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ORIGINAL PAPER

Towards an understanding of the nature of resistance to *Phytophthora* root rot in red raspberry

J. Graham · C. A. Hackett · K. Smith · M. Woodhead · K. MacKenzie · I. Tierney · D. Cooke · M. Bayer · N. Jennings

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Abstract A mapping population segregating for root rot resistance was screened under both field and glasshouse conditions over a number of seasons. Few correlations between field and glasshouse scores were significant. Final root rot scores were significantly negatively correlated with measures of root vigour. Two QTL associated with resistance were identified as were overlapping QTL for root vigour assessments. Markers significantly associated with the traits were used to identify BAC clones, which were subsequently sequenced to examine gene content. A number of genes were identified including those associated with stem cell identity, cell proliferation and elongation in the root zone, control of meristematic activity and organisation, cell signalling, stress response, sugar sensing and control of gene expression as well as a range of transcription factors including those known to be associated with defence. For marker-assisted breeding, the SSR marker Rub118b 110 bp allele from Latham was found in resistant germplasm but was not found in any of the susceptible germplasm tested.

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J. Graham (✉) · K. Smith · M. Woodhead · I. Tierney · D. Cooke · N. Jennings
Genetics Department, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland, UK
e-mail: Julie.Graham@scri.ac.uk

C. A. Hackett · K. MacKenzie
Biomathematics and Statistics Scotland, Invergowrie, Dundee DD2 5DA, Scotland, UK

M. Bayer
Bioinformatics Department, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland, UK

Introduction

Root rot, caused by *Phytophthora fragariae* var. *rubi*, (Wilcox et al. 1993) (now renamed *P. rubi*) continues to be one of the most serious and destructive diseases of raspberry (Seemüller et al. 1986; Duncan et al. 1987; Harrison et al. 1998). Although named as root rot, all parts of the plant below or at ground level can be infected, including roots, root buds before emergence, crowns and the bases of canes (primocane and fruiting cane) (Harrison et al. 1998).

The almost simultaneous outbreaks of a new disease across Europe in traditional raspberry-growing areas (e.g. raspberries have been grown in Tayside, Scotland for more than a century) suggested that the disease had spread through the propagation network and had been distributed to farms in new planting material. Uptake of new highly susceptible cultivars was a major factor in disease spread (Wilcox et al. 1993). Once established local spread of the pathogen within and between fields in run-off water or contaminated soil also contributed to the problem.

The prevention of new outbreaks must become the underpinning philosophy in control strategies for root rot. Ensuring that the planting material is free of disease, followed by planting in soil where raspberries have never been grown previously, is the best strategy (Duncan et al. 2000; Duncan and Cooke 2002). In addition, planting raspberries on ridges (hilling) is now standard practice worldwide to improve drainage and aeration, and in some soils, improves plant growth and fruit yields (Heiberg 1995, 1999; Maloney et al. 1993; Wilcox et al. 1999). Such cultural improvements are necessary due to lack of

effective fungicides. An integrated control programme involving clean planting material and fungicides where available are currently the most effective ways of preventing new outbreaks and controlling the severity of existing outbreaks of raspberry root rot. However, it seems likely that this potent disease will be managed most effectively in the future by enhanced host–plant resistance.

Screening cultivars of red and other raspberries and wild *Rubus* species have identified a few potential sources of resistance including Latham and Winkler's Sämling and species material such as *R. strigosus, R. occidentalis* and *R. ursinus* have been identified (Barritt et al. 1979). The raspberry–blackberry hybrid cv. Tayberry, also demonstrates immunity to this disease (Duncan and Kennedy 1987). However, none of the commercially important raspberry cultivars in Europe seems to have useful levels of resistance (von Scherer and Riedel 1990) nor has any resistance been identified in wild raspberry germplasm tested in the UK (Graham et al. 2009b).

Although genetic resistance through plant breeding offers a feasible and effective method of control, it has proved difficult and time-consuming due to the unreliability of predicting performance in commercial situations with selected genotypes rarely standing up to infection (Jennings pers. comm.). The inheritance of root rot resistance was thought to be a quantitative character, which has hampered the study of resistance as well as raspberry breeding. Pattison et al. (2007) demonstrated that resistance to root rot was neither monogenic nor extensively quantitative, based on observations of segregation ratios. More research on the genetic basis of resistance, breeding and screening for resistance is required.

The development of molecular maps (Graham et al. 2004, 2006, 2009a; Pattison et al. 2007) and the development of molecular markers linked to resistance should lead to a greater understanding of the nature of resistance. Results from genetic mapping and sequencing of raspberry BAC clones (Hein et al. 2005) identified within relevant QTL, will improve and accelerate selection efficiencies within breeding programmes (Graham and Smith 2002; Graham and Jennings 2009).

To enable QTL associated with resistance to be identified however, robust screening systems are required, which can produce reliable phenotypic data for mapping. Breeders have tended to screen in infection plots over 2–3 seasons and designate any plants continuing to grow well as resistant. This has proved very unreliable with cultivars designated as resistant succumbing to root rot infection soon after release (Jennings pers. comm.). Glasshouse experiments can be used to determine the resistance status of seedlings against pest and diseases, but how the glasshouse results relate to plant performance under field conditions is unclear, and for root rot in particular, this has been the cause of much discussion (Cooke pers. comm.). A hydroponic screening system has also been used to assess resistance in red raspberry (Pattison et al. 2004) and the recent development of a method of screening for resistance in a semi-hydroponic system may prove more useful in this context (Muehlchen et al. 2010).

This work set out to screen a mapping population segregating for root rot resistance under both field and glasshouse conditions and examine the correlation between the assessments, to map QTL associated with resistance, then identify and sequence BAC clones spanning the QTL to examine gene content in the regions.

Materials and methods

The population, as described previously (Graham et al. 2004, 2006, 2009a), consists of a full sib family of 350 individuals generated from a cross between the European red raspberry cv. Glen Moy and the North American red raspberry cv. Latham.

Field screening

Progeny from the Latham x Glen Moy cross and both parents were cloned by the propagation of root material collected from each mother plant. This was chilled for 6 weeks after which the root was put in trays with compost and placed in a warm glasshouse. Plants growing from the root material served as a source for the establishment of replicated field trials. Once plants were established and grown they were placed outside under rain shelters to acclimatise.

Two different trial sites were selected at SCRI. The first was known to be contaminated by *P. rubi* having previously been tested (SCRI farm records) and the second was considered disease free. Disease incidence and severity were further exacerbated at the contaminated site by spreading and rotavating contaminated topsoil from another site, irrigation on a daily basis using a tape irrigation system from June until September and planting in the absence of ridging and fungicide treatment. In contrast, the clean site was ridged as standard practice for growing raspberries to control *Phytophthora* and was treated with fungicides. Management at both sites was otherwise in line with current commercial practice.

Both field trials were planted in a randomised block design with three blocks per site, in two plants plots (6 clones per plant line at each site) with 1.5 m between each plant to minimise root growth overlap between clones. Data were collected on visual symptoms of root rot damage from 320 replicated progeny in the field in season 3 (2004), 4 (2005) and 5 (2006) after planting. A scale 1–5 (1 no sign

of disease and 5 being dead) was utilised to record symptom production 5–7 times across each growing season.

In order to investigate the impact of root viability in relation to root rot symptoms, the number of root suckers (density) and the spread of root suckers (diameter) from the mother plants were recorded on a 0–5 scale (0 being no production) under field conditions at both locations as an indicator of below ground root viability. For density scores, 0 = no root suckers, 1 = 1–4 suckers, 2 = 5–8 suckers, 3 = 9–20, 4 = 21–40 and 5 = >40 suckers. For root sucker spread, distance of root suckers from the mother plant was estimated; a score of 1 being suckers up to 10 cm from the mother plant and 5 being suckers at 1 m or greater from the mother plant.

Glasshouse screening

Twelve-week-old plants growing from the mother root material served as a source for inoculation. The glasshouse screening was limited to the 188 progeny for which molecular marker genotypes were available. Due to the number of progeny tested and the replication required, the glasshouse screening was carried out in five rounds. For each round, approximately 70 progeny were cloned to provide 9 plants for inoculation and 3 plants as non-inoculated controls (12 clones per progeny). Each round was set up in a randomised block design with four blocks, one of which remained un-inoculated. Each block had three clones per progeny. The parents Latham and Glen Moy were included in every round, enabling a joint analysis of the rounds.

