists/data/WBcolour.pdf). Cv Pearl was bred by Limagrain (formerly Nickersons Seeds) and has been recommended for growth in the UK since 1999; it has been the main winter barley malting cultivar grown by farmers over this period. It was initially rated as having a good resistance to *R. secalis*, being rated '8', on a 1–9 scale of increasing host resistance as described in the recommended list protocols (www.hgca.com). Leonie originally had the best rating of '9,' but its resistance rating had declined to '5.9' by 2010 (www.hgca.com). Cv Cocktail was first recommended for cultivation in the UK in 2003 and was formerly an accepted spring barley malting cultivar in the UK. It does not possess either of the two *R* genes for resistance to *R. secalis* found in current UK spring barley cultivars and had a moderate resistance rating of '5' when first recommended, which had increased slightly to '5.9' in the 2010 recommended list (www.hgca.com).

WB05-13 was bred to combine the resistance to *R.secalis* and BaYMV-1 of Leonie with the accepted malting quality attributes of Pearl; thus progeny from its cross with Cocktail are expected to segregate for resistance to *R. secalis* and to BaYMV-1 as well as for the *sdw1* dwarfing gene found in Cocktail. In addition, WB05-13 has the *Vrs1*.t allele at the VRS1 locus on chromosome 2H and the mapping population therefore also segregates for the *deficiens* ear type. Over 800 individual plants were derived from microspore culture of the F_1 progeny; 550 lines were fertile and produced sufficient seed for a field multiplication plot that was sown at the James Hutton Institute in autumn 2006. Immediately prior to harvest, a single plant was recovered from each of the multiplication plots. For the first 191 lines, the seed from this single plant was used as the primary seed source for agronomic trials and a reference seed stock. The remainder of the plot was harvested with a small plot combine and the seed was used as secondary seed source for agronomic trials.

A single seed was taken from the reference stock of each line and grown in the glasshouse. A 2–3 cm length of leaf tissue was harvested from the youngest leaf of each of these barley plants at the 3–4 leaf stage. Leaf material was harvested into 96 deep well blocks (VWR # AB-0932) containing a stainless steel ball bearing (Spex Centriprep Ltd #662316). Total plant DNA was extracted using the Tepnel Nucleoplex Automated DNA Isolation, according to the manufacturer's instructions using the Standard Plant Lysis and Plant Purification protocols (Tepnel #: 33300). DNA concentration was estimated using Quant-iT Picogreen dsDNA Assay kit (Invitrogen #P11496). Sufficient volume of a 1:200 working dilution of picogreen reagent in 1× TE was made up and 197 µl was pipetted into white flat bottomed assay plates (Thermo Fisher #DIS-940-010T). 3 µl of DNA samples to be measured and DNA standards that were made up at 75, 50, 25, 12.5, 6.25, 3.13, 1.56 and 0 ng/µl from Lambda DNA (Invitrogen #1363336) were added to the picogreen reagent. Plates were incubated for 2 min then absorbance readings were taken from a Flouroskan Ascent plate reader. A standard curve was created using the DNA standards (R^2 value between 0.950 and 0.999) and was then applied to the unknown samples to estimate concentrations. DNA concentrations were normalized to 50 ng/µl in preparation for genotyping.

A 5 µl aliquot of DNA from each line was used for genotyping with a custom Bead Xpress Oligo Pool Assay (Illumina), which comprised 384 single nucleotide polymorphism (SNP) markers that had been selected (based on their quality, informativeness and coverage of the barley genome) from the set of 1536 gene-based SNP markers previously developed for the first Illumina production Barley Oligo Pooled Array (Close et al. 2009). Allele calls were made as SNP bases using the Illumina Beadstudio software and validated manually.

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Map construction

The individual base calls for each marker were converted into 'a' (Cocktail), 'b' (WB05-13) and—'(missing) scores by comparison to the parental scores for input into JoinMap 4 (Van Ooijen 2006). Before conversion, monomorphic markers or markers that had a large number (>15%) of heterozygous calls were discarded, since the former are uninformative in mapping and the latter reflect poorer quality markers. There was a small proportion of remaining heterozygotes in the data and individual lines were removed where there were more than 15% of these since the DNA quality and/or quantity was suspect. The few remaining heterozygous calls were re-classified as missing. Markers that consistently remained linked to each other between LOD 2.0 and LOD 10.0 were classified into groups that were each assigned to an individual barley chromosome by comparison with previously mapped positions for each marker (Close et al. 2009). Marker order and position within each linkage group was estimated by using the regression mapping option of Joinmap 4.0 with Kosambi's mapping function. In all cases, linkage phase was identical to that predicted by the parental genotypes.

