

ORIGINAL ARTICLE

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Genetic characterization of downy mildew resistance from the hop (*Humulus lupulus* L.) line USDA 64035M

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Abstract

Downy mildew (caused by *Pseudoperonospora humuli*) resistance (DMR) will facilitate the success of hop (*Humulus lupulus* L.) production in humid, temperate climates. However, DMR in hop has a narrow genetic base, and its genetic architecture is poorly understood. In this study, we characterized the genetic control of DMR using a biparental mapping population derived from a cross between a female, the susceptible cultivar ‘Comet,’ and a male plant, the resistant line USDA 64035M. Resistance was evaluated after inoculation with the downy mildew pathogen as disease severity and percent leaf area with sporulating lesions under greenhouse conditions. Significant (p value < 0.01) genotypic differences were observed for the traits. Broad-sense heritability was 0.33 for disease severity and 0.72 for percent leaf area with sporulating lesions. Genome–phenome association between 4090 markers and 274 individuals identified three significant markers for disease severity and five for percent leaf area with sporulating lesions. Our study demonstrated that DMR was under polygenic control with small effect loci in this population. In addition, a pleiotropic locus on chromosome two was found to be associated with both disease severity and percent leaf area with sporulating lesions. Identifying the gene(s) underlying such a pleiotropic locus can help improve our understanding of hop response to pathogen infection.

1 | INTRODUCTION

Hop (*Humulus lupulus* L.) is a dioecious ($2n = 18+XX, XY$) crop species whose female inflorescence or cone is an important source of bittering, flavoring, and preserving agents for

Abbreviations: AES, additive effect size; BLUP, best linear unbiased prediction; CIM, composite interval mapping; DM, downy mildew; DMR, downy mildew resistance; GBS, genotyping-by-sequencing; LD, linkage disequilibrium; MAF, minor allele frequency; QTL, quantitative trait locus (or loci); SNPs, single nucleotide polymorphisms; STWRG, stepwise regression.

beer brewing. Hop cultivation often takes place in humid, temperate regions that are often characterized by the prevalence of the disease downy mildew (DM) (Henning et al., 2016; Royle & Krehmiller, 1981). DM is caused by *Pseudoperonospora humuli*, an obligate biotrophic oomycete (Wilson, 1914). DM causes extensive reduction in hop cone quality and overall yield (Purayannur et al., 2021; Royle & Krehmiller, 1981). The disease can manifest in several ways, including lesions on foliage and floral tissue, systemic infection of shoots, and rotting of the perennial crown and root system. The pathogen overwinters in the roots and crowns and appears

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in the spring on newly emerged shoots. The infected shoots, called basal spikes, are characterized by stunted growth and pale, downcurved leaves with a silvery upper surface (Neve, 1991; Purayannur et al., 2021). Leaf infection signs and symptoms include dense sporulation on abaxial leaf surface and chlorotic lesions (Purayannur et al., 2021).

Integrated DM management strategies have been suggested to mitigate the prevalence of DM and its devastating impact on hop production (Bordelon et al., 2018). Notwithstanding, this management approach adds to the overhead cost of production and at the same time pesticide(s) use in hop production may result in regulatory challenges during exportation. Cultivation of disease-resistant varieties is the most effective management strategy; however, there are very few cultivars that have high levels of resistance to DM (Woods & Gent, 2016), and no widely grown cultivars have complete resistance (Purayannur et al., 2021). With regard to the pathogenicity of *P. humuli* in hop, there has been no reported occurrence of any form of pathogenic variation among isolates associated with host genotype or geographic region (Purayannur et al., 2021; Royle & Krehmiller, 1981). *Pseudoperonospora humuli* has limited genetic diversity worldwide, consistent with clonal reproduction or a high degree of selfing due to homothallism (Gent et al., 2017, 2019; Kitner et al., 2021; Summers et al., 2015; Wallace & Quesada-Ocampo, 2017). Earlier studies have suggested that DM resistance (DMR) is under polygenic control (multiple genes with small effects) in hop (Henning et al., 2015; Parker, 2007). DMR, once developed, has been stable across continents even after decades of cultivation of the same cultivars (Purayannur et al., 2021; Royle & Krehmiller 1981).