Fungal cultures of *Phytophthora* were stored at 4°C on oatmeal agar slopes. These were inoculated onto French Bean Agar (FBA) plates, three inoculants per plate, and then incubated for 7–10 days at 20°C where 90% growth coverage of the plate was seen. Two isolates of *P. rubi*, Codes: SCRP333, FVR11, IMI 355974, CBS 967.95; isolated in Scotland in 1985 from a single zoospore; raspberry host (Latin binomial *Rubus idaeus*); known as *race 3*; and *P. rubi*, Code: SCRP324, FVR 67; isolated in 1991; raspberry host (Latin binomial *Rubus idaeus*); known as *race 1* were used together for inoculation. Each plant was inoculated with two mycelial plugs (7 mm in diameter) of actively growing mycelium of each isolate with each plug placed at the corner of the pot 3 cm into the compost. Plants were maintained in a cool glasshouse (max. day temperature 12°C) and saturated twice daily for the duration of the experiment to encourage disease development. Eight weeks after inoculation the plants were assessed subjectively for the extent of root rot symptoms and root density with Latham and Glen Moy as the extremes for comparison.

Analysis of the field scores

The root rot, density and diameter scores from the field trial were examined to look for differences between the clean and infected sites, differences between years, differences among the progeny and interactions among these. Root rot was assessed over the 2 years showing obvious symptom production (season 4 and 5) as repeated ordinal scores, which is challenging to analyse. One approach is to ignore the ordinal nature of the data and to treat them as integer data and analyse using ANOVA. Another approach is to calculate summary statistics more appropriate to the ordinal nature of the data. Here we used both approaches.

A principal coordinate analysis was used to summarise the series of root rot scores at the infected site, and to extract the principal sources of variation across the population. A matrix of similarities between the plots was calculated using the root rot scores for the 2 years and the city-block metric. Principal coordinates were then derived from the similarity matrix. Analysis of variance of the principal coordinates was used to test for differences among the progeny. However, exploratory plots of the first principal coordinate showed that this was strongly related to a spatial trend from top to bottom of the field site. This trend was anticipated from an aerial photograph taken in 2005 to monitor infection and was also observed in the final root rot scores for each year. This trend was approximately linear for each block, and so a linear effect of bed number for each block was included in the analysis of the principal coordinate scores to remove the spatial effect. Progeny means for the final root rot scores for 2005 and 2006 and for the principal coordinate scores were predicted from the analysis of variance model for subsequent QTL analysis, after adjustment for the spatial effect.

Root density, diameter and plant height were assessed at the clean and infected sites in 2005 and 2006. Analysis of variance was used to test for significant differences among these factors, treating the ordinal scores as integer data. Correlations between these measurements and the root rot scores were calculated for the infected and clean site, for each year separately.

Analysis of the glasshouse scores

The glasshouse root rot and root density scores were analysed using residual maximum likelihood (REML) (Patterson and Thompson 1971) to combine the five rounds, with round, progeny, treatment (control/infected) and the progeny by treatment interaction fitted as fixed effects and block within round as a random effect. A separate residual variance was estimated for each round. Progeny means were estimated for use in QTL mapping.

Linkage analysis and QTL mapping

A linkage map was available for this population, as described in Graham et al. (2006), with some groups updated in McCallum et al. (2010) and Woodhead et al. (2010). QTL analysis was carried out using the MapQTL 5 software (Van Ooijen 2004). A Kruskal–Wallis (KW) test was used as a preliminary test to identify regions of the genome linked to the root rot scores, density and diameter and height data from the root rot site to explore whether alleles from one or both parents were contributing. The threshold for the KW test was established using a small permutation test of 400 permutations (carried out in Genstat 12 for Windows). Interval mapping was then carried out using MapQTL, using a permutation test based on 1,000 permutation to establish the threshold.

Identification and sequencing of BAC clones spanning root rot QTL

Based on initial QTL mapping results, BAC clones were identified for sequencing from linkage groups (LG) 3 and 6.

Linkage Group 6

New primers were designed to the Rub 118b sequence (which was identified near the centre of the one-lod support interval for the root rot QTL on LG 6), using the Primer 3 programme (Rozen and Skaletsky 2000) to amplify a probe sequence without the SSR motif to prevent non-specific hybridisation (forward primer: tctttcgactttggcaagaa, reverse primer: gagcaagctttgagcaacac). The product was amplified in Glen Moy using 25 ng DNA, 1.0 μM primer, 0.2 mM dNTPs and 0.1 units Taq polymerase (Roche Diagnostics GmbH, Germany) on a GeneAmp 9700 PCR System Thermal cycler (Applied Biosystems, Foster City, CA, USA). Amplification was performed as follows: 5 min at 95°C, 60 s at 94°C, 60 s at 57°C, 60 s at 72°C for 35 cycles, followed by 8 min at 72°C. The PCR product was cloned into pGEM T Easy (Promega, Madison, WI, USA) and plasmid DNA isolated using the Qiagen Mini-spin kit as described by the manufacturer. Plasmid DNA was sequenced using the universal M13 forward and reverse primers as described elsewhere (Woodhead et al. 2008) to verify authenticity of the insert. The insert was then used to probe the raspberry BAC library (Hein et al. 2005) using standard protocols. Seven putative positive clones were identified and BAC DNA was prepared using the Sigma PhasePrep kit according to the manufacturer's instructions. Following PCR verification (using the original RUB 118b SSR primers and also the Leaf 97 primers (also significant in the LG 6 QTL) (Graham et al. 2004) one clone of approximately 145 kb containing both sequences, BAC Ri38J3, was taken forward for further analysis. BAC end sequencing of this clone was performed using the pIndigo BAC forward (ggatgtgctgcaaggcgattaagttgg) and reverse primers (ctcgtatgttgtgtggaattgtgagc) as described in Graham et al. (2009a). Sequences were analysed on an ABI 3730 DNA Analyser (Applied Biosystems) and data analysed manually using Sequencher 4.7 (DNA Codes Corporation). Primers were designed to the BAC ends using Primer 3, used to amplify the corresponding sequences in Glen Moy and Latham and the products cloned into pGEMT Easy (Promega) and sequenced as described above. Potential sequence polymorphisms were identified in the products and primers were designed for Pyrosequencing assays using the PSQ Assay Design Software 1.0 (Biotage, Uppsala, Sweden). Pyrosequencing assays were performed for both BAC end sequences (bes_Ri38J3F (F: tttgaagctgaagggaagga, R: catttccatcatcatccatttg), bes_Ri38J3R (F: aaccacccgactccaactaa, R: gaagttctccttccccc tga) on 188 mapping individuals using the PSQ96MA Pyrosequencing instrument (Biotage) and SNPs were scored as described in Graham et al. (2009a) to verify the BAC clone mapped into the root rot QTL on LG 6 before sequencing was carried out.

Linkage Group 3

For the QTL on LG 3, an SSR marker Ri9O22_SSR01, derived from BAC Ri9O22 previously mapped to this linkage group (Graham et al. 2009a), was found to be the marker most significantly associated with the root rot/root vigour QTL on this linkage group. This BAC clone was taken forward for complete sequencing.

BAC DNA Sequencing: BAC Ri38J3

BAC DNA was prepared using the Sigma Phaseprep Kit and DNA sequencing was performed by inserting a transposon into the BAC DNA using the EZ::TN^{TM} <KAN-2> Insertion Kit (Epicentre, Madison, WI, USA) and subsequent transformation of Transformax EP1300 Electrocompetent *E. coli* (Epicentre) as described by the manufacturer. Transformants containing the transposon were identified and grown as described (Epicentre). BAC DNA was extracted using the TEPNEL automated Nucleoplex BAC isolation kit (Manchester, UK) and sequenced using the EZ:Tn5 forward and reverse sequencing primers (F 5′-gccaacgactacgcactagccaac-3′, R 5′-gagccaatatgcgagaacacccgagaa-3′) and the Big Dye Terminator v. 3.1 cycle sequencing kit (Applied Biosystems) as described (Epicentre). Cycling was performed on a GeneAmp9700 PCR System Thermal Cycler (Applied Biosystems) as described above and data were analysed manually using Sequencher

4.7 to form contiguous sequences. Further DNA sequence was obtained by designing primers to the contigs to 'walk' along the BAC. The sequence was subjected to a BLAST based vector screening using the NCBI univec database as the target (http://www.ncbi.nlm.nih.gov/VecScreen/UniVec.html) to identify vector contamination. The vector sequences were manually removed using BioEdit software (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) to produce a single contig totalling 149,804 bp.

