1   **Genomic regions associated with chocolate spot (*Botrytis fabae* Sard.) resistance in faba bean (*Vicia faba* L.)**

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13   **Key message**

14   QTL mapping identified key genomic regions associated with chocolate spot resistance on faba bean chromosomes 1

15   and 6, which may serve as novel genetic tools to develop disease-resistant faba bean cultivars.

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17   **Abstract**

18   Chocolate spot (CS), caused by *Botrytis fabae* Sard., is an important threat to global faba bean production. Growing

19   resistant faba bean cultivars is, therefore, paramount to preventing yield loss. To date, there have been no reported

20   quantitative trait loci (QTLs) associated with CS resistance in faba bean. The objective of this study was to identify

21   genomic regions associated with CS resistance using a recombinant inbred line (RIL) population derived from resistant

22   accession ILB 938. A total of 165 RILs from the cross Mélodie/2 × ILB 938/2 were genotyped and evaluated for CS

23   reactions under replicated controlled climate conditions. QTL analysis identified five loci contributing to CS resistance

24   on faba bean chromosomes 1 and 6, accounting for 5.0–23.4% of the total phenotypic variance. The sequences of SNP

25   markers linked to resistance QTLs on chromosome 1 that have the largest effects encode multiple classes of putative

26   disease and/or defense-related genes. The results of this study not only provide insight into disease-resistance QTLs,

27   but also can be used as potential targets for marker-assisted breeding in faba bean genetic improvement for CS

28   resistance.

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30   **Keywords**: *Botrytis fabae*; chocolate spot; faba bean; disease resistance; QTL mapping; candidate gene

Introduction

Faba bean (*Vicia faba* L., 2n=2x=12) is a widely grown diploid cool-season legume (pulse) crop with a genome size of approximately 13 Gb (Soltis et al. 2003). It is the fifth most important pulse crop, after common bean (*Phaseolus vulgaris* L.), chickpea (*Cicer arietinum* L.), pea (*Pisum sativum* L.), and lentil (*Lens culinaris* Medik.), with an annual production of 5.4 Mt (FAOSTAT 2021). It provides an affordable source of dietary protein, fiber, carbohydrates, and other valuable nutrients (Duc et al. 2015; Khazaei and Vandenberg 2020). The faba bean seed contains about 30% protein (Warsame et al. 2018), making it one of the best alternative sources for the plant-based protein industry for food, feed, and extractable protein. The crop plays an important role in a sustainable agriculture system, due to its symbiotic fixation of atmospheric nitrogen and its use as a break crop in cereal-dominated rotations (Angus et al. 2015). Global faba bean production is generally challenged by foliar diseases such as ascochyta blight (*Ascochyta fabae* Speg.), chocolate spot (*Botrytis fabae* Sard.), rust (*Uromyces viciae fabae* Pers.), downy mildew (*Peronospora viciae* (Berk.) Caspary), and gall disease (*Physioderma viciae*) (Adhikari et al. 2021).

Chocolate spot (CS), caused by the necrotrophic fungal pathogen *Botrytis fabae* (Sard.), is one of the most destructive diseases of faba bean plants worldwide and can cause up to 90% yield reduction on susceptible cultivars when conducive environmental conditions prevail (Gorfu and Yaynu 2001; Bouhassan et al. 2004; Tivoli et al. 2006; Beyene et al. 2018). CS can manifest itself on the above-ground parts of the plant at any growth stage, mainly on leaves. It initially appears on leaves as reddish-brown spots that develop into chocolate-colored lesions. These can enlarge into extensive necrotic zones that lead to severe premature defoliation. If the disease develops at flowering time, it can cause complete crop failure. The pathogen survives on crop residues or lodged stubble of the previous year's growing season and on contaminated seeds (Harrison 1988). Its spores are spread primarily between fields by wind and rain. Variation in virulence among *B. fabae* isolates has been demonstrated (Hutson and Mansfield 1980), although no race classification has been described so far. The strategies used to control chocolate spot on faba bean include crop rotation, reduced planting density, timely fungicide applications, use of clean seeds, and host-plant resistance (Stoddard et al. 2010). Other species of *Botrytis* contribute to chocolate spot disease, but *B. fabae* is the main one (Fan et al. 2015).

Genetic resistance is a key part of any integrated disease management approach to prevent yield loss caused by CS. Fungal evolution and migration leads to the need for continuous incorporation of new sources of resistance genes or

58   alleles into elite breeding materials. A few sources of partial genetic resistance to CS have been identified in faba bean

59   germplasm (Hanounik and Robertson 1988; Bouhassan et al. 2004; Villegas-Fernández et al. 2009; Maalouf et al.

60   2016). Partial resistance to CS was transferred into elite breeding lines and resulted in the release of several cultivars

61   with moderate levels of resistance (Temesgen et al. 2015; Maalouf et al. 2019). Incorporation and pyramiding of CS

62   resistance genes from multiple sources into elite cultivars could be facilitated with marker-assisted selection (MAS).

