

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

Introduction 33

Rhynchosporium 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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52 to major gene factors on chromosomes 3H and 7H, 53
54 are available. However, breakdown of such sources
55 of host resistance is generally rapid, if they are used
56 in widespread commercial deployment of resistant
57 cultivars, as *R. secalis* populations are able to evolve
58 rapidly (Abang et al. 2006; Newton et al. 2001).
59 Therefore, novel sources of resistance to *R. secalis*
60 represent a valuable resource for plant breeders. In
61 particular, the identification of quantitative resistance
62 loci, which have previously been shown to be more
63 durable than major resistance loci in other host-
64 pathogen systems (Brun et al. 2010), is of consider-
65 able importance. To ensure food security, particularly
66 for subsistence farmers who cannot afford to use
67 fungicides, it is essential to breed for resistance that is
68 not rapidly rendered ineffective by changes in
69 pathogen populations.

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

87 A problem associated with the use of populations
88 segregating for major developmental genes to address
89 such questions is the extent to which field resistance
90 QTLs are simply pleiotropic expressions of broader
91 morphological differences. For example, in a cross
92 between the spring barley genotypes B83-12/21/5 and
93 Derkado, two known semi-dwarfing genes (*sdw1* and
94 *ari-eGP*) were consistently associated with QTL for
95 resistance to *R. secalis* (Thomas et al. 2010), with
96 semi-dwarf types showing more disease symptoms.
97 Given that secondary infection is mediated by splash
98 dispersal of *R. secalis* spores (Fitt et al. 1988), this
99 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 develop-
ment 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). Under-
standing 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 culti-
vars with durable resistance. It is therefore necessary
to determine whether suppression of disease symp-
toms (or more generally, the level of disease symp-
tom 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 epidemi-
ological 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 asymptom-
atic and symptomatic colonisation). Therefore a
better understanding of the genetic basis of resistance
in UK barley crops will also facilitate more appro-
priate 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 rhynchos-
porium 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.

148 **Materials and methods**

149 Plant material, mapping population and markers

150 A doubled-haploid mapping population was produced
 151 by microspore culture from the F₁ progeny of a cross
 152 between the spring barley cultivar Cocktail and the
 153 winter barley inbred line WB05-13, derived from a
 154 cross between the winter cultivars Leonie and Pearl. Cv
 155 Leonie was bred by Nordsaat in Germany and was the
 156 most resistant cultivar on the UK recommended list
 157 during its period of special recommendation from 2001
 158 to 2003. As it also had resistance to barley yellow
 159 mosaic virus strains BaMVV and BaYMV-1 and good
 160 malting quality, it had a special recommendation for the
 161 UK (<http://www.hgca.com/varieties/2003/common/200212/recommendedlists/data/WBcolour.pdf>). Cv Pearl
 162 was bred by Limagrain (formerly Nickersons Seeds)
 163 and has been recommended for growth in the UK since
 164 1999; it has been the main winter barley malting culti-
 165 var grown by farmers over this period. It was initially
 166 rated as having a good resistance to *R. secalis*, being
 167 rated '8', on a 1–9 scale of increasing host resistance as
 168 described in the recommended list protocols (www.hgca.com). Leonie originally had the best rating of '9,'
 169 but its resistance rating had declined to '5.9' by 2010
 170 (www.hgca.com). Cv Cocktail was first recommended
 171 for cultivation in the UK in 2003 and was formerly an
 172 accepted spring barley malting cultivar in the UK. It
 173 does not possess either of the two *R* genes for resistance
 174 to *R. secalis* found in current UK spring barley cultivars
 175 and had a moderate resistance rating of '5' when first
 176 recommended, which had increased slightly to '5.9' in
 177 the 2010 recommended list (www.hgca.com).

180 WB05-13 was bred to combine the resistance to
 181 *R.secalis* and BaYMV-1 of Leonie with the accepted
 182 malting quality attributes of Pearl; thus progeny from
 183 its cross with Cocktail are expected to segregate for
 184 resistance to *R. secalis* and to BaYMV-1 as well as
 185 for the *sdw1* dwarfing gene found in Cocktail. In
 186 addition, WB05-13 has the *Vrs1.t* allele at the VRS1
 187 locus on chromosome 2H and the mapping popula-
 188 tion therefore also segregates for the *deficiens* ear
 189 type. Over 800 individual plants were derived from
 190 microspore culture of the F₁ progeny; 550 lines were
 191 fertile and produced sufficient seed for a field
 192 multiplication plot that was sown at the James Hutton

Institute in autumn 2006. Immediately prior to
 195 harvest, a single plant was recovered from each of
 196 the multiplication plots. For the first 191 lines, the
 197 seed from this single plant was used as the primary
 198 seed source for agronomic trials and a reference seed
 199 stock. The remainder of the plot was harvested with a
 200 small plot combine and the seed was used as
 201 secondary seed source for agronomic trials.