BAC DNA Sequencing : BAC Ri9O22

BAC DNA (5 µg) was subject to 454 sequencing at the University of Liverpool (UK). An assembly was generated using approximately 29,000 reads using Newbler (v.1.1.03.24). Visual inspection of the contigs produced was performed using the assembly viewer 'Tablet' (Milne et al. 2010), which indicated that SSR sequences might be preventing the formation of larger contigs. Sequences previously derived from Sanger sequencing (both BAC ends: 1,481 and 5,833 bp long), and sequence (17,704 bp) containing a MYB-11-like gene with an SSR (Ri9O22SSR01) located within the BAC, were added to the assembly using 'phrap' (http://www.phrap.org/phredphrapconsed.html), which yielded a single sequence representing the BAC. Finally the sequence was subjected to a BLAST based vector screening using the NCBI univec database as the target (http://www.ncbi.nlm.nih.gov/VecScreen/UniVec.html) to identify vector contamination. The vector sequences were manually removed using BioEdit software (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) to produce the final, cleaned version of the BAC sequence of 156,699 bp.

Gene prediction

Open reading frames from both BAC sequences were identified using the Softberry gene structure prediction program FGENESH (http://linux1.softberry.com/berry.phtml) using *Vitis vinifera* as the reference.

Confirmation of BAC sequence locations on linkage groups

To confirm BAC locations, primers were designed to putative open reading frames and SSRs identified from BAC Ri38J3 and Ri9O22 sequences using Primer 3 (Supplementary Table 1) and the corresponding regions amplified in Glen Moy, Latham and progeny DNA as described previously (Graham et al. 2009a) to determine polymorphisms. Where size polymorphisms were observed, primers were end-labelled with FAM or HEX and PCRs performed on the mapping population and analysed on the ABI3730 DNA Analyser (Applied Biosystems) as described in Graham et al. (2004) using Genemapper v 3 (Applied Biosystems). Where SNPs were identified, primers were designed for Pyrosequencing assays which were performed as described above.

Results

Disease symptom production: field data

Data from the field assessment of disease symptoms showed that the parents Latham (the resistant parent) and Glen Moy (the susceptible parent) behaved as predicted (Table 1). At the root rot site, Latham had a mean score of 1, showing no symptoms of root rot, and Glen Moy being very susceptible had a mean final score of 4.5 in 2005 and 4.6 in 2006. The progeny median, mean and standard deviation for 2005 and 2006 are also shown. No root rot symptoms were detected at the clean site in either season. Figure 1 shows the distribution of final progeny scores at the infected site for the two seasons. The distribution of disease scores shifts as the symptoms worsen with time with little symptom production by 2004, but severe symptoms by 2006.

A principal coordinate analysis was performed based on all the 2005 and 2006 scores to summarise the root rot scores into a small number of uncorrelated factors. The first principal coordinate (PCO1) explained 43.4% of the variation in the similarity matrix, while coordinates 2–5 explained 6.6, 5.3, 4.3 and 3.3%, respectively. We excluded smaller components from the analysis. Exploratory analysis of PCO1 showed this to be related to a strong spatial trend from the top to the bottom of the site, especially in the first block of the experiment. To remove the effect of this spatial trend on the estimates of the progeny means, a linear model including a bed by block interaction was used to test for significant differences among the progeny and to estimate their means for QTL analysis. The

Table 1 Mean, median (med) and standard deviation (SD) of the final field root rot score for the parents cv. Latham and cv. Glen Moy and the progeny in season 2005 and 2006, and for the glasshouse data (GH)

	Glen Moy			Latham			Progeny mean		
	Med	Mean	SD	Med	Mean	SD	Med	Mean	SD
2005 Root Rot	4.7	4.5	0.77	1.0	1.0	0	2.5	2.7	1.07
2006 Root Rot	5.0	4.6	0.62	1.0	1.0	0	3.0	3.4	0.92
GH	4.0	3.86	1.25	2.0	1.5	1.00	2.0	1.7	1.40

Root rot was scored on a 1–5 scale (1 = no symptoms, 5 = dead)

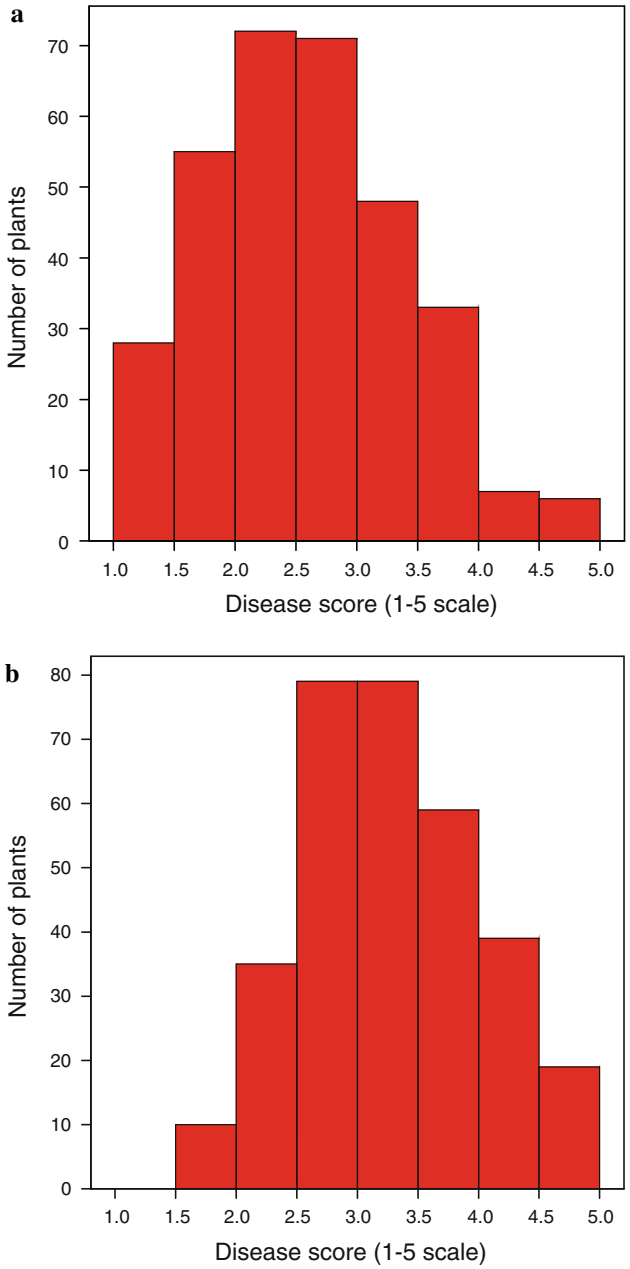

Fig. 1 **a** Distribution of final field progeny disease scores in 2005. **b** Distribution of final field progeny disease scores in 2006

same linear model was used to test for significant differences among the progeny for principal coordinates 2–5. Only PCO1 and PCO3 showed significant differences among the progeny, as determined by analysis of variance: the mean squares, significances and estimates of broad sense heritability are shown in Table 2. PCO1 was significantly positively correlated with all the root rot scores, i.e. it represents an overall measure of disease in the population. PCO3 had positive correlations with the 2006 scores but negative correlations with the 2005 scores, so that it will have high scores for the progeny that show below average root rot in 2005 but above average in 2006.

Table 2 Analysis of variance of the principal coordinates PCO1 and PCO3 of the combined root rot scores from 2005 and 2006, adjusting for a spatial effect by linear regression on Bed number

Source	*df*	PCO1 m.s.	PCO3 m.s.
Block	1	1.711	0.000
Bed	1	10.690***	0.249
Block.Bed	1	2.669***	0.060*
Progeny	319	0.127***	0.020***
Residual	317	0.066	0.014
Heritability		48.3%	31.9%

df degrees of freedom, *m.s.* mean square

* $p < 0.05$; ***$p < 0.001$

The same spatial trend was observed in the final root rot scores for each year. Analysis of variance of the final score in each year, including a linear term for the spatial effect, showed that, overall, there was a significant increase ($p < 0.001$) in root rot from 2005 to 2006. There were also significant differences among the progeny and a significant interaction between progeny and year, both with $p < 0.001$.