Field trials

Seed from the primary and secondary seed sources was used to sow the 190 lines of the mapping population in field trials over two winter barley growing seasons (2007/2008 and 2008/2009) at the James Hutton Institute rhynchosporium disease nursery (Table 1). Cocktail, Leonie and Pearl together with seven other controls were included in the trial to give a trial with 200 entries. Field trials were arranged in a row and column design with two replicates and plot sizes of 2 m × 1.5 m at a seed density estimated to produce 250 established plants m^{-2}. The plots were combine drilled with fertiliser applied at a rate of 30.5P and 87 K kg ha^{-1} and received an *N* application of 51 N kg ha^{-1} at average growth stage (GS) 30 (Zadoks et al. 1974) and 69 N kg ha^{-1} at GS 40. Weeds were controlled by applying a herbicide but no fungicides were applied. Primary inoculum was from residual barley crop debris from the previous harvest and overhead irrigation was applied on alternate days to encourage secondary disease spread, commencing when soil moisture levels decreased sufficiently to avoid water logging (late April or early May). Assessments of rhynchosporium disease symptoms (visible lesions) for whole plots were made at several growth stages (Table 1) using a 1–9 scale (Newton and Hackett 1994), where 1 represented no visible symptoms in the entire plot and 9 indicated complete leaf death due to rhynchosporium. In addition, the upper three leaves from five randomly selected plants from each plot were taken for qPCR quantification of *R. secalis* DNA at GS 50 in 2008 and at GS 26 (where five whole plants were sampled) and 50 in 2009. For each of the three leaves (and for whole plants) samples from within a plot were combined for subsequent qPCR analysis. Total DNA was extracted from the samples using a high salt extraction protocol according to Bearchell et al. (2005). *R. secalis* DNA was quantified from 50 ng of the sample of total DNA using a qPCR protocol described by Fountaine et al. (2007). Plot disease scores were normalized using a natural logarithm transformation prior to further analysis in order to normalize the data.

Relative disease expression scores (i.e. the differences between areas of visual symptoms that would be

Table 1 Times of operations during field trials on development of rhynchosporium on two replicate plots of each of 191 lines of a spring × winter barley mapping population grown in the James Hutton Institute disease nursery in the 2007/2008 and 2008/2009 growing season. Where known, the growth stage corresponding to the date is given in parentheses

Operation	2007/2008	2008/2009
Sowing	26 Oct 2007	28 Oct 2008
Plot disease assessments[a]	19 May 2008	22 April 2009
	04 June 2008 (GS 50)	01 May 2009 (GS 31–50)
	17 June 2008	14 May 2009
	30 June 2008	19 May 2009
	11 July 2008	09 June 2009 (GS 35–60)
		23 June 2009
Samples for qPCR[b]	05 May 2008 (GS 26–30)	01 April 2009 (GS 26–30)
	06 June 2008 (GS 50)	28 May 2009 (GS50)

[a] Assessment of area of visual disease symptoms across entire plots measured on a 1–9 scale (Newton and Hackett 1994)

[b] Date at which leaf samples from selected plants were taken for qPCR estimation of amount of *R. secalis* DNA

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expected, given the amounts of pathogen colonisation, and the observed areas of visual symptoms) were obtained by fitting a standardised major axis linear regression model to the relationship between the amount of *R. secalis* DNA (GS50) and visual plot disease score (GS50) using the lmod2 package in R (http://www.R-project.org). Residuals (defined as orthogonal distances from the fitted line) were calculated and taken as a measure of relative disease expression. This method was used rather than taking the residuals from a least squares linear regression to account for the presence of significant measurement error in both variables.

Statistical analysis

Statistical analyses were made using GenStat software (Payne et al. 2009). Trait means for each of the DH lines for each season were estimated using a REML mixed model, fitting barley DH line as a fixed effect, and a random model comprising replicate. The analysis was repeated using random models with additional terms to account for spatial effects (selected from: random row, random column, correlated row, correlated column). The simplest model for which there was no significantly better, more complex, model was used to estimate line means. Phenotypic variance (V_p) and additive genetic variance (V_a) for each trait were estimated by REML, fitting the effect of environment (season), replicate (within environment) and DH line as the random model. Additive genetic variance was estimated as half of the between DH lines variance component (equivalent to $2V_a$). Heritability estimates were calculated as the ratio between V_a and V_p. For each pair of traits, additive genetic covariances (cov_a) were estimated by a REML analysis of the sum of the two traits. cov_a was calculated as half of the additive genetic variance of the sum of the two traits minus V_a for each of the two traits.