Breeding for DMR in hop has been challenging. There is a narrow genetic base for DMR, as known resistance sources are limited to European gene pools (Purayannur et al., 2021). However, resistant European hop varieties often lack the unique aroma and flavor characteristics demanded by craft brewers in North America (Woods & Gent, 2016). There is a phenotypic correlation between the foliar phase and systemic shoot infection phase of the disease, but tolerance to crown rot is not necessarily correlated with resistance to the foliar phase of the disease (Purayannur et al., 2021). Introgression of disease resistance from wild relatives has been employed in other dioecious species like asparagus (Garcia et al., 2021; Nothnagel et al., 2022) and appears promising in hop. However, germplasm enhancement by introgressions from wild relatives using classical breeding methodologies takes a considerable amount of time. In hop, DMR has been stable, and race-specific resistance is currently not known (Purayannur et al., 2021). Nonetheless, novel approaches of expedited identification and introgression of disease resistance are required.

Marker-assisted breeding (MAB) has facilitated disease resistance introgression using marker-assisted selection

Core Ideas

- Downy mildew resistance (DMR) derived from 64035M is under polygenic control.
- Small effect and pleiotropic loci underlie DMR in this population.
- Biparental mapping is limited in both power and genetic diversity for the characterization of DMR in hop.
- Extensive linkage disequilibrium limits mapping resolution in our biparental population.

(MAS) for bacterial blight, blast, and sheath blight diseases in rice (*Oryza sativa* L.) (Khan et al., 2018; Ramalingam et al., 2020; Tanweer et al., 2015), and DMR in grapes (*Vitis vinifera* L.) (Saifert et al., 2018). Despite progress in MAB in major crops, there are limited studies on both MAS and genomic selection (GS) targeting improved disease resistance in hop. The successful implementation of MAS for disease resistance is dependent on knowledge of the genetic architecture of host disease resistance. Studies have shown that MAS is most suitable for vertical resistance, which is often monogenic or oligogenic, while GS might be more suitable for quantitative or horizontal disease resistance (Miedaner & Korzun, 2012; Olatoye et al., 2019). Therefore, deciphering the genetic architecture of host resistance to a particular pathogen is essential for informed decision-making in MAB.

The recent development of a hop draft genome (Padgitt-Cobb et al., 2022) has paved the way for the applications of functional and quantitative genetics research to improve our understanding of the genetic architecture of agronomically important traits. Henning et al. (2015, 2016) used genotyping-by-sequencing (GBS) datasets to dissect the genetic basis of DMR in hop. Their results supported the polygenic genetic architecture that was previously suggested. In addition, using a combination of metabolomics and genome-wide association studies (GWASs), Feiner et al. (2021) showed that DMR is mediated by pre-formed metabolites. Their GWAS results identified loci that were colocalized with genes involved in the phenylpropanoid pathway. These studies were based on biparental mapping populations with sample sizes < 200. It is unclear whether the loci identified previously are familyspecific or more broadly predictive of reaction to DM infection. In this study, our objective was to characterize the genetic architecture of percent leaf area with sporulating lesions and disease severity as indicators of DMR in hop using a large biparental mapping population ($n = 284$). We evaluated resistance to the foliar phase of DM under greenhouse conditions and report on genome-phenome mapping using composite interval mapping (CIM) and stepwise regression.