63   However, the use of MAS in faba bean CS resistance breeding is limited by lack of knowledge of the genetic basis of

64   the resistance so-far identified, by the presence of pathogen variability in the major faba bean diseases (Hanounik and

65   Robertson 1988; Gorfu 1996), and by the lack of adequate genomic resources.

66   In recent years, progress has been made in developing single nucleotide polymorphism (SNP)-based genetic maps of

67   faba bean (e.g. Ellwood et al. 2008; Cruz-Izquierdo et al. 2012; Kaur et al. 2014; Webb et al. 2016; Carrillo-Perdomo

68   et al. 2020). Among these maps, the densest SNP-based consensus maps are reported by Webb et al. (2016),

69   constructed from six mapping populations using 687 SNP markers, and Carrillo-Perdomo et al. (2020), a consensus

70   map of three mapping populations consisting of 1,728 SNP markers distributed in six linkage groups. Concurrently,

71   several QTL studies for various faba bean improvement traits have been carried out using different genetic maps (see

72   reviews by O'Sullivan and Angra 2016; Maalouf et al. 2019; Khazaei et al. 2021a) for major foliar diseases such as

73   ascochyta blight (e.g. Avila et al. 2004; Kaur et al. 2014; Atienza et al. 2016; Sudheesh et al. 2019) and rust (e.g.

74   Adhikari et al. 2016; Ijaz et al. 2021). Despite the importance and widespread global nature of CS, no QTL studies

75   have been published to our knowledge. In addition, breeding for CS resistance has been difficult because screening

76   for the disease under field conditions is unreliable, especially in dry environments where faba bean is grown mostly

77   as a rain-fed crop (Adhikari et al. 2021). Humid conditions, which are uncommon in many faba bean-growing areas,

78   provide the opportunity to screen in the field. Identification of reliable molecular markers for use in MAS systems for

79   improving CS resistance is essential for future faba bean breeding. The objective of this study was to identify QTLs

80   associated with CS resistance using an advanced RIL mapping population developed from accession ILB 938 which

81   has partial resistance to CS (Khazaei et al. 2018) under climate-controlled conditions.

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Material and methods

Plant material

Genetic resistance to *B. fabae* was evaluated using 165 RILs from the Mélodie/2 × ILB 938/2 cross (Khazaei et al. 2014a). The RILs were advanced using single seed descent to the F_8 generation. Then the F_8-derived bulked seeds of the RILs were selfed for at least two additional generations before disease phenotyping. Mélodie/2 was bred at INRA (France) as a source of low vicine-convicine (Khazaei et al. 2014a). ILB 938 (IG 12132) originates from the Andean region of Colombia and Ecuador and has resistance to several biotic and abiotic stresses, including CS (Maalouf et al. 2016; Khazaei et al. 2018). It has high water use efficiency (Link et al. 1999; Khazaei et al. 2014a) and carries a gene that decouples pigmentation in flowers from that in stipules (Khazaei et al. 2014b). It was the first identified source of resistance for CS in faba bean (Hanounik 1982; Khalil et al. 1984; Robertson 1984). ILB 938 resulted from mass selection within ILB 438 (IG 11632). ICARDA's registered BPL (pure line) derivatives of ILB 438 and ILB 938 are BPL 710 and BPL 1179, respectively.

Inoculation and phenotyping for chocolate spot reactions

A culture stock of *B. fabae* isolate FB-7 was obtained from the pulse pathology laboratory, University of Saskatchewan for mass spore production. Conidia were cultured on faba bean extract media plates (18 g of agar, 20 g of dextrose, 20 g of NaCl, and 1 L of faba bean extract which is prepared by boiling 400 g of faba bean seeds in 1 L of distilled water for 45 minutes and diluting with 1 L of distilled water after removing the faba bean seeds) and incubated for 14 d at room temperature in a cycle of 12 h of darkness and 12 h of light to induce sporulation. Plates were then flooded with sterile deionized water and conidia were harvested by scraping the colonies with the edge of a sterile glass microscope slide. The suspension was collected and filtered through one layer of cheesecloth into a clean Erlenmeyer flask. The concentration of the conidial suspension was adjusted to 5×10^5 spores mL^{-1} using a haemocytometer. The surfactant Tween 20 (polyoxyethylene sorbitan monolaurate) was added at the rate of 1 to 2 drops per 1000 mL of suspension, and the suspension was shaken well before inoculation.

The parents and 165 RILs were grown in a climate-controlled growth chamber (GR48, Conviron, Winnipeg, Canada) at the University of Saskatchewan's College of Agriculture and Bioresources phytotron facility, Saskatoon, Canada. The growth chamber conditions were adjusted to 18 h light and 6 h dark, with the temperatures maintained at 21 °C

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110   (day) and 18 °C (night), and the photosynthetic photon flux density was set to 300 µmol m^{-2} s^{-1} during the light period

111   at the crop canopy level. Three seeds of each genotype were sown in individual 3.8 L pots (15.5 cm in diameter)

112   containing a soil-less mixture (Sunshine Mix No. 3, Sun Grow Horticulture® Ltd., Vancouver, BC, Canada) and

113   fertilized once per week using 3 g L^{-1} of soluble N:P:K (20:20:20) PlantProd® fertilizer (Nu-Gro Inc., Brantford, ON,