QTL analysis

Composite interval mapping was done by using the Biometris QTL mapping procedure library (Boer et al. 2007) found in GenStat 12 (Payne et al. 2009). This methodology enables the correct variance/covariance model to be used to account for the relationships between genotype and environment in 'multi-environment' trials. The two growing seasons were treated as separate environments and the VGESELECT procedure was used to identify the most appropriate model. The marker genotypes and their map positions were used to estimate genetic predictors at 2 cM intervals using the QIBDPROBABILITIES procedure. These predictors were then included in a simple interval mapping genome scan using the procedure QMQTLSCAN with a minimum distance of 30 cM between QTL maxima. The threshold value ($-\log_{10}P$) for identifying a QTL was 3.36, estimated to be the genome wide error rate at $P < 0.05$ by the method of Li and Ji (2005). The predictors associated with the maximum value for each QTL were then included as cofactors in a composite interval mapping scan using QMQTLSCAN and the procedure was repeated iteratively until there was no change in the selected co-factors. The final list of cofactors was used in the procedure QMBACKSELECT to iteratively eliminate any non-significant loci. Finally, the effects and type of action (QTL main effect or QTL× environment interaction) of those remaining were estimated using the QCANDIDATES procedure.

Results

Genotyping and genetic map construction

Of the original 190 lines, six were discarded because they had a high proportion of missing or heterozygous allele calls. Additionally, 161 markers were discarded because they were monomorphic or highly skewed and a further 48 were discarded during the construction of the genetic map due to a high proportion of predicted genotyping errors. Therefore, the final genetic map was based on 184 lines and 175 markers. Marker chromosome allocation and order were highly consistent with the barley consensus map (Close et al. 2009) but the map size was larger for all chromosomes.

Traits

The severity of the rhynchosporium epidemic (based on visual disease score) was substantially greater in 2008/2009 than in 2007/2008, particularly during later growth stages (Fig. 1). Disease scores for QTL analysis (symptoms and *R. secalis* DNA) were made at approximately GS50. Estimated line means (DH lines only) for log-transformed disease symptom

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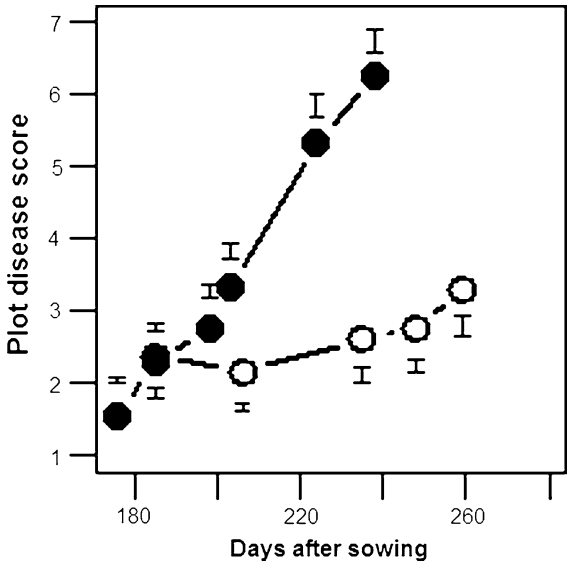

Fig. 1 Progress of rhynchosporium epidemics assessed visually as a proportion of plot area affected by leaf lesions on a 1 (symptomless) to 9 (100% leaf area covered by lesions) scale (Newton and Hackett 1994) with time (days after sowing in plots) in the James Hutton Institute disease nursery in the 2007/2008 (*open symbols*) and 2008/2009 (*filled symbols*) growing seasons. Data presented are the estimated mean plot score across all 191 DH lines in the spring × winter barley mapping population. Standard errors for each time point are indicated by *vertical bars* (located below the points for 2007/2008 and above the points for 2008/2009

scores had a mean of 0.81 (SD = 0.51) in 2007/2008 and 1.08 (SD = 0.50) in 2008/2009. Amount of *R. secalis* DNA had a mean of 435 ng (SD = 2042 ng) in 2007/2008 and 12486 ng (SD = 10731 ng) in 2008/2009. Relative disease expression was defined from a regression on standardised primary traits and as such (by definition) had a mean of 0 and standard deviation of 0.56 in both years. In the parental lines, estimated line means for the two winter barley parents had smaller disease scores than those of the spring barley parent in 2007/2008 (Leonie = 0.2, Pearl = 0.2, Cocktail = 1.15) but in 2008/2009 only one winter barley parent had a smaller disease score than the spring barley parent (Leonie = −0.02, Pearl = 0.4, Cocktail = 1.6) Fig. 2.