2 | MATERIALS AND METHODS

2.1 | Plant material

A biparental mapping population was developed from a cross between a DM-susceptible female hop ('Comet') (Zimmermann et al., 1975) and a DM-resistant male hop (USDA 64035M) (Haunold et al., 1985). USDA 64035M was developed by mass selecting for DM resistance in both field and greenhouse environments among open-pollinated seedlings from a DM-resistant female line (7K491) (Haunold et al., 1985). The female line (7K491) originated from the German Hop Research Institute in West Germany (Haunold et al., 1985), and the open-pollinated seeds were collected at Wye College in England before they were transferred to Corvallis, OR, for selection (Haunold et al., 1985). The resulting pseudo F_2 population from this cross was grown at 18–25 °C in a replicated block design in a greenhouse located in Corvallis, OR, from March to November in 2019 and 2020. In this study, our pseudo F_2 population was derived from controlled crosses between two parents as suggested by Fehr (1987) for highly heterozygous species. In heterozygous species, parents are highly heterozygous at many loci due to repeated outcrossing, and progenies from such crosses are not true F_1 s but pseudo F_2 s (Allard, 1999). This approach has been used for both phenotypic evaluation and genetic mapping in several heterozygous crop species like cassava (Aravanopoulos, 2010), mulberry (Banerjee et al., 2012), and switchgrass (Taylor et al., 2018). For each genotype, at least two independent inoculations (replications) of 4- to 5-week-old plants were conducted over time.

In each inoculation, the abaxial sides of leaves from at least three plants of each genotype were sprayed using a Preval sprayer (Nakoma Products) unit until they were saturated just before run-off with 50000 sporangia ml^{-1} of a heterogeneous population of *P. humuli* isolates derived from breeding plots in Oregon. As stated previously, *P. humuli* displays limited genetic diversity in the United States and worldwide in studied populations, and pathogenic variation associated with races, host genotype, and geographic region is unknown in *P. humuli*. Therefore, the mix of isolates used should be generally predictive of other isolates of the pathogen. The inoculum was maintained on infected leaves at –80 °C for long-term storage and revived and increased on the cultivar 'Pacific Gem' when needed for experiments using standard methods (Purayannur et al., 2020). Following inoculation, the plants were deprived from light and kept at 90%–100% relative humidity for 24 h. After 24 h, plants were returned to normal greenhouse conditions. Sporulation was induced 5 days postinoculation (dpi) by depriving the plants of light and holding them at 90%–100% humidity overnight for 12–16 h, temperature dependent. Disease severity and percent leaf area with sporulating lesions were estimated on the abaxial surface of the four youngest, fully expanded leaves from

each plant (nodes 3 and 4). Assessments of disease severity and percent leaf area with sporulating lesions were estimated visually without magnification. A highly susceptible cultivar (Pacific Gem) was included as a positive control in every experiment to ensure that the inoculum was infective and conditions were permissible for infection. Noninoculated controls ('Symphony') were also included to ensure that DM was due to the inoculation and not pre-existing on plants.

2.2 | Phenotypic analysis

Analysis of the phenotypic data was performed using both *lm* function in R (RStudio Team (2019) and *lmer* function in the *lme4* R package (Bates et al., 2017). A best linear unbiased prediction (BLUP) model was fitted using the *lmer* function described as follows:

$$y_{ijlrs} = \mu + \delta_i + \theta_j + \phi_{il} + \varphi_r + \Phi_{is} + e_{ijlrs}$$

where y is the vector of phenotypic trait values indexed on the i th genotype, j th level of disease on the positive control plants, on the l th inoculation date, in the r th run of an experiment, of the s th replicate plant. μ is the grand mean (intercept), δ_i is the term for genotypes, θ_j is the term for checks, ϕ_{il} is the interaction term for genotype and inoculation date, φ_r is the term for run nested within inoculation date, Φ_{is} the interaction term for genotypes and replicate plant, and e is the residual. Terms were fitted as random effects in order to obtain BLUPs and variance components used for estimating heritability. The BLUPs of the trait values were used to plot the distribution of phenotypic trait values and estimate the phenotypic correlation between the two traits using *ggscatter* function from the *ggpubr* R package (Kassambara, 2020). The variance components obtained from BLUP estimation were used to estimate the broad-sense heritability (Holland et al., 2010; Rife et al., 2019) as follows:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{gb}^2}{b} + \frac{\sigma_e^2}{b}}$$

where σ_g^2 is the genotypic variance, σ_{gb}^2 is the genotype-block interaction variance, σ_e^2 is the residual error variance, and b is the number of replicate blocks (i.e., greenhouse benches).

