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Quantitative Trait Loci Controlling *Phytophthora cactorum* Resistance in the Cultivated Octoploid Strawberry (*Fragaria* x *ananassa*)

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Running Title: *Phytophthora cactorum* QTL in strawberry

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ABSTRACT [187 WORDS]

The cultivated strawberry, *Fragaria x ananassa* (*Fragaria* spp.) is the most economically important global soft fruit. *Phytophthora cactorum*, a water-borne oomycete causes economic losses in strawberry production globally. A bi-parental cross of octoploid cultivated strawberry segregating for resistance to *P. cactorum*, the causative agent of crown rot disease, was screened using artificial inoculation. Multiple resistance quantitative trait loci (QTL) were identified and mapped. Three major effect QTL (*FaRPc6C*, *FaRPc6D* and *FaRPc7D*) explained 36% of the variation observed and in total, the detected QTL explained 86% of the variation observed. There were no epistatic interactions detected between the three major QTLs. Testing a subset of the mapping population progeny against a range of *P. cactorum* isolates revealed no major differences in host response, however, some lines showed higher susceptibility than predicted, indicating that additional undetected factors may affect the expression of some quantitative resistance loci. Using historic crown rot disease score data from strawberry accessions, a preliminary genome-wide association study of 114 individuals revealed additional loci associated with resistance to *P. cactorum*. Mining of *Fragaria vesca* Hawaii 4 v1.1 genome revealed candidate resistance genes in the QTL regions.

KEYWORDS (3-8 keywords)

Fragaria, *Phytophthora cactorum,* quantitative resistance, quantitative trait locus

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INTRODUCTION

The cultivated strawberry, *Fragaria x ananassa* (*Fragaria* spp.) is the most economically important global soft fruit and is an integral part of the diet of millions of people [1]. In a recent modelling study, a reduction in fruit and vegetable production or a reduction in affordability due to climate change has been predicted to be a key driver of food insecurity. Twice as many climate-related deaths were associated with reductions in fruit and vegetable consumption than with climate-related increases in the prevalence of underweight individuals [2].

Traditionally, the major strategy for disease control in strawberry production relied heavily upon pre-plant fumigation and chemicals. The withdrawal of methyl bromide along with other active chemicals, including fungicides and soil fumigants are increasing the challenges in field strawberry production, resulting in a rise of occurrences and severities of some once well controlled diseases [3]. A switch to producing strawberries in soilless substrate is now common practice across the world. The soilless substrate system offers many advantages, including the benefit of separating the strawberries from the infected soil [4]. This has resulted in a reduction in the prevalence of some soil-borne diseases, but not for water-borne pathogens such as the hemibiotrophic oomycete, *Phytophthora cactorum*. *P. cactorum* (Lebert and Cohn) Schröeter is a destructive pathogen, that can infect a wide variety of plant species, causing serious damage in both ornamental and agricultural crops [5]. It is the causative agent of strawberry crown rot [6] and strawberry leather rot [7], affecting the fruit. Both diseases are reported to cause economic losses in strawberry production globally; in Norway in 1996/97 there were reports of plants losses of up to 40% caused by crown rot [8] and in 1981 reports of commercial

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71 farms in Ohio described crop losses from leather rot of 20-30% [9]. Amplified fragment
72 length polymorphism (AFLP) analysis of *P. cactorum* isolates of crown rot and
73 leather rot showed they are distinctly different from each other and from *P. cactorum*
74 isolated from other hosts [10]. No correlation has been found between resistance to
75 crown rot and resistance to leather rot [11].
76
77 Strawberry plants infected with *Phytophthora* crown rot develop initial symptoms in
78 spring and summer, frequently during hot periods. Plants can often appear stunted;
79 the youngest leaves are usually the first to wilt, followed by the older leaves,
80 eventually resulting in the collapse and death of the plant [12]. Red-brown lesions and
81 longitudinal splits can be observed within the crown [13]. Sexually produced oospores
82 are the primary source of inoculum; these are the resting spores that can persist in
83 the soil or infected plants for many years [12]. Under the conducive conditions of
84 saturated soil, oospores germinate to produce sporangia which release the motile
85 asexual life stage, zoospores. Zoospores are chemotactically attracted to nearby
86 roots [14], where they attach to the root surface, encyst and penetrate the root
87 epidermis.
88
89 The public breeding programme at East Malling (NIAB EMR, Kent, UK), since its
90 establishment in 1983, has successfully released 43 strawberry cultivars to the
91 Northern European market. Efforts have focused on combining excellent fruit quality
92 with high yield, low percentage waste and resistance to filamentous diseases.
93 Breeding for disease resistance is a high priority for many breeding programmes
94 across the world. There has been extensive research investigating qualitative (major
95 gene) resistance to *Phytophthora* species (for a selection of *R* gene - *Avr* gene

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interactions see Table 2 in Vleeshouwers *et al.* [15]), however, much less is known about quantitative resistance (multiple genes, each of partial effect) to *Phytophthora* species. Quantitative trait loci (QTL) mapping is a routine technique for pinpointing genes controlling complex polygenic traits to specific regions of the genome, through statistical analysis. Previous studies have identified resistance to *P. cactorum* in the octoploid strawberry and it appears to be under polygenic control [16-18], with a major locus, *FaRPc2*, recently reported on linkage group 7D [19]. Variation in resistance has been also observed in the wild progenitors of *F*. x *ananassa*; *Fragaria chiloensis* and *Fragaria virginiana* populations [20].

The cultivated strawberry (2*n* = 8*x* = 56) is an allo-polyploid outbreeder with a genome comprised of four comparable homeologous sets of diploid chromosomes [21,22]. The octoploid genome is estimated to be 698 Mb; 80% of the size of quadrupling the diploid genomes (~200 Mb each) [23]. The development of the 90K SNP (single nucleotide polymorphism) Affymetrix® IStraw90 Axiom® Array [24] has aided genetic studies and marker-assisted breeding. The genomes of 19 octoploid and six diploid strawberry accessions were sequenced to serve as resources for SNP discovery and interpretation. The octoploids used were ‘Holiday’, ‘Korona’, ‘Emily’, ‘Fenella’, ‘Sweet Charlie’, ‘Winter Dawn’, ‘CA65.65.601’ and ‘NH-SB480’, six F_1 progeny of ‘Holiday’ x ‘Korona’, one F_2 progeny of ‘Dover’ x ‘Camarosa’, one *F. virginiana* and three *F. chiloensis* [24]. The diploids used were the known progenitor *Fragaria vesca* and two probable progenitors *Fragaria iinumae* and *Fragaria mandshurica* [24]. A high percentage of SNP markers were designed sub-genome-specific to detangle the complexity of the allo-octoploid genome and allow accurate scoring. However, its widespread use was limited by cost. A smaller, cheaper

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121 version of the array, Axiom® IStraw35 384HT, has been developed by combining
122 mapped SNP probes from multiple groups from across the world and contains just
123 over 34 000 markers [25,26].
124
125 The genus *Phytophthora* comprises of numerous destructive crop pathogens [27]. The
126 most extensively studied are *Phytophthora infestans* (late blight of potato and
127 tomato) and *Phytophthora sojae* (root and stem rot of soybean). The majority of *R*
128 genes identified in potato against *P. infestans* belong to the coiled-coil, nucleotide-
129 binding, leucine-rich repeat (CC-NLR) class of intracellular immune receptors [28].
130 The corresponding avirulence (*Avr*) genes identified belong to the RxLR class of
131 effectors [28]. These secreted, modular effectors have an RxLR motif for translocation
132 into the host cell with a quickly evolving effector domain at the C-terminus. Fewer
133 extracellular resistance genes have been characterised, however, the few that have,
134 have been associated with resistance to multiple *Phytophthora* pathogens. Cell
135 surface L-type-lectin-RLKs (receptor-like kinases) have been associated with
136 resistance to *Phytophthora brassicae*, *Phytophthora capsici* and *P. infestans* [29-31].
137 An extracellular receptor-like protein (RLP) ELR (elicitin response) was identified in a
138 wild species of potato. ELR was found to recognise elicitin proteins from a diverse
139 set of *Phytophthora* species, including *P. infestans*, *P. sojae* and *Phytophthora*
140 *cryptogea* [32,33].
141
142 In this study, the genetic basis of quantitative resistance to *P. cactorum* was
143 investigated in a bi-parental cross of the cultivated octoploid strawberry (*F.* x
144 *ananassa*). The mapping of resistance in controlled glasshouse experiments and the
145 identification of QTL associated with resistance is reported. Furthermore, using