Correlations and heritabilities

There was a good genetic correlation between plot visual disease score at GS50 and amount of *R. secalis* DNA in leaves (at GS50) ($r_A = 0.91$). The heritability of plot disease score at GS50 (0.59) was substantially greater than that of amount of *R. secalis* DNA (0.08). Relative expression of disease symptoms also had a small heritability (0.03). Phenotypic correlations (2008/2009 only) between early growth stage (GS26) amount of *R. secalis* DNA and later (GS50) plot disease score were small ($r_p = 0.18$) compared to the phenotypic correlation between early and later growth stage plot disease score ($r_p = 0.67$).

QTL genome scans

The final QTL model based on visual plot disease scores identified three significant QTL effects (Table 2). These QTL effects were on chromosomes 2H, 3H and 7H. The position of the QTL on 3H is identical to that of a height QTL (data not shown) at the known position of the semi-dwarfing gene *sdw*1 (between markers 11_10515 and 11_20612). Given what is known about the epidemiology of rhynchosporium and that crop height has previously been reported as a mechanism of disease escape, this QTL very probably represents a pleiotropic effect of *sdw1*. Whilst the QTL effect on 7H (located between markers 11_11098 and 11_10169) is in a similar position to Vrn-H3 (a determinant of flowering time located on the short arm of chromosome 7H), Vrn-H3 is more distal than the 7H resistance QTL and it is inferred that it is flanked by markers 11_20162 and 11_11014 (44–84 cM) on the current map. Similarly, whilst an *R* gene for resistance to *R. secalis* (*Rrs2*) has been mapped to the short arm of chromosome 7H (Hanemann et al. 2009), its mapped position is distal to the QTL effect identified here, being between markers 11_11179 and 11_20245 (0–7 cM on this Map) (unpublished data). Similarly, for the resistance QTL on 2H (located between markers 11_10791 and 11_10085), a QTL affecting flowering time (*Flt-2L*) has been reported on the long arm of chromosome 2H (Chen et al. 2009). However, this locus does not appear to be segregating in this population, with no significant QTL effects for ear emergence or height detectable (data not shown). In addition, the position of *Flt-2* is likely to be proximal to that of this resistance QTL, with the rice region that is collinear to the region containing Flt-2 (Chen et al. 2009) being located between markers 11_21459 and 11_10383 on this map. Likewise, the final QTL model for amount of *R. secalis* DNA identified three resistance QTL

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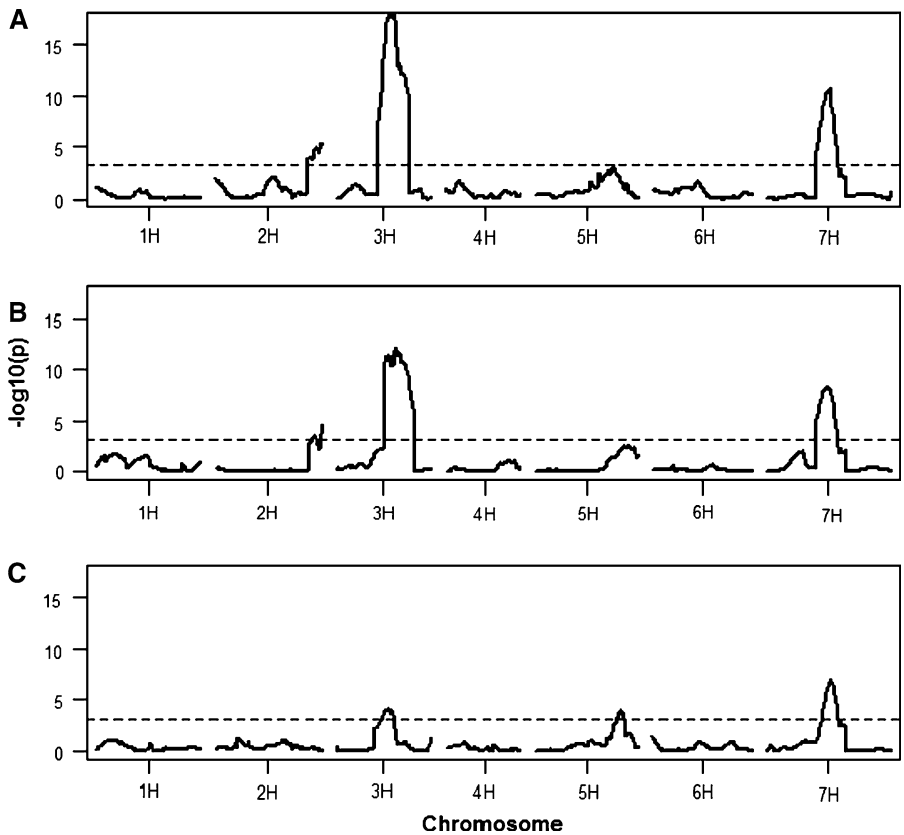