202 A single seed was taken from the reference stock
 203 of each line and grown in the glasshouse. A 2–3 cm
 204 length of leaf tissue was harvested from the youngest
 205 leaf of each of these barley plants at the 3–4 leaf
 206 stage. Leaf material was harvested into 96 deep well
 207 blocks (VWR # AB-0932) containing a stainless steel
 208 ball bearing (Spex Centriprep Ltd #662316). Total
 209 plant DNA was extracted using the Tepnel Nucleo-
 210 plex Automated DNA Isolation, according to the
 211 manufacturer's instructions using the Standard Plant
 212 Lysis and Plant Purification protocols (Tepnel #: 33300). DNA concentration was estimated using
 213 Quant-iT Picogreen dsDNA Assay kit (Invitrogen
 214 #P11496). Sufficient volume of a 1:200 working
 215 dilution of picogreen reagent in 1× TE was made up
 216 and 197 µl was pipetted into white flat bottomed
 217 assay plates (Thermo Fisher #DIS-940-010T). 3 µl of
 218 DNA samples to be measured and DNA standards
 219 that were made up at 75, 50, 25, 12.5, 6.25, 3.13, 1.56
 220 and 0 ng/µl from Lambda DNA (Invitrogen
 221 #1363336) were added to the picogreen reagent.
 222 Plates were incubated for 2 min then absorbance
 223 readings were taken from a Flouroskan Ascent plate
 224 reader. A standard curve was created using the DNA
 225 standards (*R*² value between 0.950 and 0.999) and
 226 was then applied to the unknown samples to estimate
 227 concentrations. DNA concentrations were normalized
 228 to 50 ng/µl in preparation for genotyping.

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

242 Map construction

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

266 Field trials

267 Seed from the primary and secondary seed sources
 268 was used to sow the 190 lines of the mapping
 269 population in field trials over two winter barley
 270 growing seasons (2007/2008 and 2008/2009) at the
 271 James Hutton Institute rhynchosporium disease nurs-
 272 ery (Table 1). Cocktail, Leonie and Pearl together
 273 with seven other controls were included in the trial to
 274 give a trial with 200 entries. Field trials were
 275 arranged in a row and column design with two
 276 replicates and plot sizes of 2 m × 1.5 m at a seed
 277 density estimated to produce 250 established plants
 278 m⁻². The plots were combine drilled with fertiliser
 279 applied at a rate of 30.5P and 87 K kg ha⁻¹ and
 280 received an N application of 51 N kg ha⁻¹ at average
 281 growth stage (GS) 30 (Zadoks et al. 1974) and
 282 69 N kg ha⁻¹ at GS 40. Weeds were controlled by
 283 applying a herbicide but no fungicides were applied.
 284 Primary inoculum was from residual barley crop
 285 debris from the previous harvest and overhead
 286 irrigation was applied on alternate days to encourage
 287 secondary disease spread, commencing when soil

Table 1 Times of operations during field trials on develop-
 ment 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

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

312 expected, given the amounts of pathogen colonisation,
 313 and the observed areas of visual symptoms) were
 314 obtained by fitting a standardised major axis linear
 315 regression model to the relationship between the amount
 316 of *R. secalis* DNA (GS50) and visual plot disease score
 317 (GS50) using the lmod2 package in R ([http://www.
 318 R-project.org](http://www.R-project.org)). Residuals (defined as orthogonal dis-
 319 tances from the fitted line) were calculated and taken as a
 320 measure of relative disease expression. This method was
 321 used rather than taking the residuals from a least squares
 322 linear regression to account for the presence of signifi-
 323 cant measurement error in both variables.