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146 historic crown rot disease score data, a genome-wide association study was
147 conducted to investigate the presence of QTL within the wider germplasm.
148 Subsequent to the identification of resistance QTL, the diploid strawberry reference
149 genome (*F. vesca* Hawaii 4 v1.1) was mined for candidate resistance genes.
150
151 **MATERIALS AND METHODS**
152 **Strawberry plant material and *Phytophthora cactorum* isolates**
153 The mapping population used in this study was a cross between the cultivated June
154 bearing strawberry cultivars 'Emily' x 'Fenella'. 'Emily' is an early season variety with
155 resistance to powdery mildew (*Podosphaera aphanis*), bred by NIAB EMR (formally
156 HRI-East Malling) and released in 1995. It is moderately susceptible to *P. cactorum*.
157 'Fenella' is a mid-late season variety with good resistance to Verticillium wilt
158 (*Verticillium dahliae*) and crown rot (*P. cactorum*), bred by NIAB EMR (formally East
159 Malling Research) and released in 2009. The F_1 full sib family of 181 individuals
160 were clonally propagated by pinning down runners; the 181 progeny were planted in
161 a field at the East Malling site in May 2014 and grown under netting. The strawberry
162 runners were pinned down in beds and grown on for five months, from July 2014 –
163 January 2015. The clones were then dug up, the excess soil was shaken off and the
164 bare-rooted plants were transferred into a 2 °C cold-store for 1 week, before being
165 transferred to a -2 °C cold-store for at least two months. Plants were brought out of
166 cold-storage and potted into 9 cm diameter pots (Soparco) and dead leaves were
167 removed. Plants were grown in a glasshouse compartment maintained at 20 ºC
168 during the day and 15 ºC at night on a 16/8 hour, day/night light cycle, for three
169 weeks before inoculation with *P. cactorum* isolates.
170

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171 The main *P. cactorum* isolate used in this study for the bi-parental QTL mapping was
172 P414. Isolates P404, P415 and P416 were used to screen the 15 representative
173 (five 'resistant', five 'intermediate' and five 'susceptible') genotypes from the bi-
174 parental cross. The isolates used in the genome-wide association study were P371,
175 P372, P404, P407, P412, P413 and P416. All isolates are known to be pathogenic
176 to *F.* x *ananassa,* having been isolated from infected strawberry plants. Isolates of
177 *P. cactorum* were maintained on V8-juice (Arnotts Biscuits Limited) agar (200 ml V8-
178 juice, 8-9 ml 1M KoH (Sigma-Aldrich), 20 g Agar (Fisher BioReagents) and 800 mL
179 distilled water, autoclaved) at 20 ºC in the dark.
180
181 **_Phytophthora cactorum_ zoospore production**
182 Ten mm discs were cut from the margins of actively growing cultures of *P. cactorum*
183 on V8-juice agar and placed into empty 9 cm triple-vented petri dishes (five per plate;
184 Thermo Scientific). The plates were then carefully flooded with diluted compost
185 extract (50 g compost in 2 L dH_2O; and dH_2O 1:1) and sealed with Parafilm (Bemis
186 Company). Plates were placed in an incubator set at 20 ºC with lights on
187 continuously for 48 hours to stimulate sporangia development. After 48 hours, the
188 diluted compost extract was poured off and replaced with fresh diluted compost
189 extract. The plates were placed into a fridge (~4 ºC) for 45 min and then moved onto
190 the bench at room temperature for 45 min. The inoculum suspension was then
191 vacuum filtered and kept on ice. The concentration of zoospores was calculated
192 using a haemocytometer and the concentration was adjusted to 1 x 10^4 zoospores
193 per mL [34].
194
195 **Strawberry inoculation assays**

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196 The pathogenicity screens were carried out under controlled conditions in a
197 glasshouse. Compartments were maintained at 20 ºC during the day and 15 ºC at
198 night on a 16/8 hour, day/night light cycle for four weeks after inoculation with *P.*
199 *cactorum*. The 'Emily x 'Fenella' progeny screens were performed in six
200 experiments. The first two experiments comprised of one replicate mock inoculated
201 and one replicate artificially inoculated with *P. cactorum* isolate P414. The other four
202 experiments were comprised of two replicates artificially inoculated with *P. cactorum*
203 isolate P414. The 15 representative (five 'resistant', five 'intermediate' and five
204 'susceptible') genotypes screened with three other *P. cactorum* isolates (P404, P415
205 and P416) were carried out in a separate experiment and inoculated separately.
206 Plants for all screens were arranged in a randomised block design for each set of
207 replicates. In total, ten replicates of each strawberry genotype were artificially
208 inoculated with a suspension of *P. cactorum* zoospores. Fifteen mm wounds were
209 made using a scalpel on one petiole per plant and strawberry plants were sprayed
210 with ~5 mL of 1 x 10^4 zoospore suspension. For each strawberry genotype, two
211 plants were mock inoculated by wounding in the same way and inoculated with ~5
212 mL diluted compost extract. To maintain humidity, plants were completely covered
213 with clear plastic sheeting for 48 hours. Plants were scored following a slightly
214 modified version of Bell *et al.*'s disease scale [35]. Foliage was assessed visually for
215 the presence of wilting symptoms once a week. The scores 8, 7, 6, 5 were assigned
216 if the plant died during the first, second, third or fourth week after inoculation,
217 respectively. After four weeks, the plants were cut open longitudinally and the
218 crowns were assessed on a scale of 1-5; 1 – healthy (0% infection), 2 – up to 25%
219 infection, 3 – 26-50% infection, 4 – 51-75% infection, 5 – 76-100% infection.
220

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221 **Analysis of disease scores**

222 The data for the ten replicates of each genotype was averaged and a mean crown
223 rot disease score was used for further analysis. Statistical analyses were performed
224 using R (v3.2.2, "Fire Safety" [36]). The mean crown rot disease data for the 'Emily' x
225 'Fenella' progeny was tested for normality using the Shapiro-Wilk normality test.
226 Broad sense heritability (H^2) was calculated, $H^2 = V_G/V_P$, where V_G is the total
227 genetic variance and V_P is the total phenotypic variance.

228

229 **DNA extraction and genotyping**

230 Young emerging leaf samples were collected in 2 mL microcentrifuge tubes along
231 with two ball bearings and flash frozen in liquid nitrogen. Frozen leaf samples were
232 ground to a fine powder for 2 mins at 60 o/m using a TissueLyser (Qiagen).
233 Genomic DNA (gDNA) was extracted using the DNeasy kit (Qiagen) following the
234 manufacturer's protocol and eluted in 60 µL Buffer AE. gDNA quantity and purity
235 were determined using the NanoDrop (ND-1000, Thermo Scientific)
236 spectrophotometer. gDNA of 'Emily' and 'Fenella', the 181 progeny and 59
237 strawberry accessions, were sent to Oxford Genomics Centre for genotyping on the
238 Affymetrix® IStraw90 Axiom® Array [24]. Later, a further 55 strawberry accessions
239 were genotyped on the Affymetrix® IStraw35 Axiom® Array [26].