114   Canada). Three weeks after seeding, the plants were inoculated with a spore suspension (5 × 10^5 spores mL^{-1}) at ~3

115   mL per plant until runoff using a pressurized knapsack sprayer. Plants were placed in an incubation chamber for seven

116   days. Two humidifiers (Vicks Fabrique Paz Canada, Inc., Milton, ON, Canada) were placed in the incubation chamber

117   to ensure 90–100% relative humidity for infection and disease development. The disease severity data were collected

118   per individual plant at seven days post-inoculation (dpi) using a 0 to 10 rating scale with 10% increments. The

119   experiment was repeated four times under the same growing conditions during 2020-2021, and each time disease

120   scores were recorded from three replicates (one plant per replicate). Data were converted to percentage disease severity

121   using the class midpoints for data analysis and analyzed as a randomized complete block design.

122   **Statistical analysis**

123   Disease score data were analyzed using SAS v.9.4 (SAS Institute, Cary, USA). Normality and variance homogeneity

124   of the residuals were analysed using the Shapiro-Wilk normality test and Levene's test for homogeneity, respectively.

125   Genotypes were treated as fixed effects and blocks as random effects; significance of variances were declared at 5%

126   significance level. Least square means were estimated for genotype using LSMEANS statements and used for QTL

127   analysis. Multivariate relationships between RILs and experimental repeats were investigated by principal component

128   analysis (PCA) and biplots of autoscaled (i.e., each repeat mean and grand mean centred at the origin) data in R (R

129   Core Team 2020) with the 'factoextra' package.

130   **Genotyping**

131   Genomic DNA was extracted from three-day-old germinated embryo axes of the parents and 188 RILs from the

132   mapping population using the CTAB method, as described previously (Björnsdotter et al. 2021). DNA quality was

133   assessed by agarose gel electrophoresis; concentrations were determined with a Quant-iT PicoGreen dsDNA Assay

134   Kit (ThermoFisher Scientific, UK) following the manufacturer's guidelines. DNA samples were brought to a

135   concentration of 35 ng/µl in 40 µl aliquots and genotyped using the Axiom 'Vfaba_v2' 60K array that was developed

136   from metatranscriptome data (O'Sullivan et al. 2019).

Linkage map construction

138   Genotypic data for the RIL population was filtered against markers showing significant segregation distortion

139   (deviating from the expected 1:1 ratio) using a chi-square (χ^2) test. Markers missing in 1% or more of the data were

140   removed from the analysis. Draft maps were generated from the remaining 180 RILs using ASMap software (Taylor

141   et al. 2017) with a p-value of $1E^{-10}$ and a maximum distance between SNP markers of 15.0 cM for grouping them into

142   linkage groups. This linkage map was refined using MapDisto v. 1.7.7.0.1 (Lorieux 2012) with a logarithm of odds

143   (LOD) score of 3.0 and a cut-off recombination value of 0.35. The best marker order was estimated using both

144   "AutoCheckInversions" and "AutoRipple" commands in MapDisto. Distances between markers were calculated using

145   the Kosambi function (Kosambi 1943). Linkage groups (LGs) were scanned and corrected for double recombinants

146   using MapDisto v. 1.7.7.0.1 (Lorieux 2012). The final LGs were assigned to chromosomes according to the NV644 ×

147   NV153 genetic map developed at the University of Reading, UK (unpublished).

QTL mapping of disease resistance

149   Windows QTL Cartographer 2.5 software (Wang et al. 2012) and multiple QTL mapping (MQM, Manichaikul et al.

150   2009), run in R/qtl software (Broman et al. 2003), were used to detect QTLs. For Windows QTL Cartographer,

151   composite interval mapping (CIM) was implemented using the Kosambi map function with Ri1 cross type

152   (recombinant inbred line, derived by selfing) at a 1.0 cM interval walk speed. Cofactor selection was performed using

153   forward and backward regression in the standard CIM model with a probability of in and out of 0.1 and a window size

154   of 5 cM. QTL significance thresholds were determined by permutation tests (1000 permutations) at a significance

155   level of $P = 0.05$. Multiple QTL mapping was completed with the "*stepwiseqtl*" function (Broman et al. 2003), using

156   the Haley-Knott regression (Haley and Knott 1992) methods. The optimal QTL model was chosen based on the highest

157   penalized LOD score (Manichaikul et al. 2009) after forward and the backward selection and elimination modeling.

158   Penalties for model selection and the genome-wide significance threshold (= 0.05) were determined through 1000

159   permutations of the "*scantwo*" function for two-dimensional QTL scanning. The percentage of the phenotypic

160   variance explained (PVE) and effects of QTLs were obtained by fitting a mixed linear model using the "*fitqtl*" function.

161   The confidence intervals for each QTL were estimated using the "*lodint*" function that calculates the 1.5 LOD support

162   intervals. To identify putative candidate genes, the sequences of the SNP markers in the QTL intervals were searched

163   by BLASTn in Phytozome v13 (Goodstein et al. 2012) on the *Medicago truncatula* reference genome (Mt4.0v1). QTL

164   positions on the linkage map were drawn by MapChart v. 2.2 (Voorrips 2002).