2.3 | Genetic analysis

The biparental population (parents and offspring) was raised under glasshouse conditions for tissue collection for DNA extraction. An adapted version of the Qiagen DNAeasy DNA extraction protocol (Miniextraction kits; Qiagen Incorporated) was implemented using young hop leaves

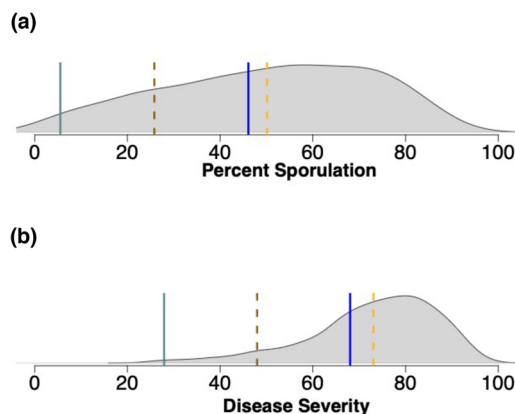


FIGURE 1 Asymmetric distribution of trait values. Phenotypic distribution of line means in the hop biparental population for (a) percent area leaf covered in sporulating downy mildew lesions (percent sporulation) and (b) disease severity caused by *Pseudoperonospora humuli*. Solid lines represent parental trait values for female parent (blue) and male parent (gray). Broken lines show mean trait values for both parents (brown) and offspring (yellow)

(Henning et al., 2016). DNA samples were sent to the Oregon State University Center for Quantitative Life Sciences Core Facility for GBS library development using ApeKI restriction enzyme (Elshire et al., 2011). Sequencing was performed using an Illumina HiSeq 3000. SNP-calling was performed using TASSEL v5.0 pipeline as described by Padgitt-Cobb et al. (2022) using the Dovetail Hi-C assembled “Cascade” genome used as reference genome. Postfiltering of markers, tag SNP identification, and development of the genetic map were described by Padgitt-Cobb et al. (2022). The resulting genetic data used for this study were made up of 4090 SNP markers across 10 chromosomes.

Linkage disequilibrium (LD) decay analysis for the population was conducted using PopLDdecay software implemented in the Linux terminal (Zhang et al., 2019). Genome–phenome mapping was conducted using both quantitative trait locus (QTL) mapping R-package *rqtl* (Broman, 2010) and stepwise regression QTL mapping approach implemented in TASSEL 5.0 software (Bradbury et al., 2007). First, the genomic data in genetic distance (cM) were used to perform CIM using the *cim* function in the *rqtl* package. The *cim* function parameters were specified accordingly with five marker covariates, 10 cM window size, the Haley–Knott regression method, 0.0001 error probability, and the Haldane mapping function. Second, the genomic data in physical distance were used to perform stepwise regression. The stepwise method works by performing both forward inclusion, refitting the model to ensure all variables entered remain significant, and backward elimination steps. This model facilitates efficient QTL detection by accounting for the effect of major effect loci and reducing collinearity among markers. The entry and exit *p* value parameters were set at 0.001 and 0.002, respectively.

In addition, a permutation of 100 was used during QTL analysis. Furthermore, the additive effect size of each significant marker identified using both methods was estimated as the difference between the mean of the two homozygous classes of each QTL divided by two, while the proportion of phenotypic variation explained by each QTL was estimated as described in the following equation:

$$y_{ij} = \mu + \gamma_i + \varepsilon_{ij}$$

where y_{ij} is the phenotype; γ_i is the term for a significant marker coded as 0, 1, and 2, where 0 and 2 are for the homozygous classes and 1 is for the heterozygous class; and ε_{ij} is the residual. A test of pleiotropy between a significant marker in common between the two traits was conducted using the *pleio.q.sequential* and *pleio.q.test* functions in the *pleio* R-package (Schaid & Sinnwell, 2018). The *pval.threshold* parameter for sequential tests of pleiotropy in *pleio.q.sequential* function was set to 0.05. Data were visualized using custom R scripts.