240

241 **Linkage analysis of the bi-parental cross of 'Emily' x 'Fenella'**

242 Initial genotype calls were made using Affymetrix Power Tools (version 1.16.1) and
243 the R package SNPolisher (version 1.5.0). Further filtering used custom Python
244 scripts, part of the Crosslink package (https://github.com/eastmallingresearch/
245 crosslink) to remove markers with strong segregation distortion [37]. A bi-parental

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246 genetic map of 'Emily' x 'Fenella' was produced using the 181 progeny using the
247 Crosslink software [37]. The same pipeline was also used to generate bi-parental maps
248 from IStraw90 data from four additional crosses: 'Redgauntlet' x 'Hapil', 'Flamenco' x
249 'Chandler', 'Capitola' x 'CF1116' (INRA) and 'Camarosa' x 'Dover' (CRAG). Custom
250 Python and R scripts were used to create a consensus genetic map from all five bi-
251 parental maps. Further custom scripts adjusted the fine scale marker ordering of the
252 consensus map to match the *F. vesca* genome v2.0 [22] whilst identifying and
253 correcting probable *F. vesca* genome assembly errors. The resulting hybrid
254 consensus map was used to inform the ordering of the 'Emily' x 'Fenella' map.
255
256 The four sub-genomes of *F*. x *ananassa* were assigned the letters A, B, C and D in
257 the 'Emily' x 'Fenella' linkage map. The letter denotes the similarity of the sub-
258 genome to *F*. *vesca*, as described by van Dijk *et al.* [38]. The most similar sub-
259 genome was named A, the second most similar was named B (similar to the wild
260 diploid *F. iinumae*), the third most similar was named C and the least similar was
261 named D.
262
263 **QTL mapping**
264 Histograms of mean crown rot disease scores were visualised and were tested for
265 normality (QQ-plot). The raw mean data (W=0.96877, p<0.0004) was used for QTL
266 analysis. QTL mapping was performed using Kruskal-Wallis (K-W) non-parametric
267 ANOVA on the combined map of 'Emily' and 'Fenella'. Identification of QTL specific
268 to one parent and QTL that are present in both parental genotypes were estimated
269 with the K-W ANOVA method, eliminating the need to perform separate QTL
270 analysis on the two parental linkage maps. K-W analysis identifies markers linked to

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271 single traits/QTL individually and produces a *K* statistic. QTL associated with
272 resistance to *P. cactorum* were identified if $p<0.01$, and the most significant marker
273 was selected. A permutation test consisting of 10 000 iterations of randomly
274 selected data was performed to verify that significance selection was not needed.
275
276 A stepwise linear regression model was performed to estimate the effect of each
277 QTL. Non-significant QTL were removed from the model ($p>0.05$). The combination
278 of the QTL effect sizes were used to estimate predicted means for each individual.
279 The predicted means were plotted against observed average scores and their
280 coefficient of determination (r^2) was calculated. One-way analysis of variance
281 (ANOVA) was performed to test for epistatic interactions between the three major
282 effect QTL.
283
284 The Bonferroni correction was applied to the K-W *p* values to control the false-
285 positive (type I error) rate. It was calculated using: critical *p* value (α)/number of
286 comparisons being made. Only QTL with *p* values more significant than the
287 Bonferroni correction were investigated further.
288
289 **Genome-wide association study**
290 A preliminary genome-wide association study was performed using historic crown rot
291 score data of strawberry accessions, collected between 1995-2017, using a mixture
292 of two *P. cactorum* isolates each year: pre-2006 - isolates P371 and P372, 2006-
293 2009 - isolates P412 and P413, 2010-2011 - isolates P412 and P407 and 2012-
294 2017- isolates P404 and P416.
295

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296 SNPs showing at least 5% minor allele frequency were assessed for association with
297 resistance to crown rot in the 114 strawberry accessions using both PLINK [39] and
298 TASSEL [40] (p<0.00005; https://github.com/harrisonlab/popgen/blob/master/snp/
299 gwas_quantitative_pipeline.md). Population structure was taken into account and
300 the Benjamini-Hochberg Procedure was used to reduce the false discovery rate and
301 p values that lower than p=0.05 were considered to be potential QTL.
302
303 **Mining of candidate resistance genes in *Fragaria vesca***
304 The most significant SNP markers for each QTL were plotted on the *F. vesca* Hawaii
305 4 v1.1 genome [41] in Geneious (v10.1.2), along with GFF (generic feature format) files
306 with the positions of NLR, RLK and RLP gene models. The number of genes in each
307 of these classes within 1 Mbp either side of the most significant marker for each QTL
308 were determined.
309
310 **RESULTS**
311 **Variation observed in resistance to *Phytophthora cactorum* in the bi-parental**
312 **cross**
313 Crown rot disease severity was found to vary in a genotype-dependent manner. In
314 the most susceptible individuals, total plant collapse occurred one or two weeks after
315 inoculation and 100% necrosis of the crown was observed (crown rot disease scores
316 7/8, respectively). The mock-inoculated plants remained disease-free. The
317 distribution of crown rot disease severity for the 181 individuals of the 'Emily' and
318 'Fenella' mapping population had a unimodal distribution pattern, with a slight skew
319 towards resistance (Figure. S1). The means of the raw data were normally

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320 distributed and were used for QTL mapping of loci involved in resistance to *P.*
321 *cactorum* (Figure. S1). The broad sense heritability was calculated to be $H^2 = 0.58$.
322
323 **Linkage mapping**
324 A whole-genome linkage map comprising of 11 598 SNP markers was assembled
325 using the IStraw90 markers of the 'Emily' x 'Fenella' progeny and the program
326 Crosslink [37]. The map was resolved into 28 linkage groups, representing the four
327 sub-genomes of each, of the seven chromosomes of *F.* x *ananassa*. Using only the
328 IStraw35 markers to produce the map, 8 348 SNP markers were assembled into 28
329 linkage groups.
330
331 **QTL analysis**
332 QTL mapping revealed 15 QTL significantly associated with resistance to *P.*
333 *cactorum* isolate P414, located on chromosomes 1, 2, 3, 5, 6 and 7 (p<0.01; Figure
334 1a and Table 1). The most significant marker associated with each QTL is shown in
335 Table 1. Comparing the K-W analysis between the markers from the IStraw90 array
336 and the subset on the IStraw35 array, the same 15 QTL are still significant (p<0.01;
337 Figure 1b and Table 1) before stepwise linear regression was performed. Only one
338 focal SNP changed; IStraw35 SNP NE5FF79A1403E4 (2 868 559 bp) became the
339 most significant marker on LG1B as the IStraw90 marker N5192084E36892 (2 433
340 670 bp) was removed, a distance of 434 889 bases is between the two markers on
341 the *F. vesca* Hawaii 4 v1.1 genome [41] (Table 1).
342
343 Stepwise linear regression was performed and three of these QTL were found to be
344 non-significant, leaving 12 QTL associated with resistance to *P. cactorum* (Table

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S1). Seven QTLs were present in 'Fenella' only; named *FaRPc1B* (*Fragaria* x *ananassa* Resistance to *P. cactorum* linkage group 1B), *FaRPc2C*, *FaRPc3B*, *FaRPc3C-A*, *FaRPc5B*, *FaRPc6C* and *FaRPc7A*, located on linkage groups 1B, 2C, 3B, 3C, 5B, 6C and 7A, respectively (Table 1). Three QTLs were present in 'Emily' only; named *FaRPc3C-B*, *FaRPc6D* and *FaRPc7D*, located on linkage groups 3C, 6D and 7D, respectively (Table 1). Two QTL were present in both 'Emily' and 'Fenella', named *FaRPc6A* and *FaRPc6B*, located on linkage groups 6A and 6B, respectively (Table1). Of these significant resistance QTL, two were identified on the A sub-genome (the most similar to *F. vesca*), four resistance QTLs were identified on the B sub-genome (the most similar *F. iinumae*), four resistance QTLs were identified on the C sub-genome and two resistance QTLs were identified on the D sub-genome.

All but one of the QTL behave in a dominant nature. *FaRPc6B* is over-dominant in nature (Table 1). If the individual is homozygous for either parent (AA or BB), it has a predicted crown rot disease score of 2.8/2.7. However, if the individual is heterozygous (AB/BA) at that locus then it has a predicted crown rot disease score of 3.4/3.2. The individuals that are heterozygous at this locus are more susceptible than either homozygous individuals.