165   **Results**

166   **Phenotypic analysis of the parental lines and RIL population**

167   Individuals in the RIL population showed significant variation in CS resistance (F-value = 2.21, P = 0.0001). The

168   parental lines Mélodie/2 and ILB 938/2 exhibited significant differences in resistance to CS under growth chamber

169   conditions (Fig 1). ILB 938/2 had a partial resistant reaction with mean disease severity of 30.4%, while Mélodie/2

170   showed a moderately susceptible reaction with a mean of 53.3%. The distribution of disease severity as a measure of

171   CS response for RILs showed continuous variation ranging from 20.8% to 62.9%, with a mean of 44.5% (Fig 1),

172   suggesting polygenic regulation of CS severity. Similarly, the autoscaled PCA indicated that the data had no

173   underlying structure for the first two principal components (PC1 vs PC2), which explained approximately 66.1% of

174   the variance. The PCA-biplot graph also displayed a strong positive correlation among the four screening repeats,

175   which were represented well by the graph (i.e., the vector with equal length), demonstrating that the data could be

176   used for accurate mapping of CS resistance QTLs (Fig 2).

177   **Linkage map construction**

178   A total of 35,363 SNP markers were filtered for polymorphism between the parents, significant segregation distortion,

179   and missing data. A final genetic map was constructed from 4,089 SNP markers, which mapped to six linkage groups

180   (LGs) representing the six chromosomes of faba bean (Table 1). The LGs were numbered to match the respective faba

181   bean consensus map based on where the markers lie (Webb et al. 2016). The linkage map spanned a total genetic

182   distance of 1229.5 cM with an average marker interval of 0.3 cM. The genetic distance within LGs varied from 124.2

183   cM for LG 4 to 396.1 cM for LG 1. LG 4 contained the fewest SNP markers, whereas LG 1 contained the most SNP

184   markers (Table 1; Fig S1).

QTL analysis

Five QTLs associated with chocolate spot resistance were detected in the RIL population (Table 2 and Figure 3). Among these, two QTLs (qBF1.1 and qBF1.2) were identified on LG 1 by both QTL mapping methods, CIM and MQM, implemented in WinQTLCart and R/qtl software, respectively. The QTL qBF1.1 accounted for 8.9–10.7% of PVE, whereas qBF1.2 explained 12.2–12.8% of the PVE (Table 2). These QTLs were derived from the resistant line ILB 938/2. On the other hand, the remaining three QTLs, one on LG 1 (qBF1.3), explained 7.3% of the PVE, and two on LG 6 (qBF6.1 and qBF6.2), accounted for 6.2 and 6.3 % of the PVE, respectively, were detected solely by CIM methods. The resistance allele for qBF6.1 was contributed by ILB 938/2, whereas the moderately susceptible parent Mélodie/2 was the donor of qBF1.3 and qBF6.2 (Table 2).

Identification of putative candidate genes

Analysis was conducted of the sequences of the SNP markers in the region of the two stable resistance loci, qBF1.1 and qBF1.2, which were identified by both QTL mapping approaches. A similarity search using BLASTn in the *M. truncatula* genome found a number of potential genes similar to the markers linked to the observed resistance against *B. fabae*. The sequence of SNP markers linked to qBF1.1 showed similarity with seven genes on chromosome 2 and those linked to qBF1.2 with 16 genes on chromosome 5 of *M. truncatula*, all of which were annotated as genes possibly associated with plant disease resistance and/or defence (Table 3).

Discussion

Resistance breeding is the most cost-effective and environmentally friendly strategy for controlling CS. Many studies have reported ILB 938 as a source of resistance to CS (e.g. Hanounik and Robertson 1988; Rhaïem et al. 2002; Villegas-Fernández et al. 2009; Maalouf et al. 2016). The introduction of CS resistance from this source into elite cultivars could be accelerated using MAS. To implement MAS in faba bean breeding programs, identification of markers linked to QTLs or genes conferring resistance to CS is the first step. In this study, the analysis of QTLs was conducted using a RIL population derived from ILB 938/2. The RIL population showed significant variation in pathogen response, as expected from the presence of resistance genes or alleles. The frequency distribution of the CS disease score in RILs showed a continuous distribution, indicating polygenic inheritance of CS resistance in ILB

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210   938/2. To our knowledge, this study provides the first identification of genomic regions linked to CS resistance in this

211   species.

212   Here we report the first linkage map, comprised of 4,089 SNP markers in six linkage groups that correspond to the six

213   chromosomes of faba bean, generated for the ILB938-derived RIL population. The genetic linkage map spanned

214   1,229.5 cM with an average marker density of 0.3 cM. According to Stange et al. (2013), an increase in marker density

215   from 5 to 1 cM could increase the power sufficiently to precisely localize and resolve closely linked QTLs. Therefore,

216   the genetic map developed in this study has sufficient marker density to provide adequate power for QTL mapping.

217   The total map size and the marker distributions along the LGs of the current genetic map are consistent with the

218   consensus map previously published by Webb et al. (2016). It is shorter than the recently published SNP-based

219   consensus map (1547.71 cM) by Carrillo-Perrillo et al. (2020). Like previous marker-based genetic maps (Webb et al.