3 | RESULTS AND DISCUSSION

3.1 | Significant genotypic variation underlies DMR

Phenotypic measurements were taken as disease severity and percent leaf area with sporulating lesions across 274 genotypes (including biparental genotypes, parents, and check varieties). The biparental population showed significant genotypic differences ($p < 0.01$) for both disease severity and percent sporulation. Broad sense heritability was moderate for disease severity (0.33) and high for leaf area with sporulating lesions (0.72). Similar levels of heritability estimates were reported in previous studies. For instance, Feiner et al. (2021) reported a high H^2 of 0.81 for leaf area with sporulating lesions under greenhouse conditions. In another study, DMR showed moderate heritability (H^2 range from 0.38 to 0.57) under field conditions, where resistance was measured as the proportion of shoots with systemic DM relative to the total shoots per hill (Henning et al., 2015). Regardless of the approach for DMR evaluation, our findings corroborate previous studies that DMR heritability ranges from moderate to high for the foliar phase of the disease.

3.2 | Asymmetric phenotypic variation for DMR

Phenotypic distribution showed asymmetric transgressive variation, though disease severity was found to be more asymmetric than leaf area with sporulating lesions (Figure 1a,b).

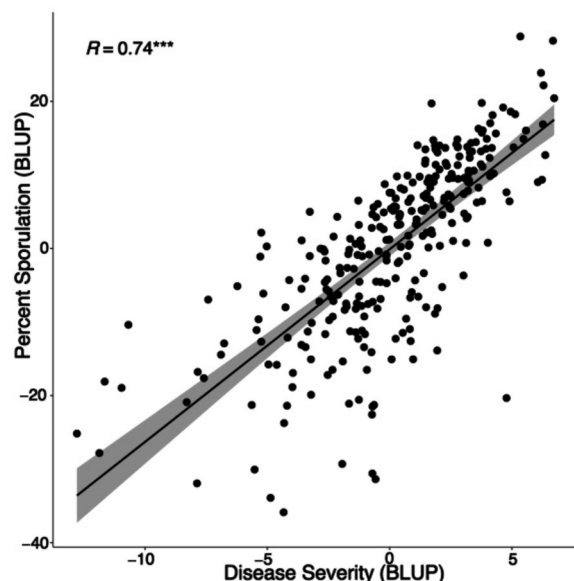


FIGURE 2 Positive relationship between downy mildew traits in the progeny of a biparental hop mapping population. Phenotypic correlation between best linear unbiased prediction (BLUP) for percent area leaf covered in sporulating downy mildew lesions (percent sporulation [y axis] and disease severity [x axis])

The proportion of leaf area with sporulating lesions for the resistant male (USDA 64035M) parent was 6% and 46% for the female parent ('Comet'). Disease severity for the male parent was 28% and 68% for the female parent. Both traits exhibited a pronounced shift in the progeny's mean trait value from their parental trait mean values (percent sporulation parental mean values = 26%, progeny mean value = 50%; disease severity parental mean values = 48%, progeny mean value = 73%). This showed a departure from additivity, signifying possible nonadditive gene(s) action underlying phenotypic variation in DMR in this population. Nonadditive causes of genetic variation like epistasis have been suggested to be reflected by asymmetric transgressive variation (Falconer & Mackay, 1996; Gaertner et al., 2012; Rieseberg et al., 1999). Furthermore, disease severity was positively correlated with percent sporulation ($R = 0.74$; p value < 0.0001 ; Figure 2). High phenotypic correlation or relationship between two traits often suggests genetic linkage or pleiotropy (Lynch & Walsh, 1998).