The linear regression also allowed the calculation of a contributing crown rot disease score to each of the 12 significant resistance QTL (Table S1). The estimate of effect sizes were combined to produce each individual's predicted score (Table S2). Individuals with no resistance QTL were predicted to have a crown rot score of 5.237 (Table S1). The detected QTL explained 85.7% of the genetic variation observed;

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the combination of all remaining 12 QTL explain a reduction in crown rot score of 4.527, leaving a score of 0.71 unexplained by genotype within this analysis. The percentage effect of the 12 individual QTLs ranged from 4.2 – 13.3% (Table S1). The three largest effect QTL, *FaRPc6C*, *FaRPc6D* and *FaRPc7D*, accounted for 10.1%, 13.3% and 12.4%, respectively and explained a total of 35.8% of the variation observed. The predicted means were plotted against observed average scores and were found to be positively correlated, $r^2 = 0.63$ (Figure 2).

The Bonferroni correction was calculated to be 0.01/15 = 0.00066 and plotted as -$\log_{10}$(0.00066) on the QTL analysis results (Figure 1a and 1b, grey dashed lines). Using the IStraw90 markers, five QTL cross this threshold; *FaRPc1B*, *FaRPc3C-B*, *FaRPc6C*, *FaRPc6D* and *FaRPc7D*, whereas only four cross the threshold using only the IStraw35 markers; *FaRPc3C-B*, *FaRPc6C*, *FaRPc6D* and *FaRPc7D* (Figure 1a and 1b). Focusing on the three major effect QTL, one-way ANOVA revealed there are no epistatic interactions between these QTL, *FaRPc6C*, *FaRPc6D* and *FaRPc7D*. Each marker has an effect on its own, is additive in nature and there are no pairwise interactions (Table S3).

Analysis of the representatives of the 'Emily' and 'Fenella' population and their response to three further *Phytophthora cactorum* isolates

A subset of 15 individuals from the progeny; five resistant individuals (EF011, EF021, EF101, EF147 and EF184) with average disease scores ranging from 1 to 2, five intermediate response individuals (EF041, EF060, EF141, EF164 and EF187), with average disease scores ranging from 2.7 to 4.3 and five susceptible individuals (EF035, EF040, EF084, EF120 and EF166) with average disease scores ranging

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395 from 5.2 to 5.8, were tested for their response to three further isolates of *P.*
396 *cactorum*, P404, P415 and P416 (Table 2). No major differences in host response
397 were observed, individuals possessing few QTL were more susceptible than
398 individuals possessing multiple QTL. However, some lines showed higher
399 susceptibility than predicted (Table 2). Genotypes that possessed the three major
400 effect QTL (*FaRPc6C*, *FaRPc6D* and *FaRPc7D*) or combinations of them were more
401 resistant than those genotypes that did not possess any (Table 2).
402
403 **Presence of known classes of resistance genes in and around QTL regions**
404 **and genome-wide association study**
405 Focusing on the three major effect QTL (*FaRPc6C*, *FaRPc6D* and *FaRPc7D*)
406 identified from the bi-parental cross, at least one NLR gene was identified within 1
407 Mbp either side of the most significant SNP, located on *F. vesca* Hawaii 4 v1.1
408 genome [41] (Table 3). At least one RLK was also identified in the same regions, as
409 well as six RLP genes in the *FaRPc6D* region (Table 3).
410
411 A genome-wide association study identified 10 SNPs significantly associated with
412 resistance to *P. cactorum* on linkage groups 5D, 6C, 7A, 7B and 7D (Table 4). None
413 of these markers overlapped with the QTL discovered in the bi-parental cross,
414 although all linkage groups except 7B had QTL on them. The two SNPs identified on
415 LG7D (ND6F3E0ED7894D and N3BEC0F68E0AF2) are located within the QTL
416 region of *FaRPc2* identified by Mangandi *et al.* [19], based the on the positions on the
417 *F. vesca* Hawaii 4 v1.1 genome [41]. There is ~0.1 Mbp between the three SNPs
418 identified on LG5D, with two RLKs located in this region. There is an RLP close to
419 the SNP identified on LG6C (NB5EE8EA0593C0) and this SNP is ~7 Mbp upstream

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420 from *FaRPc6C* identified in the bi-parental cross. There were three SNPs identified
421 on LG7A, two were close together and the was a RLK gene between them
422 (N8BC9A1515F06E and N1F8AD596FD52B). There is also an RLK gene close to
423 the other marker (N7AB0326E81D69).
424
425 **DISCUSSION**
426 Resistance to *P. cactorum* in octoploid strawberry is known to be under complex
427 genetic control, with multiple QTL involved in resistance [16,18]. In this study, single
428 QTL provide small effects, but combined, in the bi-parental study explain 86% of the
429 variation observed. A total of 15 putative QTLs for resistance to crown rot were
430 identified in this study, of which five were found to still be significant after Bonferroni
431 correction (*FaRPc1B*, *FaRPc3C-B*, *FaRPc6C*, *FaRPc6D* and *FaRPc7D*). In a
432 previous study, five putative QTLs were identified in the *F. x ananassa* 'Capitola' x
433 'CF1116' progeny, with the QTL effects ranging from 6.5 to 10.2% and coming from
434 both parents [16]. Our results are comparable, with the QTL identified from both
435 parents and with effect sizes ranging from 4.2 to 13.3%. The three major effect QTL,
436 *FaRPc6C*, *FaRPc6D* and *FaRPc7D*, had an effect size of 10.1%, 13.3% and 12.4%,
437 respectively and were highly significant with *p* values of 9.77e-08, 1.57-e07 and
438 1.91-e06, respectively.
439
440 Comparing the QTL analysis of the bi-parental cross using markers from the
441 IStraw90 and IStraw35 arrays, there were no differences between the QTL identified,
442 just a difference between the focal SNP for LG1B. The informative markers from this
443 cross were used in the development of the IStraw35 array, along with informative
444 markers from many other bi-parental crosses [26]. The cheaper IStraw35 array

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445 provides sufficient markers to identify resistance QTL within the bi-parental cross as
446 well as in the genome-wide association study, as most of the markers were present
447 on the IStraw35 array.
448
449 Resistance (*R*) genes effective against *Phytophthora* typically contain NLR domains,
450 which can directly or indirectly perceive pathogen effectors [42]. NLR proteins are
451 abundant in most plant genomes and are involved in the detection of a wide range of
452 pathogens, oomycetes, fungi, viruses, bacteria, nematodes and insects. The diploid
453 *F. vesca* has been well studied and at least 144 NLR genes have been identified [43].
454 Along with this class of resistance gene, RLK and RLP genes have been associated
455 with resistance to *Phytophthora* pathogens [29-33]. Within 1 Mbp either side of the
456 most significant marker of the three major effect QTL (*FaRpc6C*, *FaRPc6D* and
457 *FaRpc7D*) from the bi-parental cross there is at least one NLR candidate gene in the
458 *F. vesca* genome. There is also at least one RLK candidate gene within 1 Mbp
459 *FaRpc1B*, *FaRpc3C-B*, *FaRpc6D*, and *FaRpc7D* and multiple RLP candidate genes
460 within and around *FaRpc1B*, *FaRpc3C-B*, *FaRpc6D*, and *FaRpc7D*. Disease
461 resistance genes are often found in clusters in the genome [44]. Further work is
462 required to identify the genes underlying the resistance QTL.
463
464 No race structure has been reported for *P. cactorum*. The consistency between the
465 response of the 15 progeny to the four isolates of *P. cactorum* is promising in that
466 the resistance identified in the 'Emily' x 'Fenella' population would be useful against
467 different isolates of *P. cactorum*. If the three major effect QTL (*FaRpc6C*, *FaRpc6D*
468 and *FaRpc7D*; combined score reduction of 2.016) could be introgressed into elite
469 strawberry germplasm, then a good base level of resistance to *P. cactorum* could be

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provided, with the other QTL, such as *FaRpc1B* supplementing them. Interestingly 'Emily' possesses two of the major effect QTL (*FaRPc6D* and *FaRPc7D*), but is more susceptible than 'Fenella'. Possibly there are additional undetected factors affecting the overall resistance/susceptibility status of the plant. Similarly, some genotypes showed higher susceptibility than predicted (EF120 and EF167; Table 2) indicating that additional undetected factors may affect the expression of some QTL.