220   2016; Carrillo-Perrillo et al. 2020) and cytogenetic studies (Lucretti et al. 1993; Doležel and Lucretti 1995), LG 1,

221   corresponding to chromosome 1, contained the highest number of SNP markers and a larger size.

222   Using this genetic map and the CS phenotypic data for the RIL population, we mapped five QTLs associated with CS

223   resistance on LG 1 and LG 6. Two QTLs on LG 1 (qBF1.1 and qBF1.2) with high LOD scores were detected by both

224   WinQTLCart and R/qtl. These QTLs were derived from ILB 938/2 and explained a total phenotypic variation of 22%.

225   The other three QTLs, one on LG 1 (qBF1.3) and two on LG 6 (qBF6.1 and qBF6.2), passed the threshold after

226   permutation and were detected only by WinQTLCart. Interestingly, two of these three QTLs were inherited from the

227   moderately susceptible parent Mélodie/2. In QTL studies for various traits in different crops, the inheritance of the

228   resistance allele from a susceptible parent is not unusual, and a good example is the large effect QTL for improved

229   yield in rice under drought conditions derived from the susceptible parent (Bernier et al. 2007).

230   When the sequences of the SNP markers identified in the regions of the two stable QTLs (qBF1.1 and qBF1.2) were

231   used as BLASTn queries of the *M. truncatula* genome, the corresponding regions included several candidate genes

232   that may play a role in disease resistance or defence against biotic stress. Of these, several are promising candidates:

233   an alpha/beta-hydrolase protein, known as the Arabidopsis (*Arabidopsis thaliana*) Enhanced Disease Susceptibility 1

234   (EDS1) gene (Wittek et al. 2014; Vossa et al. 2019); Bax inhibitor-1 protein (Watanabe and Lam 2006); clathrin

235   adaptor complex protein (Qiao et al. 2010); NAD-dependent epimerase/dehydratase (Islam et al. 2019);

236   glyceraldehyde 3-phosphate dehydrogenase (Henry et al. 2015); NPH3 protein (Yang et al. 2016); spermine synthase

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237 (Janse van Rensburg et al. 2021); F-box protein (Hedtmann et al. 2017); and copper amine oxidase (Rea et al. 2002).

238 All these genes are known to be involved in regulating programmed cell death against pathogens and in controlling

239 cell death proliferation in response to reactive oxygen species (ROS). Recently, Castillejo et al. (2021) reported that

240 resistance to *B. fabae* is linked to a more efficient photosystem II repair cycle in the resistant faba bean accession BPL

241 710. In response to *B. fabae* infection, different levels of ROS, lipid peroxidation, and activity of the enzymatic ROS

242 scavenging system were detected in resistant and susceptible faba bean genotypes (El-Komy 2014). However, the

243 antioxidative system is more effective in removing excess ROS produced during infection in the resistant genotype,

244 and pathogenesis-related protein gene transcripts are expressed earlier and higher (El-Komy 2014; Castillejo et al.

245 2021). Genes encoding leucine-rich repeat-containing proteins such as NBS-LRR are typically disease-resistance (*R*)

246 genes that detect the pathogen and activate downstream signaling, leading to pathogen resistance (Dodds and Rathjen

247 2010). We also identified jasmonate ZIM-domain proteins, which are involved in the responses to plant pathogens

248 and abiotic stresses by regulating jasmonic acid signaling pathways and the cross-talk between various phytohormones

249 (Zhou et al. 2015). Furthermore, the expression of genes encoding xyloglucan induces resistance against necrotrophic

250 (*B. cinerea*) in Arabidopsis (Claverie et al. 2018).

251 Despite the fact that CS is one of the most damaging faba bean diseases worldwide, progress remains slow in breeding

252 for CS resistance. Among the challenges related to faba bean improvement, the restricted appearance of the disease

253 only during the humid climate cropping season makes traditional field-based phenotypic selection unreliable,

254 particularly in dry areas like the Western Canadian prairies and parts of Australia (Adhikari et al. 2021; Khazaei et al.

255 2021b). This might explain why there were no QTL studies of CS previously reported. Therefore, conventional faba

256 bean breeding could be augmented with molecular marker technologies to develop CS-resistant cultivars that are

257 suitable for fulfilling the growing global demand for faba bean generated by its high seed protein content and great

258 ecological service in cropping systems. Our study provides the first QTL identification for CS resistance using high-

259 resolution SNP markers. Some of the identified QTLs in this work can be used as potential targets for further studies,

260 and the linked markers can enable the possibility of using MAS in faba bean CS resistance breeding after validation

261 in appropriate germplasm.

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270   **Author contribution statement**

271   HK, AV, FLS and AHS conceived and designed the experiments. TSG and MB performed the experiments. WC

272   performed DNA extraction and library preparation. TSG and HK analyzed the data. AV, HK, FLS and AHS provided

273   resources and acquired the funding. TSG wrote the first draft of the manuscript. All authors read and reviewed the

274   manuscript.