Table 3 shows the median, mean and standard deviation of the root density, root diameter and plant height for the progeny in 2005 and 2006, at the clean and infected sites. These traits also showed spatial trends, and this was included in the analysis of variance as above. Table 4 shows the mean squares from the analysis of variance and the broad sense heritability, calculated over the sites and years, for these traits. Overall, root density, root diameter and plant height were all significantly lower at the infected site than the clean site, and root density and diameter were lower in 2006 than in 2005 (all with significance $p < 0.001$). However, there were significant interactions between the year, site and progeny for root density and diameter ($p < 0.01$ and $p < 0.001$, respectively). The difference between the years was less significant for the plant height measurements, but there was a highly significant interaction between progeny and site ($p < 0.001$).

All correlations between root measurements and root damage at both sites were highly significant ($p < 0.001$). The final root rot score on each plot showed significant negative correlations ($p < 0.001$) with the corresponding root density, root diameter and plant height ($r = -0.33$, -0.37 and -0.20, respectively, for 2005, and $r = -0.45$, -0.40 and -0.29 in 2006).

Glasshouse data

Residual maximum likelihood was used to analyse the glasshouse data as there were found to be significant differences in the residual variation among the five rounds. For the density scores, there was a significant interaction

Table 3 Mean, median (med) and standard deviation (SD) of the field root sucker density, diameter and height (cm) for the progeny of the cv. Latham × cv. Glen Moy cross in season 2005 and 2006, on the clean and root rot infected field sites

	Clean site 2005			Root rot site 2005			Clean site 2006			Root rot site 2006		
	Med	Mean	SD	Med	Mean	SD	Med	Mean	SD	Med	Mean	SD
Density	2.0	2.2	0.73	1.0	1.4	0.55	1.0	1.5	0.75	1.0	1.0	0.85
Diameter	2.0	2.4	0.54	2.0	1.7	0.55	2.0	1.6	0.69	1.0	1.1	0.92
Height	195.0	183.9	44.13	165.0	156.7	47.01	198.0	186.1	39.31	168.0	153.9	48.87

Table 4 Analysis of variance of the root density, root diameter and plant height for 2005 and 2006 in the cv. Latham × cv. Glen Moy mapping population, adjusting for a spatial effect by linear regression on Bed number (not shown)

Source	*df*	Density m.s.	Diameter m.s.	Height m.s.
Between plots				
Site	1	318.947***	286.885***	665,541.9***
Progeny	319	1.659***	1.066***	14,007.2***
Site.Progeny	319	0.559*	0.570**	2,095.7***
Residual	944	0.465	0.450	800.9
Within plots				
Year	1	259.684***	382.553***	2,054.7**
Year.Site	1	10.807***	8.705***	818.1*
Year.Progeny	319	0.328**	0.324***	81.5^{ns}
Year.Site.Progeny	319	0.337**	0.367***	89.8^{ns}
Residual	930	0.260	0.231	192.6
Broad sense heritability		60.8%	46.4%	83.4%

df degrees of freedom, *m.s.* mean square, *ns* not significant

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

($p = 0.01$) between the progeny and the infected/control treatments. For the root rot scores, levels of infection on the control plants were very low and these were excluded from the analysis. Like the field scores, the parents behaved as expected and there were significant ($p < 0.001$) differences in root rot scores among the progeny. The broad sense heritabilities were 46.7% for root rot and 45.1% for density. The progeny means were estimated for use in QTL analysis.

Correlation of disease data from glasshouse and field experiments

Correlations were calculated between the glasshouse and field measurements, using the progeny means for the 188 lines in the mapping population. Few correlations were significant. The density score on the uninfected glasshouse pots was correlated with the density at the infected field site in 2005 ($r = 0.24$, $p = 0.001$) and the density on the infected glasshouse pots was correlated with the diameter at the infected field site in 2006 ($r = 0.18$, $p = 0.018$).

QTL mapping

Based on permutation tests, thresholds for the KW statistic with one degree of freedom of 13.0 and 13.8 were used, corresponding to a genome-wide significance of $p = 0.1$ and $p = 0.05$, respectively. For interval mapping, the corresponding thresholds were 3.7 and 4.1.

Field root rot scores

For the field root rot scores, the KW analysis of the final root rot scores of 2005 identified two regions with significance above the permutation threshold, on linkage groups (LG) 3 and 6. The most significant marker on LG 3 was Bac9022SSR01 (which encodes a MYB11-like gene), at 78 cM, a marker segregating in both parents, which has a KW statistic of 18.1 with 3 degrees of freedom. This was also the most significant marker on LG 3 for the final root rot score in 2006 and for the first principal component of the root rot scores over the years, PCO1, with KW statistics of 36.4 and 30.7, respectively. The final root rot score in 2005 also showed significant associations with markers in the region between 62 and 87 cM on LG 6 (Rub118b as mid-point), with KW statistics up to 17.0 (with one degree of freedom). These markers, as for most markers on LG 6, segregate in Latham only. The final root rot score in 2006 and PCO1 also showed significant associations with this region of LG 6, with KW statistics of up to 18.4 and 27.1 (with one degree of freedom), respectively. No markers were significant for PCO3 using the permutation threshold.

Glasshouse root rot score

The glasshouse root rot score showed no significant associations with LG 3, but was significantly associated with the same region of LG 6, with KW statistics up to 17.6 on one degree of freedom.

The genotype means associated with each genotype at Bac9022SSR01 on LG 3 and RUB118b at 80 cM on LG 6, respectively, for the final field and the glasshouse root rot scores were examined. For Bac9022SSR01, genotype ab is associated with significantly more root rot than the other genotypes for the field data, but the pattern is less clear for

Table 5 Final root rot scores in field (Final_rr_ 05 or 06) and glasshouse (GH), and field root density and diameter scores at clean (CL) and infected (Inf) sites, associated with the genotypes at Bac9022SSR01 on LG 3

Trait	aa	ab	ac	bc	R^2 (%)
Final_rr_05	2.6 (0.11)	3.1 (0.12)	2.4 (0.08)	2.6 (0.09)	11.5
Final_rr_06	3.3 (0.11)	3.9 (0.12)	3.1 (0.08)	3.5 (0.09)	16.8
GH	2.2 (0.13)	2.3 (0.15)	2.7 (0.10)	2.3 (0.11)	5.9
Density_05_Cl	2.2 (0.08)	1.9 (0.08)	2.7 (0.06)	2.0 (0.07)	31.9
Density_06_Cl	1.5 (0.09)	1.3 (0.10)	1.8 (0.07)	1.5 (0.07)	11.5
Density_05_Inf	1.5 (0.06)	1.2 (0.07)	1.6 (0.05)	1.4 (0.05)	11.6
Density_06_Inf	1.0 (0.10)	0.6 (0.11)	1.4 (0.08)	1.0 (0.09)	14.1
Diameter_05_Cl	2.5 (0.06)	2.2 (0.06)	2.5 (0.04)	2.3 (0.05)	11.3
Diameter_06_Cl	1.7 (0.08)	1.4 (0.09)	1.7 (0.06)	1.6 (0.07)	4.9
Diameter_05_Inf	1.7 (0.07)	1.6 (0.07)	1.8 (0.05)	1.6 (0.06)	3.9
Diameter_06_Inf	1.1 (0.11)	0.7 (0.12)	1.5 (0.08)	1.0 (0.09)	15.7

Standard errors are in brackets. R^2 (%) gives the percentage of the trait variance explained by regression on this marker

Table 6 Final root rot scores in field and glasshouse (GH) associated with the genotypes at RUB118b on LG 6

Trait	aa	ab	R^2 (%)
Final_rr_05	2.4 (0.06)	2.7 (0.07)	7.6
Final_rr_06	3.2 (0.07)	3.5 (0.08)	5.1
GH	2.2 (0.08)	2.6 (0.09)	9.9

Standard errors are in brackets. R^2 (%) gives the percentage of the trait variance explained by regression on this marker

the glasshouse data (Table 5). For RUB118b, genotype ab is associated with significantly more root rot in both the field and the glasshouse (Table 6).