In *F. vesca* a single major gene locus was identified on the proximal end of linkage group 6, named *RPc-1* (*Resistance to Phytophthora cactorum 1*). *RPc-1* explained 74.4 % of the variation observed and was identified as spanning a region of 801 genes and 69 potential plant disease resistance genes, including multiple different classes of resistance gene, in a 3.3 Mb region of linkage group 6 from position 5 151 532 to 9 201 791 bp [45]. *RPc-1* would be on *F. x ananassa* LG6A, based on the convention of van Dijk *et al.* [38]. In the bi-parental cross a minor QTL was identified on LG6A, imputing this on *F. vesca*, it is very far away, ~29 Mbp upstream of *RPc-1*. Toljamo *et al.* observed the expression of potential resistance genes in the *RPc-1* region, in *P. cactorum* inoculated *F. vesca* Hawaii 4 roots [46]. Within the *RPc-1* locus, four NLR protein-encoding genes (101306457, 101297569, 101300750 and 101304699) were identified as being expressed, two of which were significantly down-regulated within the inoculated plants (101300750 and 101304699) [46]. The authors propose 101297569 as a strong candidate as it had the highest expression (mean expression level, 32.41) of the NLR genes in that region. Other types of resistance genes were identified within the *RPc-1* locus, two L-type-lectin-RLKs (receptor-like kinase) were significantly upregulated (101310048 and 101309756; $\log_2$ fold change 3.50 and 2.25, respectively), these were both considered strong

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candidates. Two G-type-lectin-RLKs (101305393 and 101305094) and one RLP (101290881) were also upregulated in the inoculated roots [46]. Further characterisation is required to fully understand the resistance mechanisms against *P. cactorum* in *F. vesca*, as well as *F.* x *ananassa*.

Two of our major effect QTL were identified on the D sub-genome (*FaRPc6D* and *FaRPc7D*), which is the least similar to *F. vesca* [38]. Recently, a major locus associated with resistance to *P. cactorum* in *F.* x *ananassa* was identified on linkage group 7D, named *FaRPc2* [19]. The small genome-wide association study of the wider germplasm highlighted different areas of the genome associated with resistance to *P. cactorum*, compared to the QTL identified from the bi-parental cross, indicating further loci, not captured in our study of 'Emily' and 'Fenella' to be exploited for resistance. Two of these SNPs were in the same region as the major locus *FaRPc2*, identified by Mangandi *et al.* [19]. In the same study, further QTL on LGs 1D, 3B, 5B, 6A and 6B were also identified, however, they were not consistent across the replicates or significant enough to be confirmed [19].

Despite the genome-wide association study being underpowered due to the small number of individuals investigated, several significant SNPs were identified and overall there was good concordance between the two approaches used, PLINK and TASSEL, but some SNPs were more significant in one method, compared to the other. Further individuals are required to be tested to increase the power of the analysis.

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519 Quantitative resistance provides many challenges to breeders due to the complex
520 nature of inheritance. However, this complexity can increase the durability of
521 resistance as it is harder for the pathogen to overcome [47]. To a large extent, the
522 'Emily' x 'Fenella' progeny responded similarly to each of the isolates tested (though
523 there is some slight indication of variation in some *P. cactorum* isolates to the same
524 plant genotypes), indicating that the three major effect QTL would be useful in
525 commercial cultivars to provide broad-spectrum partial resistance. In future studies,
526 it would be useful to explore the resistance in the wider germplasm, using a larger
527 genome-wide association study approach on many hundreds of accessions, using
528 the same isolate. This would provide more information about the status of resistance
529 within the population and identify parents with other desirable traits. Subsequent
530 work is also required to identify the gene(s) within the QTL regions and elucidate the
531 mechanism of resistance. Future work will address whether the quantitative
532 resistance to *P. cactorum* is a combination of multiple weak gene-for-gene
533 interactions between the host and the pathogen by studying both the host and the
534 pathogen in greater detail.
535
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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

FIGURE LEGENDS

Figure 1. Quantitative trait loci analysis (QTL) results for 'Emily', 'Fenella' and shared markers across the 28 strawberry linkage groups. The coloured dots represent -$\log_{10}(p)$ scores for the QTL analysis, while the black dashed horizontal line represents the significance threshold 2 (p=0.01) and the grey dashed horizontal line represents the significance threshold 3.2 (p=0.0006; Bonferroni correction). Regions on linkage groups 1A, 1B, 1D, 2C, 3B, 3C, 5B, 6A, 6B, 6C, 6D, 7A, 7C and 7D were significantly associated with resistance to *Phytophthora cactorum*, based on p<0.01. (**a**) IStraw90 markers, (**b**) IStraw35 markers only.

Figure 2. Predicted means are highly correlated with observed average crown rot scores, r^2 = 0.63.

Figure 3. Combined map of 'Emily' x 'Fenella', depicting the position of *Phytophthora cactorum* resistance quantitative trait loci (QTL) from the bi-parental cross and the genome-wide association study. Circles are QTL from 'Emily', squares are QTL from 'Fenella', diamonds are QTL shared in both cultivars and triangles are QTL identified from the genome-wide association study (GWAS).

Figure 4. Manhattan plot of genome-wide association study on 114 strawberry accessions across the 28 strawberry linkage groups. The coloured dots represent -$\log_{10}(p)$ scores for the QTL analysis, while the black dashed horizontal line

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572 represents the significance threshold 4.3 (p=0.00005). Regions on linkage groups
573 5D, 6C, 7A, 7B and 7D are significantly associated with resistance to *Phytophthora*
574 *cactorum*.

575

576 **SUPPLEMENTARY FIGURE LEGENDS**

577 **Figure S1.** Histogram of phenotypic distribution of average crown rot (*Phytophthora*
578 *cactorum*) disease severity data for the 'Emily' x 'Fenella' progeny; 'Fenella' (2.1)
579 and 'Emily' (4.4) average scores highlighted.