324 Statistical analysis

325 Statistical analyses were made using GenStat software
 326 (Payne et al. 2009). Trait means for each of the DH
 327 lines for each season were estimated using a REML
 328 mixed model, fitting barley DH line as a fixed effect,
 329 and a random model comprising replicate. The anal-
 330 ysis was repeated using random models with addi-
 331 tional terms to account for spatial effects (selected
 332 from: random row, random column, correlated row,
 333 correlated column). The simplest model for which
 334 there was no significantly better, more complex, model
 335 was used to estimate line means. Phenotypic variance
 336 (V_p) and additive genetic variance (V_a) for each trait
 337 were estimated by REML, fitting the effect of envi-
 338 ronment (season), replicate (within environment) and
 339 DH line as the random model. Additive genetic
 340 variance was estimated as half of the between DH
 341 lines variance component (equivalent to $2V_a$). Herita-
 342 bility estimates were calculated as the ratio between V_a
 343 and V_p . For each pair of traits, additive genetic
 344 covariances (cov_a) were estimated by a REML anal-
 345 ysis of the sum of the two traits. cov_a was calculated as
 346 half of the additive genetic variance of the sum of the
 347 two traits minus V_a for each of the two traits.

348 QTL analysis

349 Composite interval mapping was done by using the
 350 Biometris QTL mapping procedure library (Boer
 351 et al. 2007) found in GenStat 12 (Payne et al. 2009).
 352 This methodology enables the correct variance/
 353 covariance model to be used to account for the
 354 relationships between genotype and environment in
 355 'multi-environment' trials. The two growing seasons
 356 were treated as separate environments and the

VGSELECT procedure was used to identify the 357
 most appropriate model. The marker genotypes and 358
 their map positions were used to estimate genetic 359
 predictors at 2 cM intervals using the QIBDPROB- 360
 ABILITIES procedure. These predictors were then 361
 included in a simple interval mapping genome scan 362
 using the procedure QMQLSCAN with a minimum 363
 distance of 30 cM between QTL maxima. The 364
 threshold value ($-\log_{10}P$) for identifying a QTL 365
 was 3.36, estimated to be the genome wide error rate 366
 at $P < 0.05$ by the method of Li and Ji (2005). The 367
 predictors associated with the maximum value for 368
 each QTL were then included as cofactors in a 369
 composite interval mapping scan using QMQL- 370
 SCAN and the procedure was repeated iteratively 371
 until there was no change in the selected co-factors. 372
 The final list of cofactors was used in the procedure 373
 QMBACKSELECT to iteratively eliminate any non- 374
 significant loci. Finally, the effects and type of action 375
 (QTL main effect or QTL \times environment interaction) 376
 of those remaining were estimated using the QCAN- 377
 DIDATES procedure. 378

379 Results

380 Genotyping and genetic map construction

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

392 Traits

393 The severity of the rhynchosporium epidemic (based
 394 on visual disease score) was substantially greater in
 395 2008/2009 than in 2007/2008, particularly during
 396 later growth stages (Fig. 1). Disease scores for QTL
 397 analysis (symptoms and *R. secalis* DNA) were made
 398 at approximately GS50. Estimated line means (DH
 399 lines only) for log-transformed disease symptom

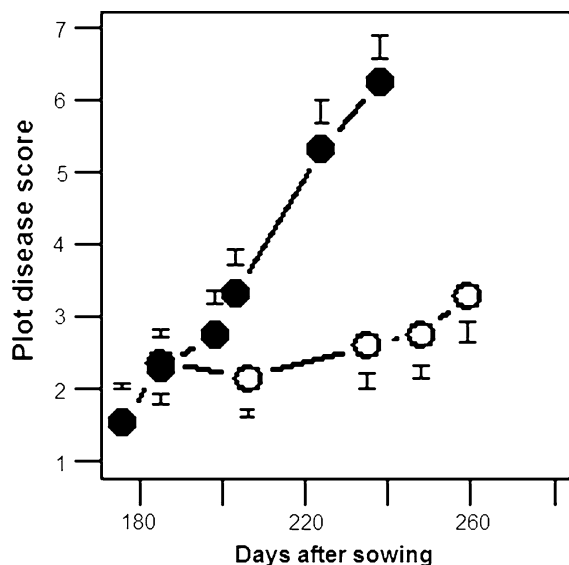


Fig. 1 Progress of rhynchosporium epidemics assessed visually as a proportion of plot area affected by leaf lesions on a 1 (symptomless) to 9 (100% leaf area covered by lesions) scale (Newton and Hackett 1994) with time (days after sowing in plots) in the James Hutton Institute disease nursery in the 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 \times 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)

400 scores had a mean of 0.81 (SD = 0.51) in 2007/2008
 401 and 1.08 (SD = 0.50) in 2008/2009. Amount of *R.*
 402 *secalis* DNA had a mean of 435 ng (SD = 2042 ng)
 403 in 2007/2008 and 12486 ng (SD = 10731 ng) in
 404 2008/2009. Relative disease expression was defined
 405 from a regression on standardised primary traits and
 406 as such (by definition) had a mean of 0 and standard
 407 deviation of 0.56 in both years. In the parental lines,
 408 estimated line means for the two winter barley
 409 parents had smaller disease scores than those of the
 410 spring barley parent in 2007/2008 (Leonie = 0.2,
 411 Pearl = 0.2, Cocktail = 1.15) but in 2008/2009 only
 412 one winter barley parent had a smaller disease score
 413 than the spring barley parent (Leonie = -0.02,
 414 Pearl = 0.4, Cocktail = 1.6) Fig. 2.