Root density and diameter

The field measurements of density and diameter were also most significantly associated with LG 3. For the density on the clean site in 2005, the most significant marker was Bac9022SSR01 (with a KW statistic of 54.0 on 3 degrees of freedom), but markers from 26 to 133 cM were also significant. No further regions were located for this trait by the KW analysis. Long regions of LG 3 were also significant for root density on the clean site in 2006 (maximum KW of 29.3 on 1 degree of freedom for RUB233a at 68 cM), for root density at the infected site in 2005 (maximum KW of 25.9 on 1 degree of freedom for Rub177a1 at 59 cM) and for root density at the infected site in 2006 (maximum KW of 29.1 on 1 degree of freedom for Rub177a1). No markers were significantly associated with the density measurements from the glasshouse. Long regions of LG 3 were also associated with most of the root diameter measurements, although not the root diameter measurements from the infected site in 2005. Table 5 gives the mean scores for each trait associated with the genotypes of Bac9022SSR01. Genotype ab, which was associated with the highest root rot scores in the field, had the lowest scores for density and diameter.

Plant height at the root rot infected site

The height measurements from the clean site were analysed by Graham et al. (2009a): here focus was on the heights at the infected sites. In 2005, the most significant association with height was for marker RiMADS_01, at 11 cM on LG 5, with a KW statistic of 17.8 on 1 degree of freedom. This marker was from Latham, and was in a similar position to a QTL reported by Graham et al. (2009a) for plant height at the clean site. This marker was also the most significantly associated with plant height at the infected site in 2006 (with a KW statistic of 18.7).

Figure 2 shows the linkage map and the one-lod confidence intervals for the above QTLs, using interval mapping. This was produced using MapChart 2.1 (Voorrips 2002).

One method to search for further QTLs affecting these traits is to use multiple QTL mapping, but for this population there were convergence problems, with the MapQTL software taking large numbers of iterations and overfitting in some regions of the map. A stepwise regression approach, testing each marker in turn together with the most significant markers above, was therefore used to search for additional associations that were significant with $p < 0.001$.

For the root rot measurements from the field trials, no further effects were found in addition to those on LG 3 and 6. For the glasshouse root rot score, the KW analysis and interval mapping identified only a region on LG 6, but the stepwise regression found a further region, on LG 3, with the most significant marker E41M31-153 at 56 cM ($p < 0.001$). Bac9022SSR01 was also significant by the stepwise regression ($p < 0.001$). However, the allele effects for these two markers for the glasshouse score showed a quite different pattern from the field score, with allele 'b' at E41M31-153 associated with a mean decrease of 0.43 (se 0.112) in the glasshouse and mean increases of 0.23 (se 0.091) and 0.35 (se 0.099) in the final field scores in 2005 and 2006, respectively.

For the field root density and diameter measurements, stepwise regression, starting from marker Bac9022SSR01 on LG 3 at 78 cM, identified several further associations as significant with $p < 0.001$, on LGs 3, 5, 6 and 2. The following markers were selected as being representative of a significant region of the chromosome and with as many

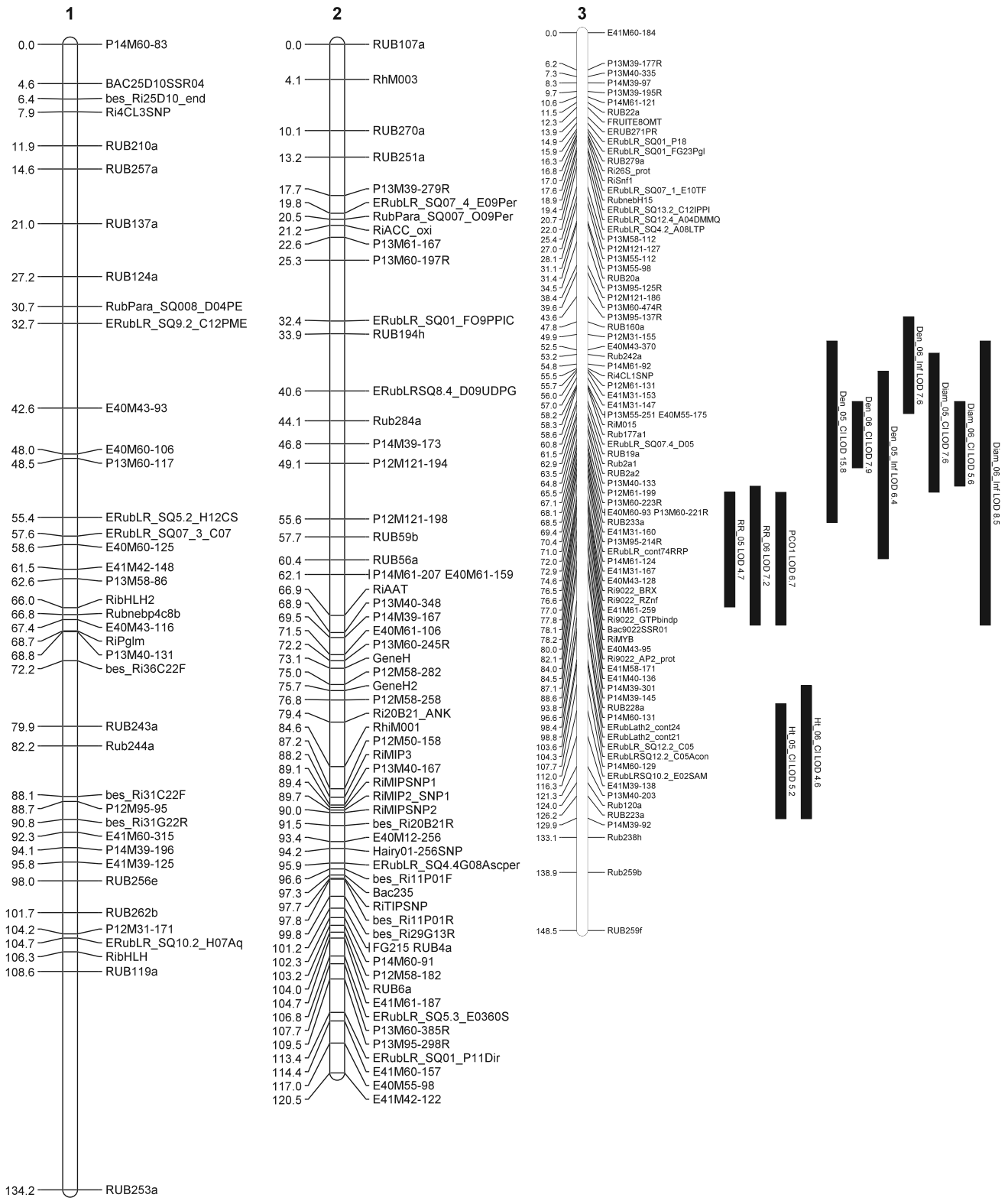

Fig. 2 Linkage groups 1–7 showing one-lod confidence intervals (to the right of the LG) for QTL locations for root rot (RR), root density (Den), root diameter (Diam) PCO1 derived from root rot scores and height (Ht) for each season at the clean (Cl) or infected (Inf) sites