580

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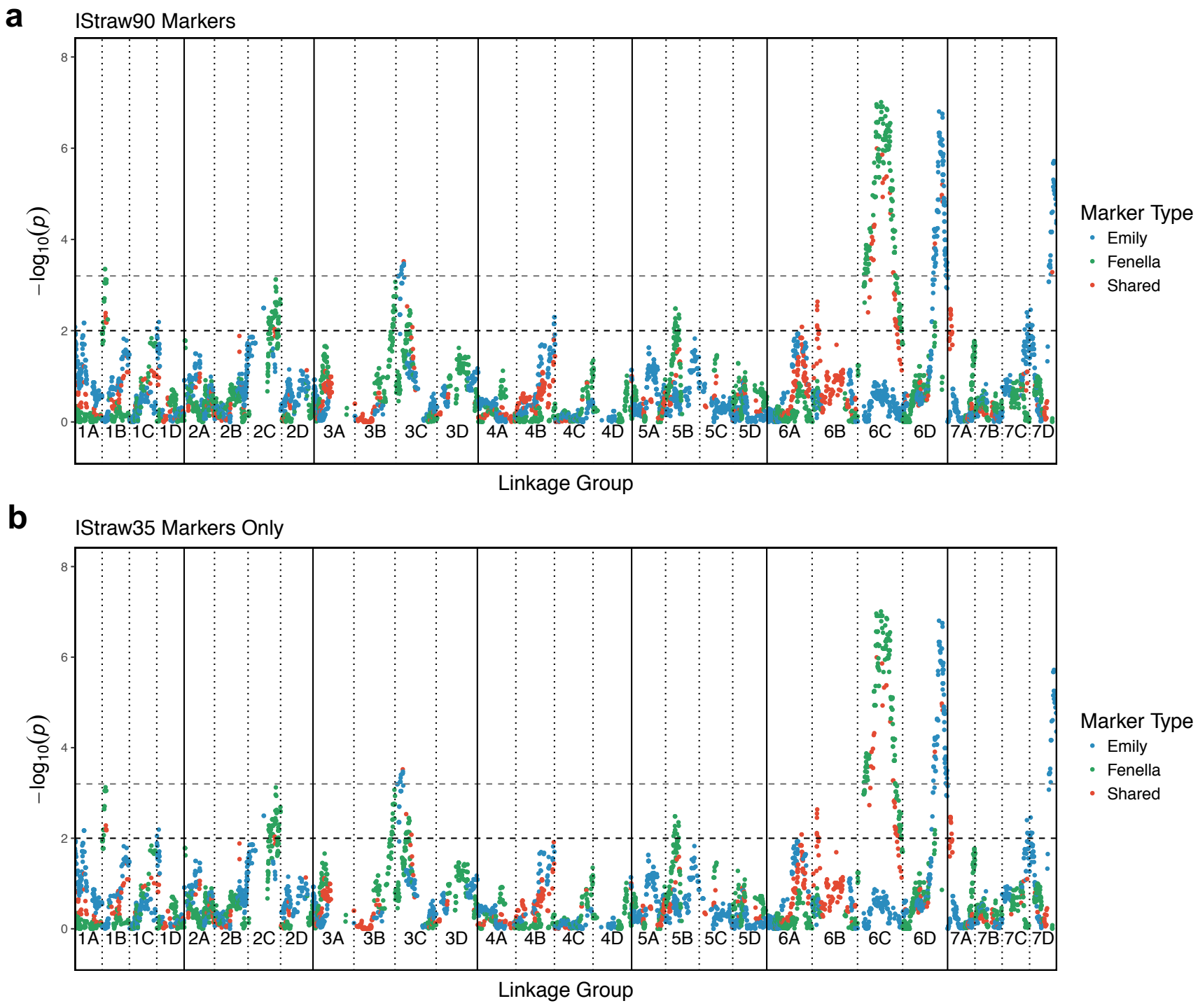
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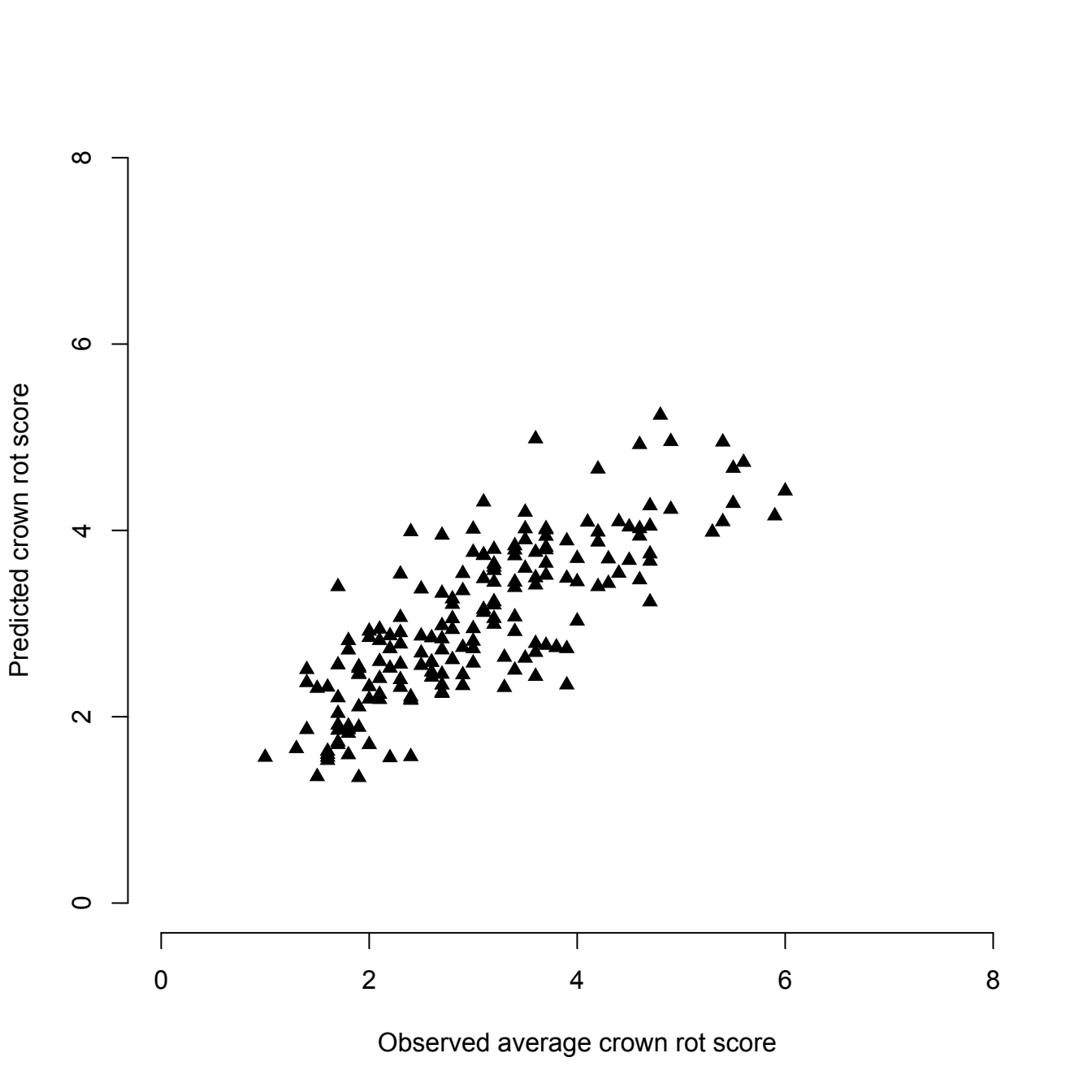
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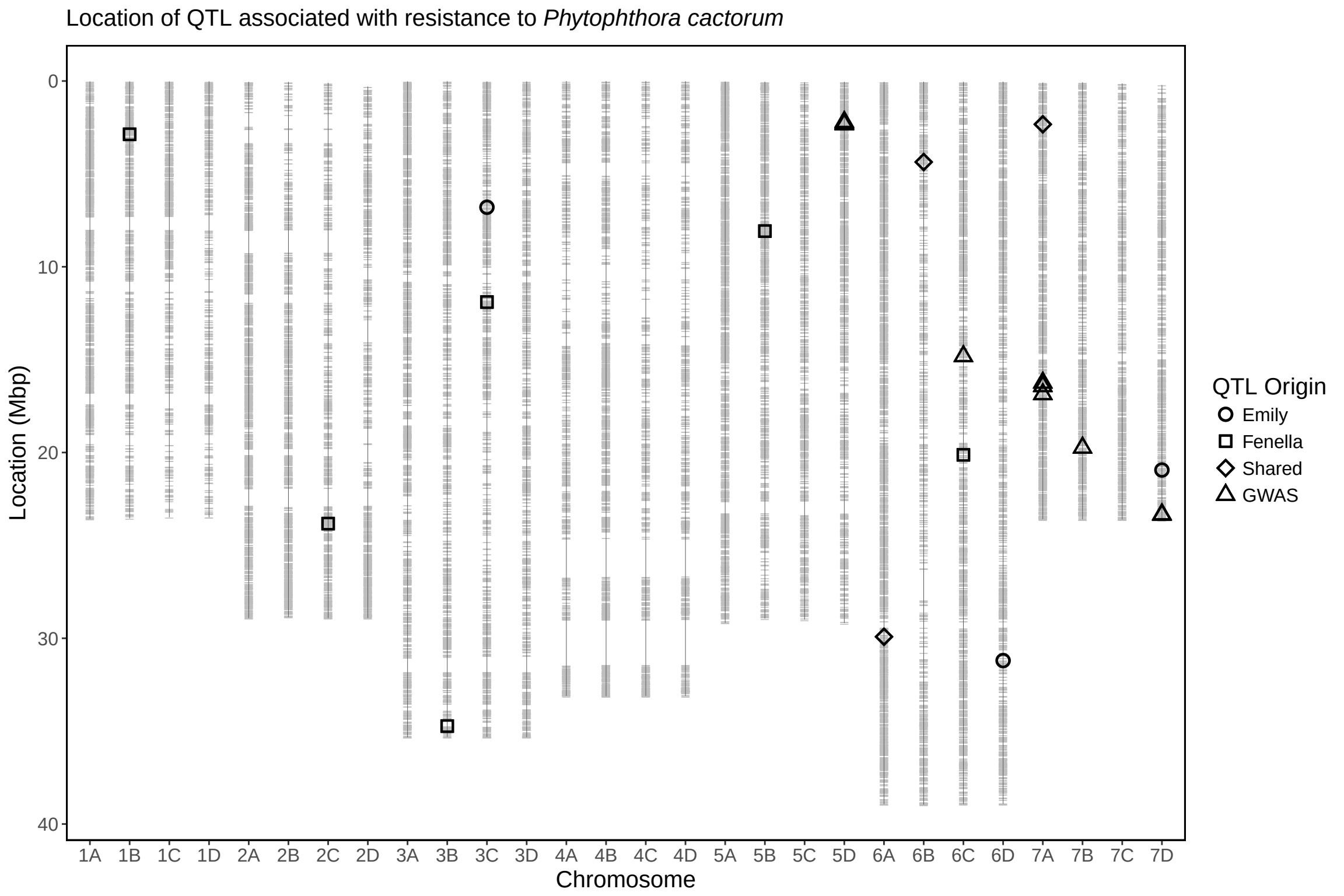
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721