275   **Conflict of interest**

276   The authors declared that they have no competing interest.

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444   **Table 1.** Characteristics of the genetic linkage map for the 165 faba bean RIL population derived from cross Mélodie/2

445   × ILB 938/2 at F8.

Chromosomes (Linkage groups)	Number of markers	Map length (cM)	Average marker interval (cM)	Maximum gap (cM)
1	1262	396.1	0.31	3.23
2	656	218.1	0.33	3.21
3	668	181.4	0.27	3.58
4	488	124.2	0.25	2.22
5	488	139.8	0.29	4.32
6	527	169.9	0.32	3.55
Total	4089	1229.5	0.30	-

446

447 **Table 2.** Quantitative trait loci (QTL) for resistance to chocolate spot in 165 RIL population derived from cross

448 Mélodie/2 × ILB 938/2.

QTL	LG[a]	Peak (cM)	2- LOD interval (cM)	CIM[d]			MQM[d]		
				LOD	R^b (%)	Add[c]	LOD	R^b (%)	Add[c]
qBF1.1	1	28.5	22.7 – 29.7	4.2	8.9	-3.0	4.5	10.7	-3.2
qBF1.2	1	127.3	125.2 –130.4	5.7	12.2	-3.5	5.3	12.8	-3.6
qBF1.3	1	191.0	189.4 –192.9	3.5	7.3	2.8	-	-	-
qFB6.1	6	109.5	104.1 –113.2	3.7	6.2	-2.5	-	-	-
qFB6.2	6	164.0	159.9 –168.8	3.1	6.3	4.1	-	-	-

449 [a]Linkage group, [b]R^2 - Percentage of phenotypic variance explained by QTL, [c]Additive genetic effect, [d]CIM - composite
450 interval mapping in WinQTLCart and MQM - multiple QTL mapping in R/qtl software

451 **Table 3**. Candidate resistance and/or defense-related genes associated with QTLs for chocolate spot resistance based on BLASTn sequence similarity searches in

452 the *Medicago truncatula* (Mt4.0v1) genome. The genes were queried using sequences of the SNP markers identified in the interval between the two stable resistance

453 loci (qBF1.1 and qBF1.2).

QTL	Gene ID#	Position#	e value	Descriptions
qFB1.1	Medtr2g019020	chr2:6098667..6117368	2.6E-21	ABC transporter c family member 11-related
qFB1.1	Medtr2g019230	chr2:6225091..6227537	2.6E-16	F-box domain (F-box) kelch motif (kelch_1)
qFB1.1	Medtr2g019740	chr2:6500045..6505955	2.0E-13	Zinc finger ccch domain-containing protein
qFB1.1	Medtr2g020190	chr2:6690504..6693742	8.8E-20	NAD dependent epimerase/dehydratase
qFB1.1	Medtr2g020520	chr2:6836280..6842446	4.3E-22	Membrane associated ring finger
qFB1.1	Medtr2g021130	chr2:7137958..7140558	1.1E-19	Bax inhibitor-1 motif-containing protein
qFB1.1	Medtr2g021260	chr2:7216245..722118	6.6E-10	Alpha/beta-hydrolases superfamily protein
qFB1.2	Medtr2g044910	chr2:19529872..19533009	7.5E-18	Jasmonate-zim-domain protein
qFB1.2	Medtr2g065470	chr2:27454062..27460627	1.4E-08	Glyceraldehyde 3-phosphate dehydrogenase
qFB1.2	Medtr5g032780	chr5:14127853..14131878	4.6E-23	NPH3 family (nph3)
qFB1.2	Medtr5g033170	chr5:14293911..14299220	2.0E-18	Copper amine oxidase
qFB1.2	Medtr5g033230	chr5:14330113..14338389	6.7E-22	Glycine transaminase / glycine aminotransferase

qFB1.2	Medtr5g033500	chr5:14425504..14431918	1.4E-19	Adaptor complexes medium subunit family (adap_comp_sub) / clathrin adaptor complex small chain (clat_adaptor_s
qFB1.2	Medtr5g033820	chr5:14595958..14601126	2.2E-14	Pprotein nsp-interacting kinase 3
qFB1.2	Medtr5g033960	chr5:14676992..14677753	3.2E-13	Ubiquitin/40s ribosomal protein s27a fusion
qFB1.2	Medtr5g034470	chr5:14952636..14957856	8.5E-14	Spermine synthase
qFB1.2	Medtr5g035070	chr5:15256253..15258244	3.2E-13	Protein altered xyloglucan 4
qFB1.2	Medtr5g035080	chr5:15260468..15263355	5.2E-08	Protein altered xyloglucan 4
qFB1.2	Medtr5g035090	chr5:15265169..15266749	1.7E-11	Protein altered xyloglucan 4
qFB1.2	Arath|AT3G14460.1 GX2357M	chr5:15319859..1532097	2.0E-04	LRR and NB-ARC domains-containing disease resistance protein
qFB1.2	Medtr5g035280	chr5:15334263..15337184	4.2E-13	Leucine-rich repeat (LRR)-containing protein
qFB1.2	Arath|AT3G14460.1_GX2362M	chr5:15345285..15358820	7.6E-10	LRR and NB-ARC domains-containing disease resistance protein
qFB1.2	Arath|AT3G14470.1_GX2447M	chr5:16412520..16415453	1.7E-05	NB-ARC domain-containing disease resistance protein

454 #Genes id and positions (chromosome, start and end base pairs) according to *M. truncatula* (Mt4.0v1) genome.