415 Correlations and heritabilities

416 There was a good genetic correlation between plot
 417 visual disease score at GS50 and amount of *R. secalis*
 418 DNA in leaves (at GS50) ($r_A = 0.91$). The

419 heritability of plot disease score at GS50 (0.59) was
 420 substantially greater than that of amount of *R. secalis*
 421 DNA (0.08). Relative expression of disease symp-
 422 toms also had a small heritability (0.03). Phenotypic
 423 correlations (2008/2009 only) between early growth
 424 stage (GS26) amount of *R. secalis* DNA and later
 425 (GS50) plot disease score were small ($r_p = 0.18$)
 426 compared to the phenotypic correlation between early
 427 and later growth stage plot disease score ($r_p = 0.67$).

428 QTL genome scans

429 The final QTL model based on visual plot disease
 430 scores identified three significant QTL effects
 431 (Table 2). These QTL effects were on chromosomes
 432 2H, 3H and 7H. The position of the QTL on 3H is
 433 identical to that of a height QTL (data not shown) at
 434 the known position of the semi-dwarfing gene *sdw1*
 435 (between markers 11_10515 and 11_20612). Given
 436 what is known about the epidemiology of rhynchosporium
 437 and that crop height has previously been
 438 reported as a mechanism of disease escape, this QTL
 439 very probably represents a pleiotropic effect of *sdw1*.
 440 Whilst the QTL effect on 7H (located between
 441 markers 11_11098 and 11_10169) is in a similar
 442 position to *Vrn-H3* (a determinant of flowering time
 443 located on the short arm of chromosome 7H), *Vrn-H3*
 444 is more distal than the 7H resistance QTL and it is
 445 inferred that it is flanked by markers 11_20162 and
 446 11_11014 (44–84 cM) on the current map. Similarly,
 447 whilst an *R* gene for resistance to *R. secalis* (*Rrs2*)
 448 has been mapped to the short arm of chromosome 7H
 449 (Hanemann et al. 2009), its mapped position is distal
 450 to the QTL effect identified here, being between
 451 markers 11_11179 and 11_20245 (0–7 cM on this
 452 Map) (unpublished data). Similarly, for the resistance
 453 QTL on 2H (located between markers 11_10791 and
 454 11_10085), a QTL affecting flowering time (*Flt-2L*)
 455 has been reported on the long arm of chromosome 2H
 456 (Chen et al. 2009). However, this locus does not
 457 appear to be segregating in this population, with no
 458 significant QTL effects for ear emergence or height
 459 detectable (data not shown). In addition, the position
 460 of *Flt-2* is likely to be proximal to that of this
 461 resistance QTL, with the rice region that is collinear
 462 to the region containing *Flt-2* (Chen et al. 2009) being
 463 located between markers 11_21459 and 11_10383 on
 464 this map. Likewise, the final QTL model for amount
 465 of *R. secalis* DNA identified three resistance QTL