a
IStraw90 Markers
b
IStraw35 Markers Only
$-\log_{10}(p)$
0
2
4
6
8
1A 1B 1C 1D 2A 2B 2C 2D 3A 3B 3C 3D 4A 4B 4C 4D 5A 5B 5C 5D 6A 6B 6C 6D 7A 7B 7C 7D
Linkage Group
Marker Type
Emily
Fenella
Shared


Predicted crown rot score
0
2
4
6
8
Observed average crown rot score
0
2
4
6
8


Location of QTL associated with resistance to *Phytophthora cactorum*
Location (Mbp)
0
10
20
30
40
1A
1B
1C
1D
2A
2B
2C
2D
3A
3B
3C
3D
4A
4B
4C
4D
5A
5B
5C
5D
6A
6B
6C
6D
7A
7B
7C
7D
Chromosome
QTL Origin
Emily
Fenella
Shared
GWAS

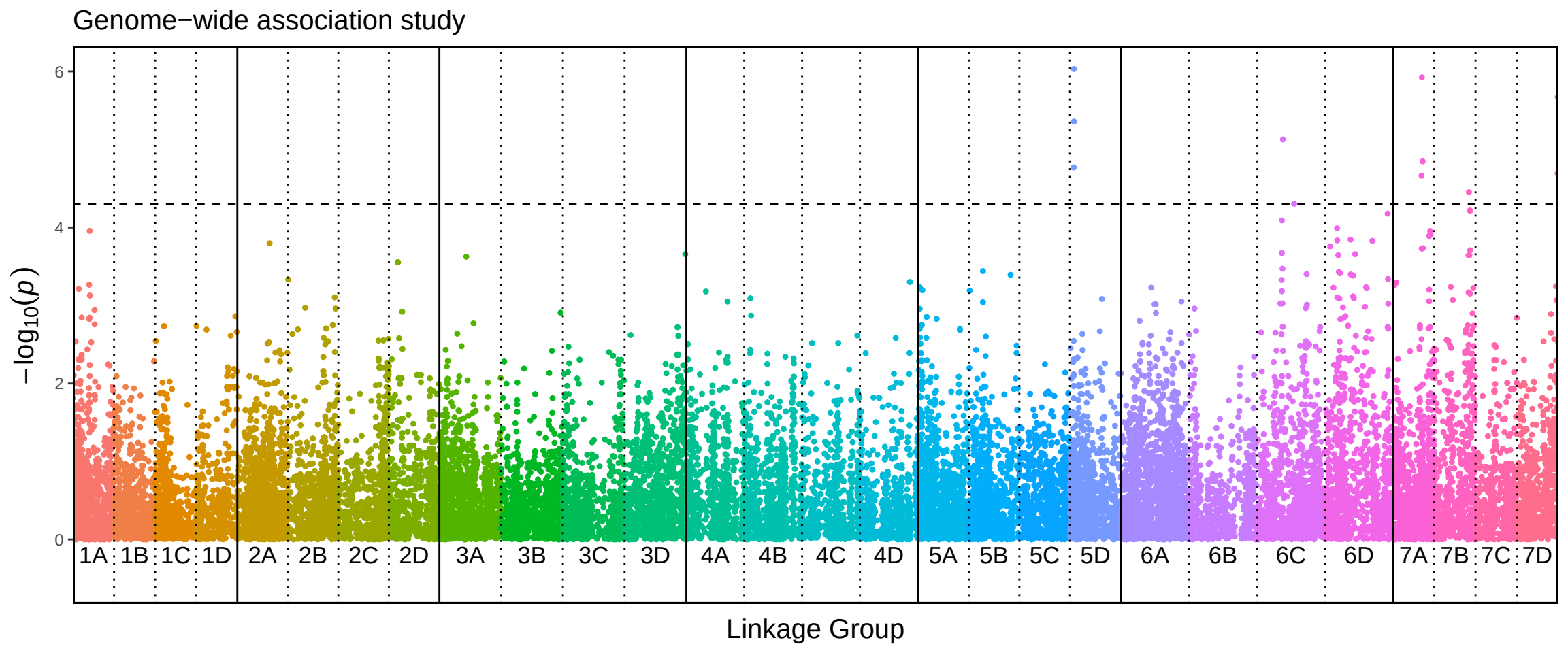

Genome−wide association study
−log10(p)
6
4
2
0
1A
1B
1C
1D
2A
2B
2C
2D
3A
3B
3C
3D
4A
4B
4C
4D
5A
5B
5C
5D
6A
6B
6C
6D
7A
7B
7C
7D
Linkage Group

Table 1. *Phytophthora cactorum* resistance quantitative trait loci (QTL) identified in the cultivated strawberry 'Emily' x 'Fenella' progeny by Kruskal-Wallis analysis using the Axiom® IStraw90 and IStraw35 SNP arrays, $p<0.01$.