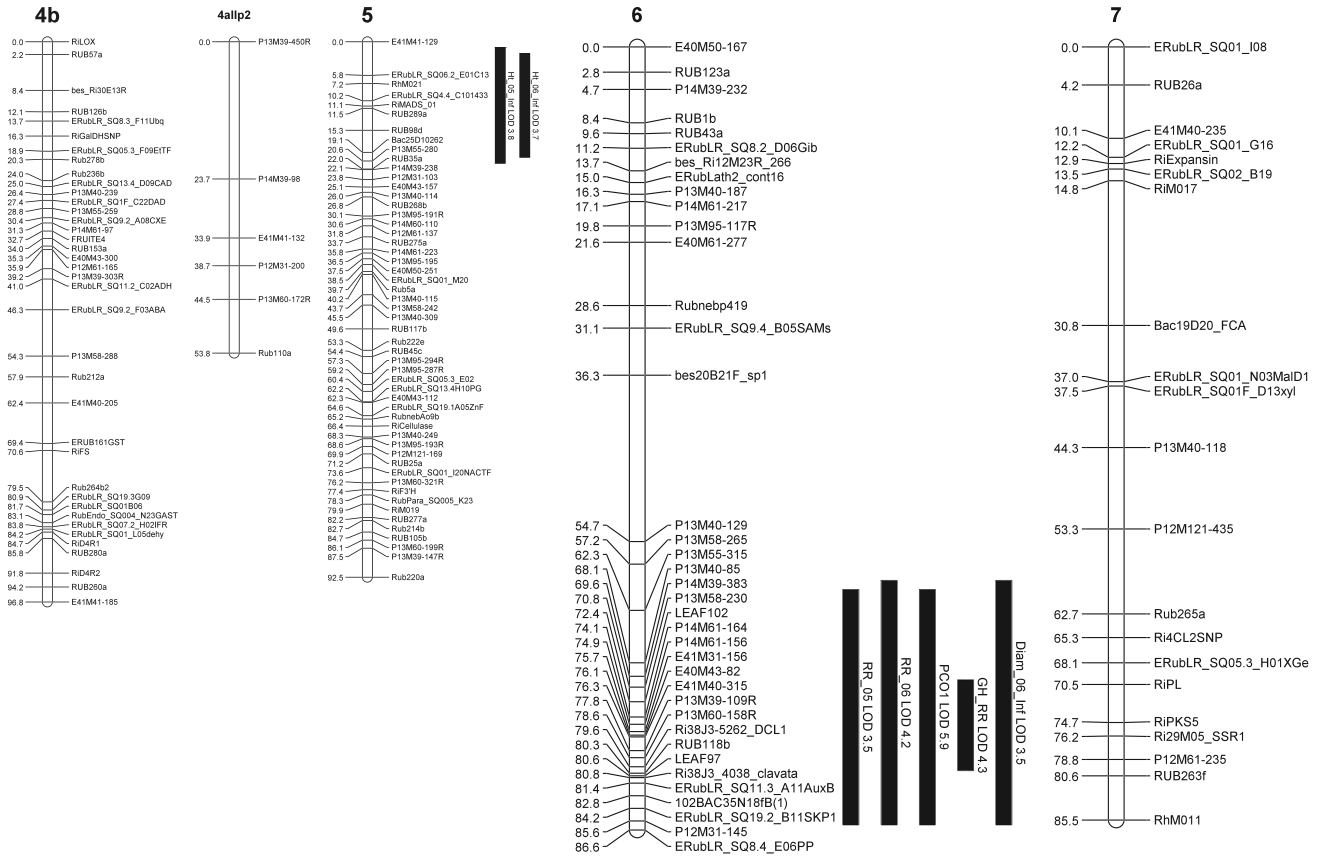

Fig. 2 continued

values present as possible: Ri26S_prot on LG 3 at 17 cM; E41M31-153 on LG 3 at 56 cM; RUB289a on LG 5 at 11 cM, ERubLR_SQ11_3_A11 on LG 6 at 81 cM and Ri20B21_Ank on LG 2 at 79 cM. RUB289a is an ab × cd marker segregating in both parents, Ri20B21_Ank segregates in cv. Glen Moy only and the rest segregate in cv. Latham only. Tables 7 and 8 shows the marker effects and the % variance of the root density and diameter scores explained by each of these markers when fitted with Bac9022SSR01. The results for the density and diameter scores have similar signs for the effects. A second association was frequently detected on LG 3, but the positioning of this was quite variable, between 18 and 58 cM. There was no further improvement of fitting both markers together. Other studies on this population, especially Graham et al. (2009a) on ripening rates, have reported that this linkage group appears to contain multiple QTL but these are hard to localise. RUB289a on LG 5 was significant ($p < 0.05$) for most of the density and diameter measurements, while ERubLR_SQ11_3_A11 on LG 6 was only significantly associated with the 2006 density and diameter measurements from the infected site. Ri20B21_Ank is most significant for the diameter measurements at the clean site, although the effects at the infected site and on the density are consistent in sign. On LG 6 at the infected site there was evidence of an effect on density and diameter in 2006.

The KW analysis of the height data identified an association with LG 5. Graham et al. (2009a) also reported an epistatic interaction for plant height at the clean site between two markers, RUB223a, a genomic SSR marker from cv. Latham on LG 3, and ERubLR_SQ07_3_C07 on LG 1, which comes from cv. Glen Moy. Regression analysis was used to test whether this was also significant for the heights at the infected sites. The same pattern was observed, with lines without either of these two marker allele combinations being significantly shorter than those with either marker. The mean heights for both sites and years are shown in Table 9.

Gene content on BAC clones

BAC Ri9O22 had previously been mapped onto LG 3 using an SSR associated with a MYB11-like (R2R3 MYB) transcription factor (Ri9O22_SSR01) implicated in ripening (Graham et al. 2009a). This was the most significant marker associated with the QTL for disease symptoms and root measures, so BAC sequencing was carried out using

Table 7 Effects on the root density measurements of markers selected by stepwise regression

Additional marker	Trait	Effect (SE)	R^2 (%)
Ri26S_prot, LG 3, 17 cM	Density_05_Cl	0.25 (0.080)**	34.3
	Density_06_Cl	0.35 (0.083)***	18.5
	Density_05_Inf	0.02 (0.063)^{ns}	9.6
	Density_06_Inf	0.29 (0.104)**	18.1
E41M31-153, LG 3, 56 cM	Density_05_Cl	−0.28 (0.112)*	31.0
	Density_06_Cl	−0.18 (0.118)^{ns}	10.1
	Density_05_Inf	−0.27 (0.084)**	16.9
	Density_06_Inf	−0.47 (0.141)**	20.7
RUB289a, LG 5	Density_05_Cl	−0.43 (0.107)***	40.1
	Density_06_Cl	−0.31 (0.118)***	17.1
	Density_05_Inf	−0.25 (0.085)**	16.8
	Density_06_Inf	−0.26 (0.15)^{ns}	15.1
ERubLR_SQ11_3_A11048, LG 6	Density_05_Cl	0.08 (0.080)^{ns}	29.2
	Density_06_Cl	0.09 (0.086)^{ns}	8.8
	Density_05_Inf	0.12 (0.061)‡	13.3
	Density_06_Inf	0.34 (0.102)***	20.4
Ri20B21_Ank LG 2	Density_05_Cl	−0.20 (0.080)*	32.6
	Density_06_Cl	−0.21 (0.086)*	11.5
	Density_05_Inf	−0.09 (0.062)^{ns}	11.0
	Density_06_Inf	−0.26 (0.103)*	17.5

All models contain the marker Bac9022SSR01. For marker RUB289a, the effect is that of genotype bd relative to the highest scoring genotype, ac. All results based on 137 lines for which all markers are present

Table 8 Effects on the diameter measurements of markers selected by stepwise regression

Additional marker	Trait	Effect (SE)	R^2 (%)
Ri26S_prot, LG 3, 17 cM	Diameter_05_Cl	0.11 (0.060)‡	12.3
	Diameter_06_Cl	0.15 (0.082)‡	6.8
	Diameter_05_Inf	0.01 (0.068)^{ns}	4.4
	Diameter_06_Inf	0.28 (0.107)*	20.0
E41M31-153, LG 3, 56 cM	Diameter_05_Cl	−0.20 (0.080)*	13.8
	Diameter_06_Cl	−0.24 (0.108)*	6.9
	Diameter_05_Inf	−0.08 (0.096)^{ns}	4.4
	Diameter_06_Inf	−0.45 (0.148)**	21.5
RUB289a, LG 5	Diameter_05_Cl	−0.32 (0.081)**	18.8
	Diameter_06_Cl	−0.24 (0.115)^{ns}	6.7
	Diameter_05_Inf	−0.39 (0.089)***	17.0
	Diameter_06_Inf	−0.29 (1.52)‡	17.7
ERubLR_SQ11_3_A11048, LG 6	Diameter_05_Cl	0.09 (0.057)^{ns}	11.8
	Diameter_06_Cl	0.04 (0.079)^{ns}	2.6
	Diameter_05_Inf	0.11 (0.067)^{ns}	6.0
	Diameter_06_Inf	0.40 (0.106)***	22.8
Ri20B21_Ank LG 2	Diameter_05_Cl	−0.22 (0.057)***	18.7
	Diameter_06_Cl	−0.23 (0.079)**	10.0
	Diameter_05_Inf	−0.11 (0.066)‡	6.3
	Diameter_06_Inf	−0.24 (0.106)*	19.1

ns not significant

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

454 sequencing (Mitreva and Mardia 2009). A single contig of 156,699 bp was obtained which contained 15 predicted genes (Table 10), when compared to *V. vinifera*. Four genes from this BAC were mapped back into the QTL on LG 3 to confirm location.