Fig. 2 Results from a multi-environment QTL genome scan for three different assessments of disease severity used to identify barley resistance to *R. secalis*. **a** Plot disease score (area of visual symptoms measured on a 1–9 scale). **b** Total amount of *R. secalis* DNA (determined by qPCR analysis of selected leaves). **c** Relative disease expression (defined as the second principal component of a principal component analysis of the two primary disease traits). *Solid lines* show how the probability (displayed on a $-\log_{10}$ scale) of an association between genotype and trait varies across each chromosome. Chromosomes are arranged sequentially along the x-axis with the 0 cM position for each chromosome at the *left* of each line. *Dotted lines* indicate the values of a test statistics equivalent to a genome-wide significance threshold of 0.05

that were all in nearly identical positions to those identified using visual disease symptoms and they were flanked by the same markers. This appears to reflect the strength of the genetic correlation between the amount of pathogen DNA and severity of visual symptoms.

Resistance QTL identified using relative disease expression generally had much smaller probabilities associated with them than those associated with QTL for primary traits. Composite interval mapping identified three resistance QTL; two are located on 3H and 7H close to the QTL effects identified using the primary disease traits (flanked by the same markers as the primary traits). The final QTL was located on chromosome 5H between markers 11_21077 and 11_11497 (Table 2).

Disease progression

An analysis of changes with time in visual plot disease scores across a single growing season (2008/2009) suggests that the heritability of plot scores remained generally consistent across all observations but that the additive genetic variance increased throughout the season (Table 3). Therefore, there was no evidence that the genetic basis of resistance varied during the course of a growing season.

Discussion

This work has identified two new QTL for resistance against *R. secalis* on barley chromosomes 2H and 7H.

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Table 2 Summary of final QTL models for the three disease traits examined (a: Visual plot rhynchosporium score; b: Amount of *R. secalis* DNA; c: Relative disease expression), showing the chromosome, map position (and flanking markers) for each QTL identified. Also shown is the estimated additive QTL effect (in the same units as phenotypic scores) in each growing season for each of the QTL included in the final QTL model

Locus	Chr	Position (cM)	Flanking markers	Effect 2007/2008 (SE)	Effect 2008/2009 (SE)
a: Visual plot score[a]					
1	2H	179.1	11_10791–11_10085	−0.13 (0.03)	−0.13 (0.03)
2	3H	90.5	11_10515–11_20612	−0.24 (0.03)	−0.24 (0.03)
3	7H	110.9	11_11098–11_10169	−0.21 (0.03)	−0.21 (0.03)
b: R. secalis DNA[b]					
1	2H	180.6	11_10791–11_10085	−305 (528)	−2,612 (517)
2	3H	99.6	11_10515–11_20612	−285 (548)	−4,008 (538)
3	7H	107	11_11098–11_10169	−120.3 (650)	−3,760 (636)
c: Relative disease expression[c]					
1	3H	86.6	11_10515–11_20612	0.18 (0.04)	0.18 (0.04)
2	5H	145	11_21077–11_11497	−0.16 (0.04)	−0.16 (0.04)
3	7H	111	11_11098–11_10169	0.35 (0.06)	0.35 (0.06)

The standard error associated with the estimated QTL effect is shown in parentheses

[a] Plot disease score at GS50 measured on 1–9 scale (Newton and Hackett 1994) and normalized using a natural logarithmic transformation

[b] Amount of *R. secalis* DNA at GS50 measured in pg

[c] Relative disease expression at GS50, defined as the residuals from a SMA regression fitting the effect of the amount of *R. secalis* DNA on area of visual disease symptoms