***Fragaria* x *ananassa* linkage group**	**Name of QTL**	**Position (bp)**	**Most significant SNP probe I90**	**Most significant SNP probe I35**	**Marker origin**	***K* statistic**	***p* value**	**Significance**	**Dominance**
LG1A		8 000 648.5	ND75E7ED7039A4	ND75E7ED7039A4	Emily	7.3	0.0068	**	Dominant
LG1B	*FaRPc1B*	2 433 670/ 2 868 559	N5192084E36892	NE5FF79A1403E4	Fenella	12.3/ 11.4	0.0004/ 0.0007	***	Dominant
LG1D		1 698 614	N513E3104CB58F	N513E3104CB58F	Emily	7.4	0.0065	**	Dominant
LG2C	*FaRPc2C*	23 827 531	N4E21F05A7F31A	N4E21F05A7F31A	Fenella	11.4	0.0008	***	Dominant
LG3B	*FaRPc3B*	34 728 523	N66F7417C27C75	N66F7417C27C75	Fenella	11.1	0.0009	***	Dominant
LG3C	*FaRPc3C-A*	11 900 441	N7437830A85CBA	N7437830A85CBA	Fenella	8.5	0.0036	**	Dominant
LG3C	*FaRPc3C-B*	6 795 148	NE05105522E8F9	NE05105522E8F9	Emily	12.8	0.0003	***	Dominant
LG5B	*FaRPc5B*	8 079 267	N2CC4D56A2F33E	N2CC4D56A2F33E	Fenella	8.6	0.0033	**	Dominant
LG6A	*FaRPc6A*	4 360 876	N1538E8B265C61	N1538E8B265C61	Shared	11.8	0.0083	**	Dominant
LG6B	*FaRPc6B*	29 917 229	N2AFD6D4292709	N2AFD6D4292709	Shared	14.5	0.0023	**	Over-dominant (negative)
LG6C	*FaRPc6C*	20 125 920	N6FAC32AE24249	N6FAC32AE24249	Fenella	28.4	9.77e-08	*******	Dominant
LG6D	*FaRPc6D*	31 199 915	N95D81BD63C375	N95D81BD63C375	Emily	27.5	1.57e-07	******	Dominant
LG7A	*FaRPc7A*	2 330 648	N949A17793D532	N949A17793D532	Fenella	13.7	0.0034	**	Dominant
LG7C		21 270 542	N6C31E61D33EA2	N6C31E61D33EA2	Emily	8.3	0.0040	**	Dominant
LG7D	*FaRPc7D*	20 941 169	NCB6B5546E8199	NCB6B5546E8199	Emily	22.7	1.91e-06	*****	Dominant

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Table 2. Evaluation of 15 representative individuals of the 'Emily' x 'Fenella' population and their resistance/susceptibility response to *Phytophthora cactorum* isolates, with presence/absence of resistance quantitative trait loci (QTL) detailed.

Individual	Predicted Score	*Phytophthora cactorum* isolate				Presence/absence of resistance quantitative trait loci (QTL)												Marker Total
		P414	P404	P415	P416	*FaRPc1B*	*FaRPc2C*	*FaRPc3B*	*FaRPc3C-A*	*FaRPc3C-B*	*FaRPc5B*	*FaRPc6A*	*FaRPc6B*	*FaRPc6C*	*FaRPc6D*	*FaRPc7A*	*FaRPc7D*	
EF147	1.3	1.9	2.6	2.7	1.4	0	1	1	1	0	1	1	1	**1**	**1**	1	**1**	10
EF011	1.6	1.0	2.3	1.6	2.3	1	1	1	1	0	0	1	1	**1**	**1**	0	**1**	9
EF101	1.6	1.6	2.6	2.8	2.0	0	1	0	1	0	1	1	1	**1**	**1**	1	**1**	9
EF184	2.2	1.7	3.0	3.6	1.7	0	1	1	1	0	0	0	0	**1**	**1**	1	**1**	7
EF021	2.5	1.9	2.9	2.3	4.0	1	1	1	0	1	1	0	0	**1**	**1**	0	0	7
EF141	2.9	3.4	2.0	4.0	4.3	1	0	1	1	1	0	0	1	0	0	1	**1**	7
EF164	2.7	3.0	3.8	2.7	4.0	1	1	0	0	0	1	0	0	0	**1**	1	**1**	6
EF041	3.4	4.0	3.6	3.4	3.7	0	0	1	0	1	1	0	0	0	0	1	**1**	5
EF060	3.8	3.2	3.2	2.0	2.7	1	0	0	0	0	1	0	0	**1**	0	1	0	4
EF187	3.7	3.1	2.7	2.7	2.3	0	0	0	1	0	0	1	1	0	0	0	**1**	4
EF120	4.4	6.0	6.4	6.4	6.6	0	0	0	1	1	0	0	1	0	0	0	0	3
EF166	4.2	5.9	6.6	6.5	6.6	0	0	0	0	0	1	0	1	**1**	0	0	0	3
EF035	4.7	5.5	5.8	5.3	4.0	0	0	1	0	0	1	0	0	0	0	0	0	2
EF084	4.7	5.6	6.3	5.0	4.0	0	0	0	0	0	1	0	1	0	0	0	0	2
EF040	4.9	5.4	5.1	5.4	3.9	0	0	0	0	0	1	0	0	0	0	0	0	1

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Table 3. Details of the number of nucleotide-leucine rich repeat (NLR) receptors, receptor-like kinases (RLK) and receptor-like proteins (RLP) within 1 Mbp either side of the most significant marker on *Fragaria vesca* Hawaii 4 v1.1.

	Name/Linkage group	Most significant SNP marker	Location on *Fragaria vesca* v1.1 (bp)	Number of genes within 1 Mbp either side of most significant SNP in *Fragaria vesca*		
				NLR	RLK	RLP
Major effect QTL from bi-parental cross	***FaRPc6C***	N6FAC32AE24249	21 039 403	1	5	0
	FaRPc6D	N95D81BD63C375	15 257 362	1	1	6
	FaRPc7D	NCB6B5546E8199	21 972 447	6	9	0
QTL from genome-wide association study		NCB944F7C5FD4D	2 343 797	4	11	0
	LG5D	N001AABE8D8B70	2 411 572	4	10	1
		ND175A23CA503B	2 471 622	4	10	1
	LG6C	NB5EE8EA0593C0	17 404 558	0	2	2
		N8BC9A1515F06E	14 759 809	7	8	2
	LG7A	N1F8AD596FD52B	14 946 159	10	8	2
		N7AB0326E81D69	16 188 363	13	6	2
	LG7B	NEA67ABA938921	18 673 991	10	11	8
	LG7D	ND6F3E0ED7894D	19 557 035	15	12	1
		N3BEC0F68E0AF2	19 586 315	15	12	1

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Table 4. Details of most significant single nucleotide polymorphism (SNP) markers associated with resistance to *Phytophthora cactorum* identified in the genome-wide association study of 114 individuals, identified by both PLINK and TASSEL analyses.

Fragaria x *ananassa* linkage group	Position on *Fragaria vesca* v1.1 (bp)	Most significant SNP probe	PLINK				TASSEL				IStraw35 marker?
			No structure		With structure		No structure		With structure		
			Raw *p*-value	FDR BH *p*-value	Raw *p*-value	FDR BH *p*-value	Raw *p*-value	FDR BH *p*-value	Raw *p*-value	FDR BH *p*-value	
LG5D	2 343 797	NCB944F7C5FD4D	1.70e-05	0.0764	9.73e-05	0.2833	1.70e-05	0.0598	0.0003	0.3408	No
	2 411 572	N001AABE8D8B70	9.31e-07	0.0214	1.06e-05	0.0956	2.56e-07	0.0061	3.24e-06	0.0673	No
	2 471 622	ND175A23CA503B	4.39e-06	0.0395	7.89e-05	0.2833	3.12e-06	0.0325	4.51e-05	0.1708	Yes
LG6C	17 404 558	NB5EE8EA0593C0	7.45e-06	0.0537	3.74e-05	0.1924	4.32e-05	0.1058	4.31e-05	0.1708	Yes
LG7A	14 759 809	N8BC9A1515F06E	2.17e-05	0.0781	1.51e-06	0.0271	2.17e-05	0.0693	2.89e-05	0.1708	Yes
	14 946 159	N1F8AD596FD52B	1.19e-06	0.0214	1.41e-06	0.0271	1.19e-06	0.0165	2.97e-06	0.0673	Yes
	16 188 363	N7AB0326E81D69	1.42e-05	0.0764	2.85e-05	0.1922	1.42e-05	0.0598	9.86e-05	0.2734	Yes
LG7B	18 673 991	NEA67ABA938921	3.53e-05	0.1157	0.0013	0.5234	3.53e-05	0.0925	0.0012	0.3775	Yes
LG7D	19 557 035	ND6F3E0ED7894D	2.12e-06	0.0255	6.98e-06	0.0839	1.11e-05	0.0598	9.72e-05	0.2734	Yes
	19 586 315	N3BEC0F68E0AF2	2.04e-05	0.0781	0.0002	0.2969	8.41e-05	0.1458	0.0014	0.3883	Yes

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