3.3 | Biparental population characterized by long distance genome-wide LD

Genome-wide LD decay rate decreased to 8% (0.43) of its initial value (0.47) at 1 Mbp (Figure 3). A long distance LD decay pattern was observed (r^2 plateauing at 0.42 around 30 Mbp), which is characteristic of biparental populations as a result of limited recombination, thus leading to low

mapping resolution as QTLs are localized to large chromosomal regions (Myles et al., 2009).

3.4 | Small effects and pleiotropic loci underlie the genetic architecture of DMR

Significant associations were found for both disease severity and percent leaf area with sporulating lesions using both CIM and stepwise regression QTL mapping approaches (Table 1, Supporting Information 1). In CIM, three QTL were detected for disease severity on chromosomes 1, 2, and 5, whereas two QTL were detected for percent leaf area with sporulating lesions on chromosomes 2 and 7, respectively (Table 1, Figure 4). In the stepwise method, three SNPs were associated with disease severity on chromosomes 1 and 2, whereas five SNPs were associated with percent sporulation on chromosomes 1, 2, 3, 4, and 7, respectively (Table 1, Figure 4). A test for pleiotropy showed that one of the identified loci (S2_380501233; proportion of phenotypic variation explained [PVE] = 5.4% for disease severity and 6% for percent leaf area with sporulating lesions; minor allele frequency [MAF] = 0.30) was significantly associated with both traits, suggesting possible pleiotropism. The additive effects of the markers ranged from 0.6% to 5.0% for disease severity and 3.0% to 6.3% for percent leaf area with sporulating lesions. At the same time, the proportion of phenotypic variation explained by the markers ranged from 3.5% to 6.0% for disease severity and from 3.0% to 6.0% for percent leaf area with sporulating lesions (Table 1). Furthermore, the MAF of all QTL ranged from 0.25 to 0.47, indicating that these loci were segregating at moderate to high frequencies in the population.

Our results showed that putative small effect loci underlie DMR in this biparental population. Studies have shown DMR to be under polygenic control, with QTL having small effect sizes (Henning et al., 2015; Parker, 2007; Woods & Gent, 2016). Feiner et al. (2021), using a biparental population of $n = 192$, reported the identification of two major loci associated with DMR; however, no information was provided about the additive effect size or proportion of variation explained by the loci. In addition, the authors did not enumerate in detail the concept of major effect versus small effect in their study. Henning et al. (2016) identified a set of four loci associated with DMR in diseased shoots under field conditions. QTL were reported to explain about 43% of phenotypic variation in a biparental population of $n = 91$. In general, biparental mapping populations are limited in both power and genetic diversity, thus making them inefficient for the characterization of the genetic architecture of complex traits. Most of the DM studies in hop were based on biparental mapping populations with $n < 200$ and generally lack sufficient power to identify small effect loci underlying complex/polygenic traits like DMR. In addition,