A single contig of 149,804 bp of the estimated 145 kb Ri38J3 BAC clone was sequenced and 18 genes predicted by FGENESH (Table 11) when compared to *V. vinifera*. Four genes from this BAC were mapped back into the QTL on LG 6 to confirm location (Fig. 2).

Table 9 Mean plant heights for each site and year associated with the genotypes at the two interacting loci RUB223a on LG 3 and ERubLR_SQ07_3_C07 on LG 1

Trait	ERubLR_SQ07_3_C07 = aa		ERubLR_SQ07_3_C07 = ab		R^2 (%)
	RUB223a = aa	RUB223a = ab	RUB223a = aa	RUB223a = ab	
Ht_05_Cl	128.4 (4.93)	201.6 (3.97)	198.0 (3.60)	201.1 (3.63)	54.0
Ht_06_Cl	134.0 (4.99)	203.2 (4.02)	200.7 (3.64)	202.9 (3.68)	50.9
Ht_05_Inf	119.4 (6.32)	162.4 (4.98)	175.7 (4.52)	164.3 (4.57)	25.8
Ht_06_Inf	122.8 (6.90)	160.6 (5.44)	176.6 (4.93)	163.4 (4.99)	20.3

Standard errors are in brackets

Table 10 The location (bp) and putative functions of the putative mRNAs encoded by BAC Ri9O22 as predicted by the Softberry FGENESH program

Predicted Gene (bp) LG3	Gene name	Putative function	Similarity *E* value
226–5,001	Reverse transcriptase	Transcription of single stranded RNA to double stranded DNA	AAA21736.1 5e−111
7,787–15,334	S locus F-box protein	Role in self incompatibility; two genes in tandem	BAD08321.1 (3e−52; 3e−40)
16,807–24,253	Brevis radix-like/Ran GTPase binding protein	Role in root growth	XP0025213848.1 1e−164
25,737–36,301	Unknown	Hypothetical protein	CAN69468.1 0.0
44,686–45,861	Unknown	Hypothetical protein	CAN69469.1 1e−77
54,160–54,660	RING zinc finger	Implicated in plant defence signalling pathways	EEF41012 2e−18
59,495–71,333	Developmentally regulated GTP-binding protein	Act as molecular switches regulating diverse cellular processes	EEF41010.1 4e−175
71,989–73,189	40S ribosomal protein	Involved in translation of RNA to protein (partial gene)	ACU19393.1 8e−08
75,327–83,373	Calmodulin binding protein	Involved in signal transduction in response to changes to intracellular Ca^{2+} levels	EEF4105.1 1e−130
83,789–84,562	Bromodomain protein	Function as targeting molecules for various transcription factors	EEF37549.1 3e−09
90,154–109,604	Transcription factor	Unknown process	XP002264494.1 0.0
117,169–118,592	MYB11-like protein	R2R3 MYB-like transcription factor	AAZ20431 3e−74
128,152–129,135	MYB-like protein	R2R3 MYB-like transcription factor	EEF09136.1 9e−60
133,759–136,834	Unknown	No hits	NA
143,344–146,081	AP2-domain-containing protein	Transcription factors involved in regulating developmental processes and response to biotic and abiotic stress	CAO45633.1 7e−134

Discussion

Root rot is clearly a devastating disease affecting all aspects of plant growth from plant height, cane number and root sucker characteristics. The field results show that screening of this nature is an effective way of differentiating progeny based on their disease resistance. Glen Moy and Latham as controls behaved absolutely as expected in

Table 11 The location (bp) and putative functions of the putative mRNAs encoded by BAC Ri38J3 as predicted by the Softberry FGENESH program

Predicted gene (bp) LG6	Gene name	Putative function	Similarity *E* value
134–5,200	C3HC4-type ring finger	Signal transduction	BAC22255.1 8e−24
6,276–24,402	Putative ethylene inducible CTR1-like protein kinase	Functions in sensing, mediating and co-ordinating cellular responses to a range of stimuli	BAD37611.1 0.0
23,582–24,100	Stress induced protein	Abiotic stress	ACN94268.1 1e−18
24,712–26,638	Knope4 /knox class 1	Signalling pathway for meristematic control	AB026062.1 1e−138
28,901–30,197	Peptidyl-prolyl cis-trans isomerase	Protein folding expressed in various tissues including root, shoot and meristem	XP002523550.1 9e−49
31,744–33,334	Unknown	Hypothetical protein	XP002523546.1 6e−10
43,983–49,794	Retrotransposon protein	Retroelement reverse transcriptase	ABA97040.1 1e−171
46,705–50,761	Unknown	Predicted protein	XP002325162.1 2e−162
65,487–73,953	Unknown	Predicted protein	XP002264906.1 0.0
74,935–78,787	Sugar/hexose transporter	Role in metabolism, growth, development and signalling	XP002523539.1 0.0
82,937–94,668	Lipid binding protein	Cell signalling	XP002523536.1 0.0
99,852–101,014	Clavata1 precurser receptor kinase	Promote differentiation of stem cells at the meristem	XP002523555.1 0.0
101,775–115,279	Dicer like protein	miRNA processing	XP_002308384.1 0.0
115,793–120,705	MCM5 protein	DNA replication	NP_178812.1 3e−105
123,471–125,984	Oxidoreductase	Oxidoreductase activity	XP002523530.1 1e−133
126,168–129,698	Transduction WD-40	Receptor for cullin based ligases	NP178186.1 4e−102
126,168–134,898	Plasma membrane ATPase	Transport eg. root epidermis	ABA18104.1 0.0
139,040–142,993	DCN1/Cullin neddylation protein	Scaffold for cullin neddylation to control root growth	XP_002527318.1 2e−58

the field. What is important to note, and what may in-fact explain why field screening in the past has had limited success in identifying resistant varieties, is to take into account the progression of the disease. The change in mean disease scores from year 4 to year 5 of trial has highlighted the fact that screening times may have been much too short in the past with 3 years usually a maximum (Jennings pers. comm.; Hoashi-Erhardt et al. 2008). An arbitrary score of 3 (as the mid-point) has generally been chosen for the cut off identifying disease resistance/tolerance. As root rot resistance is a continuous trait this cut off is somewhat artificial. Not only that, but it throws away valuable information for analysis. If employed here more progeny would have been assigned to the resistant category than the susceptible for year 4. Glasshouse trials could differentiate the progeny on the basis of resistance/tolerance against root rot. Like the field trials, the glasshouse data were normally distributed but at the lower end of the disease severity scale. This suggests that glasshouse screening will lead to a significantly higher proportion of escapes than field

screening but the recent development of a method of screening for resistance in a semi-hydroponic may prove useful in this context (Muehlchen et al. 2010). Little correlation was identified between field and glasshouse screening however, suggesting it is of limited use in breeding. Interestingly, although glasshouse and field trials disease scores were not significantly correlated, both data sets identified similar regions on LG 6 and LG 3. This suggests the underlying genetics of resistance/tolerance to the disease is the same and consequently suggests that only minor effects vary between field and glasshouse experiments.

The demonstration of a highly significant correlation between the root sucker parameters (as assessed by the density and diameter of root sucker production at both clean and infected sites) and root rot resistance is potentially of great value to breeders as it provides an easy visual screen for germplasm with some level of resistance/tolerance to root rot. The fact that root parameters and disease resistance map to the same QTL on LG 3 and to a marker ERubLR_SQ11_3_A11, an EST with similarity to an auxin receptor or germin-like protein possibly involved in initiation of new axes of growth in a defense response (Godfrey et al. 2007), close to the resistance QTL on LG 6, raised the questions of whether cv. Latham-based resistance is actual resistance genes, or the result of morphological trait(s) or a combination of both these factors, which would have consequences for breeding.