455   **Figure captions:**

456   **Figure 1.** Frequency distributions of chocolate spot disease scores in 165 RILs derived from cross Mélodie/2 × ILB

457   938/2 evaluated under growth chamber conditions. Each value is the mean of 4 screenings, each with 3 replicates. The

458   arrows indicate the average disease severity of the parents. Disease severity was rated on a 0–10 scale, where the

459   disease severity score increased in 10% increments

460   **Figure 2.** PCA-biplot graph of PC1 vs PC2 for auto-scaled and centered data for chocolate spot severity for each

461   repeat (rep) collected from a 165 recombinant inbred lines (RILs) population of a cross Mélodie/2 × ILB 938/2 cross

462   (FBR-06). Each RIL score, which is a deviation from its rep average value, was displayed as a point on the graph and

463   reps as a blue arrow (vector). The parental lines are circled with black dots. The origin of the graph represents the

464   average value for both each rep and across all repeats. The angle between any two arrows indicates the correlation

465   between repeats (if the angle is 90 degrees, it means both variables show a lack of correlation) and the length of the

466   arrow represents the variance explained by two components (all equal length means a perfect fit). The colour bar

467   indicator on the right shows the deviation of each score from the averages

468   **Figure 3.** Location of chocolate spot resistance QTLs on chromosome 1 (A, B and C) and 6 (D and E) using 165 RILs

469   derived from cross Mélodie/2 × ILB 938/2. The QTL positions are shown with a red bar, and the loci within the QTL

470   regions are shown on the right of the bar. Only portions of the linkage map related to the QTL positions are displayed
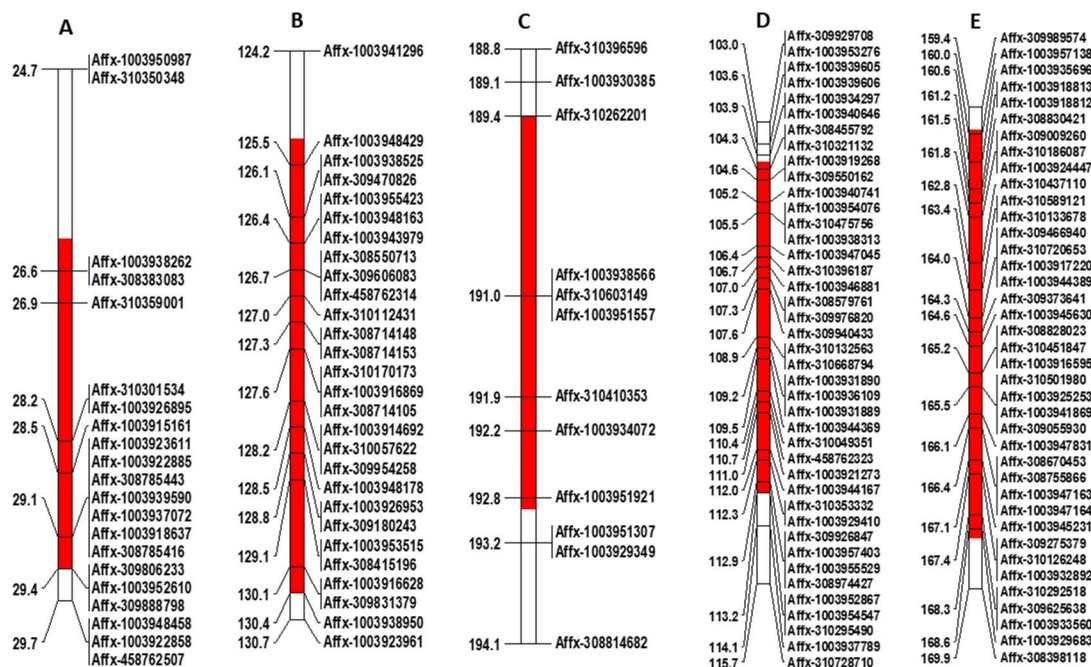
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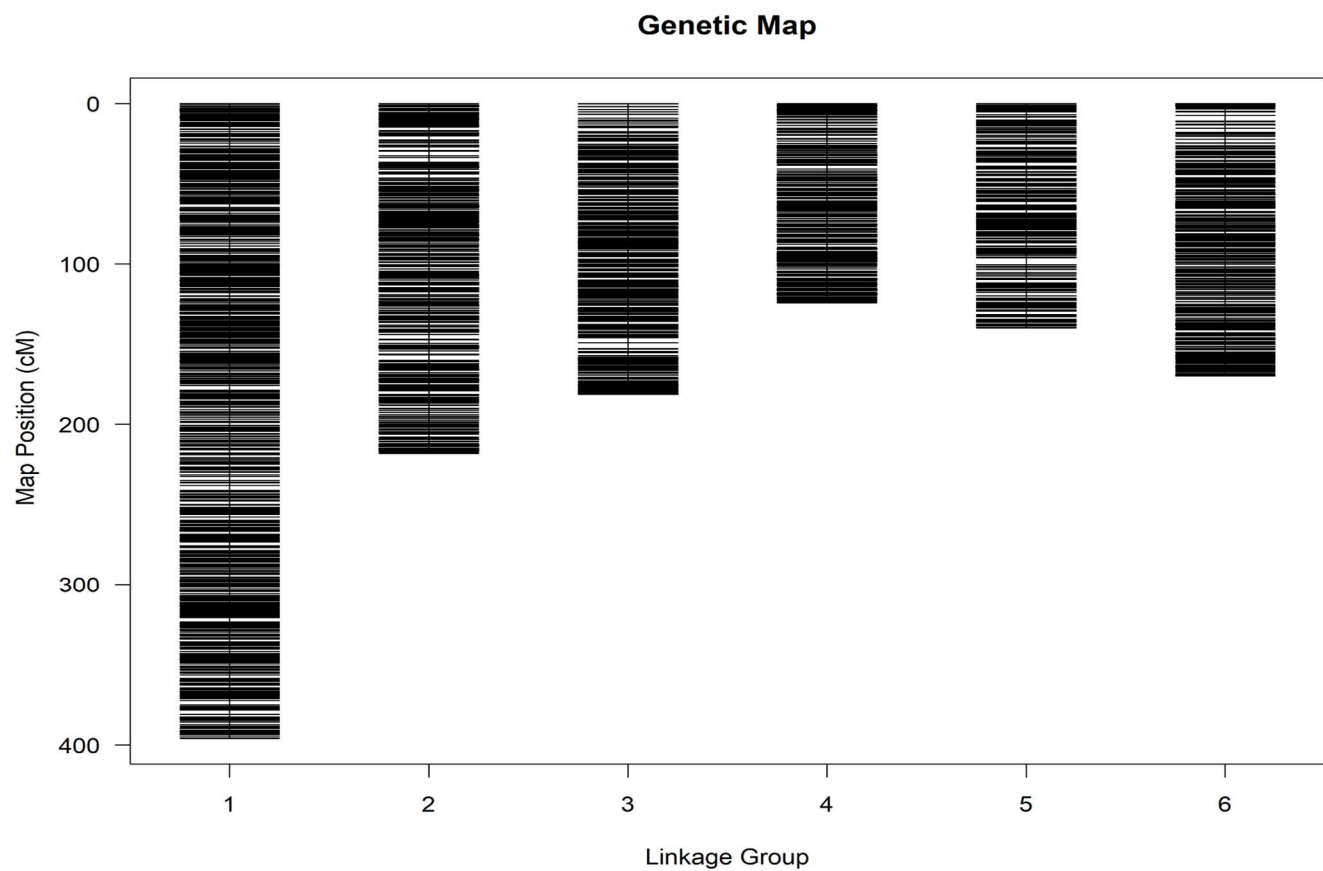
471   Fig 1