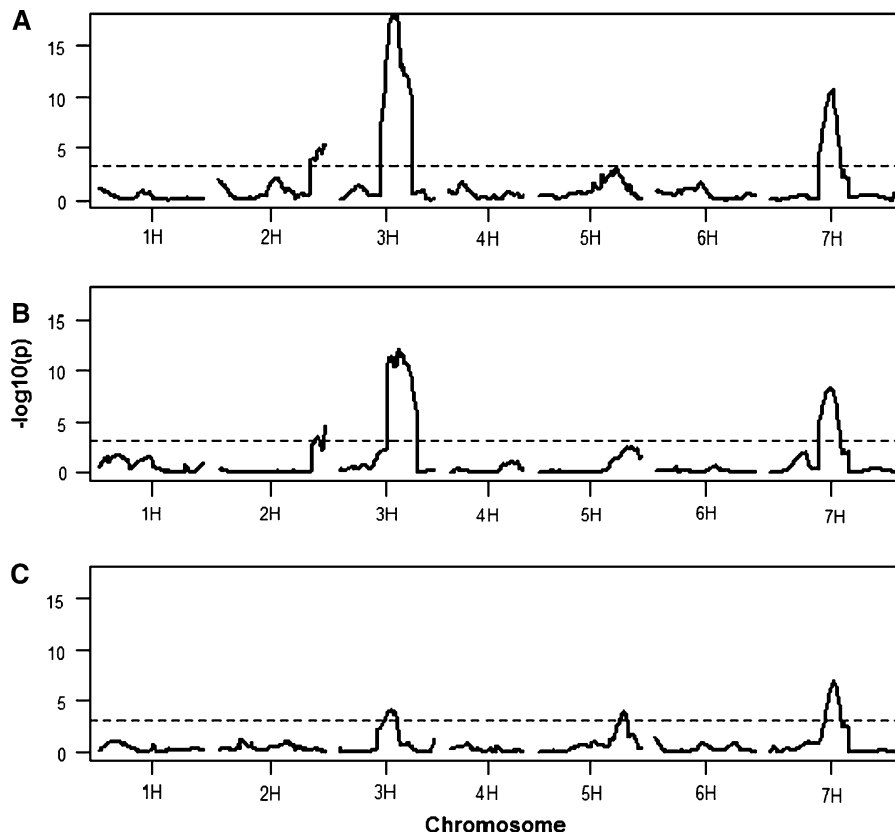


Fig. 2 Results from a multi-environment QTL genome scan for three different assessments of disease severity used to identify barley resistance to *R. secalis*. **a** Plot disease score (area of visual symptoms measured on a 1–9 scale). **b** Total amount of *R. secalis* DNA (determined by qPCR analysis 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

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

472 Resistance QTL identified using relative disease
473 expression generally had much smaller probabilities
474 associated with them than those associated with QTL
475 for primary traits. Composite interval mapping identified
476 three resistance QTL; two are located on 3H
477 and 7H close to the QTL effects identified using the
478 primary disease traits (flanked by the same markers as
479 the primary traits). The final QTL was located on
480 chromosome 5H between markers 11_21077 and
481 11_11497 (Table 2).

Disease progression 482

An analysis of changes with time in visual plot 483
disease scores across a single growing season (2008/ 484
2009) suggests that the heritability of plot scores 485
remained generally consistent across all observations 486
but that the additive genetic variance increased 487
throughout the season (Table 3). Therefore, there 488
was no evidence that the genetic basis of resistance 489
varied during the course of a growing season. 490

Discussion 491

This work has identified two new QTL for resistance 492
against *R. secalis* on barley chromosomes 2H and 7H. 493

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: <i>R. secalis</i> 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

494 These QTL effects are not associated with known
495 morphological or developmental genes. Neither do
496 positions of these QTL correspond to those of loci for
497 resistance against *R. secalis* infection that had been
498 previously identified (Zhan et al. 2008). As such, whilst
499 it is not possible to discount the possibility that these
500 loci represent morphological or physiological mecha-
501 nisms of disease escape that differ between the parental
502 lines, the lack of previously reported effects in these
503 regions suggest that they represent novel loci that will

504 be a useful resource for understanding and manipulating
505 the interaction between host and pathogen.

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

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

530 The similarity between the profiles of resistance
531 QTL identified using visual disease symptoms and
532 those identified using amount of *R. secalis* DNA
533 appears to reflect the strength of the genetic corre-
534 lation between these two traits. This result is
535 unsurprising, given the nature of the relationship
536 between them. Nevertheless, the low heritability of
537 the resistance QTL identified using amount of
538 *R. secalis* DNA suggests that the precision of the
539 qPCR method may not compare favourably to that of
540 conventional scoring of area of visual disease symp-
541 toms (this might be either due to insufficient
542 sampling, or be inherent to the assay itself). In either
543 case, it is possible that this is responsible for the
544 relative weakness of the correlation between early
545 growth stage qPCR scores and later visual symptom
546 scores. Sampling a greater number of plants from
547 within a plot, to produce a bulked sample would offer
548 the possibility of improved precision without increas-
549 ing costs associated with performing the qPCR assay.
550 Whilst improving the precision of the qPCR/sampling
551 protocol may help in making early season qPCR
552 scores a useful predictor of later disease severity,
553 other results have shown that variation in amounts of
554 rainfall may be a major determinant of subsequent
555 epidemic development (Fitt et al. 2010).

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

575 Clearly, the strength of the analysis of relative
576 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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