The set of markers identified at the clean site for root vigour traits is very similar, in location and which parent is involved, to those identified for ripening (Graham et al. 2009a), not just on LG 3 but also on LG 5 (an SSR marker Rub289a), and LG 2 (Gene H) (Graham et al. 2006). A comparison of the signs of effects show agreement throughout: the alleles associated with longer time to ripening are associated with smaller root density and diameter measures and vice versa and these may be regarded as general vigour genes.

The effect on LG 6 at ERubLR_SQ11.3_A11 on root density or diameter has only been identified at the infected site. Therefore this may be better interpreted as a resistance locus, where in the susceptible plants the root density and diameter is reduced by the disease, rather than assuming all the effects are purely vigour genes.

Sequencing the two BAC clones and what is already known from previously identified genes in neighbouring regions has provided an insight into the host of different genes within or near the QTL underlying the root vigour and resistance traits. Genes identified in these regions appear to be those shown in other species to be involved in root growth and development, signalling, metabolite transport and mRNA processing, as well as transcription factors implicated in defence.

Genes identified on the 9022 BAC on LG 3 are primarily transcription factors (Table 10) including a Brevis Radix-like gene, shown to control the extent of cell proliferation and elongation in the growth zone of the root tip in *A. thaliana* (Mouchel et al. 2004). Genes containing RING zinc finger motifs are implicated in plant growth as well as defence (Cheung et al. 2007; Perricone 2009) and calmodulin binding proteins are essential for root development (Muto and Hirosawa 1987; Yang et al. 1998) and are involved in the stress response by mediating calcium signalling (Vanderbeld and Sneddon 2007). Bromodomain containing proteins have roles in transcriptional regulation and *Arabidopsis* plants containing a mutation in bromodomain protein GTE4 exhibit plant development defects, including root development, through effects on root meristem cell proliferation (Airoldi et al. 2010). R2R3 MYB proteins are involved in many processes, including root growth in *A. thaliana* (Mu et al. 2009). At the other end of the BAC clone on LG 3 is a sequence with homology to AP2-type transcription factors which are known to trigger de novo formation of roots from shoot structures and are pivotal in the determination of stem cell identity (Aida et al. 2004). It is interesting to speculate that the contribution of LG 3 to root rot resistance is based on general plant/root growth and vigour.

QTL on LG 6, however, may be argued to be based on some form of resistance as this does not overlap with any root vigour QTL and the additional marker identified for root traits on LG 6 was only identified at the infected site. From the gene content on LG 6 (Table 11), it is possible at this time only to speculate that resistance may be based on an ability to trigger new root differentiation and growth in response to pathogen attack.

Plant development is driven by stem cells localised in apical meristems (root and shoot), which allow adaption to environmental conditions. Plants can optimise their root architecture by initiating lateral root primordia and influencing growth of primary or lateral roots (Malamy 2005). In *Arabidopsis*, primary root growth and patterning is regulated by *CULLIN3* genes with DCN1 functioning as a scaffold-type E3 ligase for cullin neddylation (Kurz et al. 2008). Genes encoding DCN1 and a WD40 with similarity to an *A. thaliana* DWD protein (Lee et al. 2008) with a role as substrate receptor for Cullin ubiquitin ligases have been identified on LG 6. The root apical meristem (RAM) constitutes the stem cells that produce all below ground organs and the different stages of development are controlled by various phytohormones with auxin playing a major role (Leyser 2006). Cell-to-cell communication maintains meristematic activities and organisation to coordinate new organ formation. An essential regulatory mechanism for meristem organisation is a regulatory loop between WUSCHEL (WUS) and CLAVATA (Miwa et al.

2009), which co-ordinates the development of distinct organs. The CLAVATA (CLV) gene products in *A. thaliana*, including the CLV1 receptor-kinase, regulate the maintenance of a population of centrally located stem cells and the differentiation of peripherally located daughter cells (DeYoung et al. 2006). A Clavata 1 precursor gene has been identified on LG 6. In raspberry new shoot apical meristems arise from below ground on the roots, and in other plants these are maintained by two signalling pathways WUSCHEL and KNOX. Organs containing tissues with meristematic activity show KNOPE expression, and a Knope 4 gene has also been identified on the BAC on LG 6. In *A. thaliana* the role of KNOX genes KNAT3, KNAT4 and KNAT5 in root development have been investigated. These genes showed root domain and cell type specific expression patterns, and their expression was regulated by hormones that influence root growth (Truernit and Haseloff 2007). Root KNOX genes have been shown to be triggered by rhizobia and nematodes and may belong to an early response process (Koltai et al. 2001) possibly signalling root growth to overcome pathogen attack (Testone et al. 2008).

A number of genes with a role in cell signalling have also been identified on both BAC clones. A putative ethylene inducible kinase and putative lipid binding protein both with roles in signalling have been identified on LG 6, as has a sugar transporter. Sugars play a role in many hormone signalling pathways such as abscisic acid and ethylene (Cheng et al. 2002; Gibson 2005; Zhou et al. 1998; Yanagisawa et al. 2003) as well as in plant growth and development, with the hydrolysis of sucrose by cell-wall invertases and the subsequent import of hexoses into target cells crucial for appropriate metabolism, growth and differentiation in plants (Ohto et al. 2005; Price et al. 2003; Roldan et al. 1999). It is predicted that *Arabidopsis* has at least 14 STP genes, which together with complex patterns of expression for each, and the possibility that each protein may have more than one physiological function, provides the plant with the potential for a multiplicity of patterns of monosaccharide utilisation to direct growth and differentiation or to respond flexibly to changing environmental conditions (Sherson et al. 2003). An H+-ATPase has been identified on LG 6. These play a central role in transport across the plasma membrane. As a primary transporter, it mediates ATP-dependent H(+) extrusion to the extracellular space, creating pH and potential differences across the plasma membrane that activate a large set of secondary transporters (Arango et al. 2003). These ATPases are actively expressed in tissues where intense transport occurs including root epidermal cells (Serrano 1989; Sussman and Harper 1989). This primary pump appears to be controlled by many factors such as hormones and environmental stresses including mechanical stress and might act as an intermediate in signal transduction pathways (Sussman and Harper 1989; Michelet and Boutry 1995; Palmgren 1998; Oufattole et al. 2000). Two zinc finger domains have also been identified within these QTLs. These are found in proteins involved in various signal transduction and regulatory pathways (Saurin et al. 1996). Work has recently been carried out to associate ring finger proteins with phenotypic consequences. Transgenic tobacco plants expressing the *Car-ZFP1* gene show enhanced growth with larger primary root and more lateral roots than control plants (Zeba et al. 2009). An FAD-dependent oxidoreductase has been identified on LG 6. Reactive oxygen species are also known to be key signalling molecules associated with growth and development as well as stress responses, and redox activation of transcription factors is widely used in animal immune systems (Dalton et al. 1999). A number of transcription factors families which may have implications in regulating defence have been identified on LG3. These include two MYB-like genes and AP2 type transcription factors (Ethylene Response Factor), which bind to promoter elements of defence-related genes (Eulgem 2005). DICER-LIKE 1 (DCL1) has been identified on LG 6. Small RNAs (miRNA and siRNA), in plants are encoded in intergenic regions and require this particular type of III RNase named DCL1 (Chapman and Carrington 2007). Non-coding RNAs such as these, play a fundamental role in developmental regulation and epigenetic modification and several miRNAs are involved in root development (Guo et al. 2005). Auxin-related transcription factors are targets of miRNA which limit the expression of these genes (Wang et al. 2005).

Currently, it is only possible to speculate on the mechanism(s) of resistance based on what is known about the genes within the QTL from work in other species, and the next step will be to utilise a microarray containing all the genes identified across both BACs as well as genes in neighbouring regions and examine gene expression in resistant and susceptible germplasm with and without infection with *P. rubi*. Long term the generation of a larger population will be used to confirm associations.

With regard to the development of markers for marker-assisted breeding, limited germplasm, specifically root rot resistant material was available for examination of the markers identified on the two linkage groups. Of the material available, germplasm was examined using Rub 118b from LG 6, potentially the resistance QTL. The 110 bp allele from Latham was never found in any of the susceptible germplasm tested only the resistant material. Further development and testing of markers for use in marker-assisted breeding is ongoing.

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