Table 3 Estimates of heritability (H^2) and additive genetic variance (V_a) of visual plot rhynchosporium scores at various measurement dates during the course of the 2008/2009 growing season

Measurement date	H^2	V_a
22 April 2009	0.33	0.08
01 May 2009	0.31	0.26
14 May 2009	0.36	0.54
19 May 2009	0.39	1.00
09 June 2009	0.41	2.25
23 June 2009	0.35	1.91

These QTL effects are not associated with known morphological or developmental genes. Neither do positions of these QTL correspond to those of loci for resistance against *R. secalis* infection that had been previously identified (Zhan et al. 2008). As such, whilst it is not possible to discount the possibility that these loci represent morphological or physiological mechanisms of disease escape that differ between the parental lines, the lack of previously reported effects in these regions suggest that they represent novel loci that will be a useful resource for understanding and manipulating the interaction between host and pathogen.

The QTL effect identified on chromosome 3H, which affected both area of disease symptoms and amount of *R. secalis* DNA, was associated closely with the known position of *sdw1* (Barua et al. 1993). This effect is probably a pleiotropic effect of height that acts by limiting effective dispersal of pathogen spores by rain-splash during secondary spread of the disease (Fitt et al. 1988); indeed, this QTL co-locates with an extremely strong QTL effect for height detected in a separate (fungicide treated) field trial (data not shown). This reinforces the importance of disease escape as a component of field resistance to *R. secalis*. For all identified QTL, the winter parent supplied the resistant allele. This is consistent with the observation that winter barley types generally have a higher resistance rating than spring types. However, the absence of strong associations between positions of major vernalistation/flowering time loci and those of resistance QTL suggests that it is not growth habit *per se* that affects resistance, but rather that desirable resistance characters are associated with winter barley types. This would appear to validate the use of winter ×

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spring crosses to identify novel sources of resistance for incorporation into spring germplasm.

The similarity between the profiles of resistance QTL identified using visual disease symptoms and those identified using amount of *R. secalis* DNA appears to reflect the strength of the genetic correlation between these two traits. This result is unsurprising, given the nature of the relationship between them. Nevertheless, the low heritability of the resistance QTL identified using amount of *R. secalis* DNA suggests that the precision of the qPCR method may not compare favourably to that of conventional scoring of area of visual disease symptoms (this might be either due to insufficient sampling, or be inherent to the assay itself). In either case, it is possible that this is responsible for the relative weakness of the correlation between early growth stage qPCR scores and later visual symptom scores. Sampling a greater number of plants from within a plot, to produce a bulked sample would offer the possibility of improved precision without increasing costs associated with performing the qPCR assay. Whilst improving the precision of the qPCR/sampling protocol may help in making early season qPCR scores a useful predictor of later disease severity, other results have shown that variation in amounts of rainfall may be a major determinant of subsequent epidemic development (Fitt et al. 2010).

The results of the QTL genome scan using relative disease expression, whilst not conclusive, suggest that the degree to which any given amount of *R. secalis* colonisation causes symptom expression has a genetic basis in barley. Interestingly, for the best QTL effect for this trait (on chromosome 7H), the spring parent (Cocktail) contributes the resistant allele, indicating that increased relative disease expression may be a pleiotropic effect of the resistant allele at this locus. Nevertheless, a weak QTL effect in the region of 3H containing *sdw1* (the effect of which is expected to be entirely due to disease escape) and the absence of identified QTL in regions not identified in the primary disease traits suggest that these effects are statistical artefacts. The other small QTL effect identified for this trait was on chromosome 5H; this QTL does not correspond to those identified with the primary traits but in this case the winter barley parent contributes the resistant allele.

Clearly, the strength of the analysis of relative disease expression is only as good as that of the method used to derive the phenotypic data. Ideally, such phenotypes would be derived by directly measuring the symptomatic response of individual lines to varying amounts of pathogen colonisation. However, this approach requires a degree of control that is not practical to obtain in large-scale field experiments. The method used (SMA regression) here has been shown to be effective on simulated data sets but a more detailed statistical consideration of the problem of measuring relative disease expression in experimental data must be considered a priority.

The identification of apparently novel resistance loci confirms the value of winter barley germplasm as a source of resistance to *R. secalis*, and illustrates that mapping populations from crosses between winter and spring barley offer a method for identifying such resistance. The results show that the suppression of disease symptoms is a component of the expression of resistance mechanisms controlled by some genes but not others.

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