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473   Fig 2

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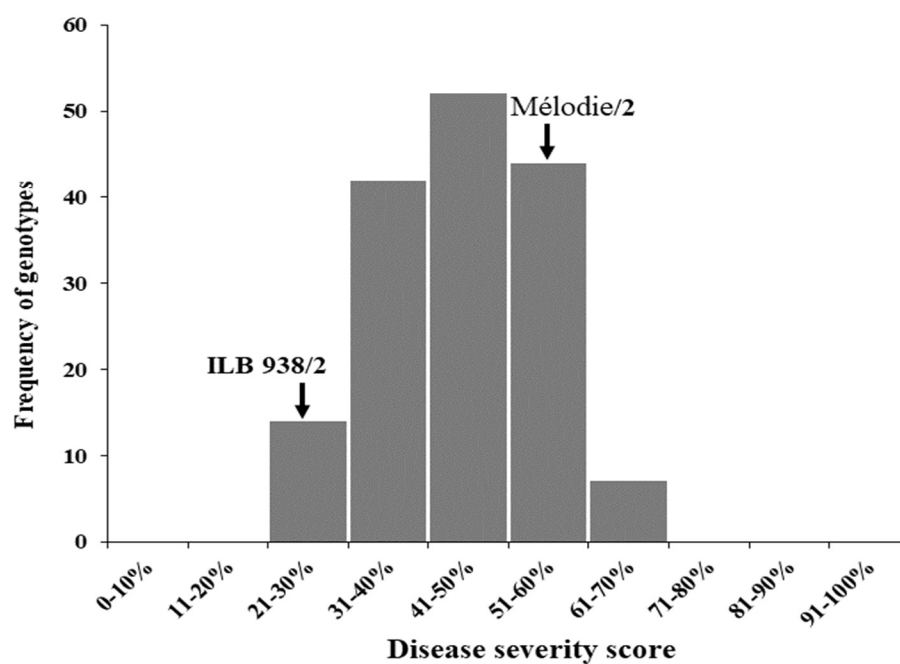
Figure S1. Genetic linkage map of cross Mélodie/2 × ILB 938/2 at F8.