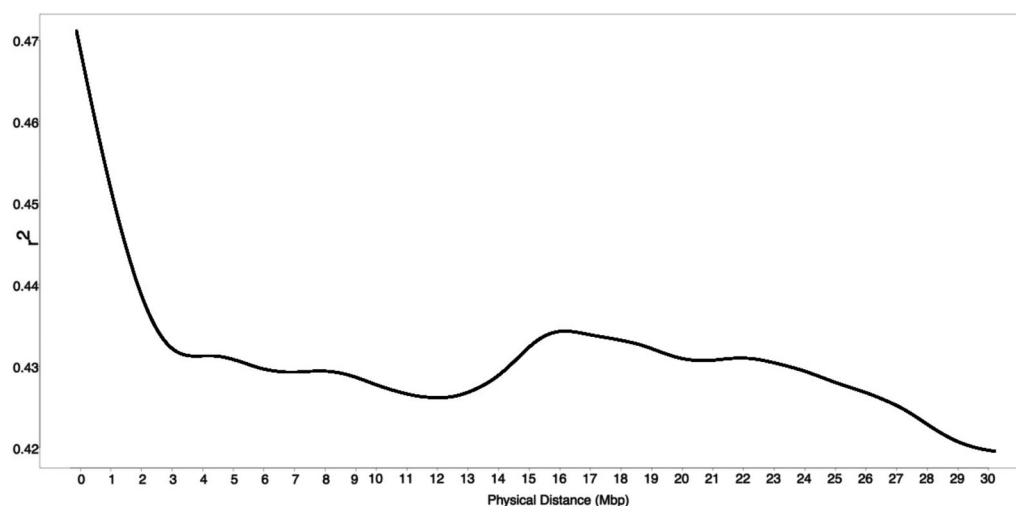


FIGURE 3 Linkage disequilibrium decay in a hop biparental mapping population. The black spline line shows the rate of linkage disequilibrium (r^2 ; y axis) decay across physical distance (x axis) in megabase pairs

TABLE 1 Downy mildew resistance loci in the bi-parental population

Model ^a	Trait ^b	Name	Chr	Physical position	Genetic position	AES (%)	PVE (%)	MAF	LOD/-log (p value)
STWREG	Disease severity	S1_206587467	1	206587467	105.20	4.00	5.30	0.47	2.96e-06 ^c
STWREG	Disease severity	S2_173203877	2	173203877	44.23	3.30	5.40	0.26	1.24e-06 ^c
STWREG	Disease severity	S2_94993080	2	94993080	51.70	4.40	3.50	0.25	3.48e-04 ^c
CIM	Disease severity	S1_2678713	1	2678713	59.66	0.60	6.00	0.29	4.00 ^d
CIM	Disease severity	S2_380501233	2	380501233	47.02	3.00	5.40	0.30	4.00 ^d
CIM	Disease severity	S5_112556980	5	112556980	84.60	5.00	5.10	0.45	4.10 ^d
STWREG	Sporulating lesions	S1_468114451	1	468114451	63.40	5.80	3.00	0.28	3.60e-04 ^c
STWREG	Sporulating lesions	S3_374908394	3	374908394	31.50	6.20	3.20	0.33	2.71e-04 ^c
STWREG	Sporulating lesions	S2_173203877	2	173203877	44.23	6.20	5.00	0.26	1.91e-05 ^c
STWREG	Sporulating lesions	S4_5069239	4	5069239	117.73	5.20	5.00	0.45	3.69e-05 ^c
STWREG	Sporulating lesions	S7_21679410	7	21679410	25.36	3.60	3.00	0.33	2.41e-04 ^c
CIM	Sporulating lesions	S2_380501233	2	380501233	47.02	3.00	6.00	0.30	4.90 ^d
CIM	Sporulating lesions	S7_18877520	7	18877520	88.60	6.30	5.00	0.37	3.60 ^d

Abbreviations: AES, additive effect size; Chr, chromosome; MAF, minor allele frequency; LOD, logarithm of the odds; PVE, proportion of variation explained.

^aModel: Stepwise regression (STWREG); composite interval mapping (CIM).

^bSporulating lesions: Percent leaf area with sporulating lesions.

^cLOD, logarithm of the odds values.

^dp values.

interpretations of QTL effect sizes are often not reliable in the context of the Beavis effect (Xu, 2003) due to the tendencies of QTL effect sizes to be inflated as a result of small population sizes.

Our study is also susceptible to such inflated effect sizes because of our small ($n = 284$) population size, though the population size is larger compared to other DMR studies reported in hop. Population sizes of $n > 1000$ are needed for robust and accurate estimation of QTL effect size (King & Long, 2017). Furthermore, our study only identified a few

loci associated with DMR comparable to the same number of loci identified by Henning et al. (2015) under greenhouse conditions. This may be attributed to limited genetic diversity segregating within the two parents used for population development. Breeding for DMR in hop is plagued with a narrow genetic base because resistance sources are limited to certain genetic pools from the United Kingdom and continental Europe (Woods & Gent, 2016). It is our recommendation that large diversity association panels and multiparental mapping populations involving diverse founders be developed to

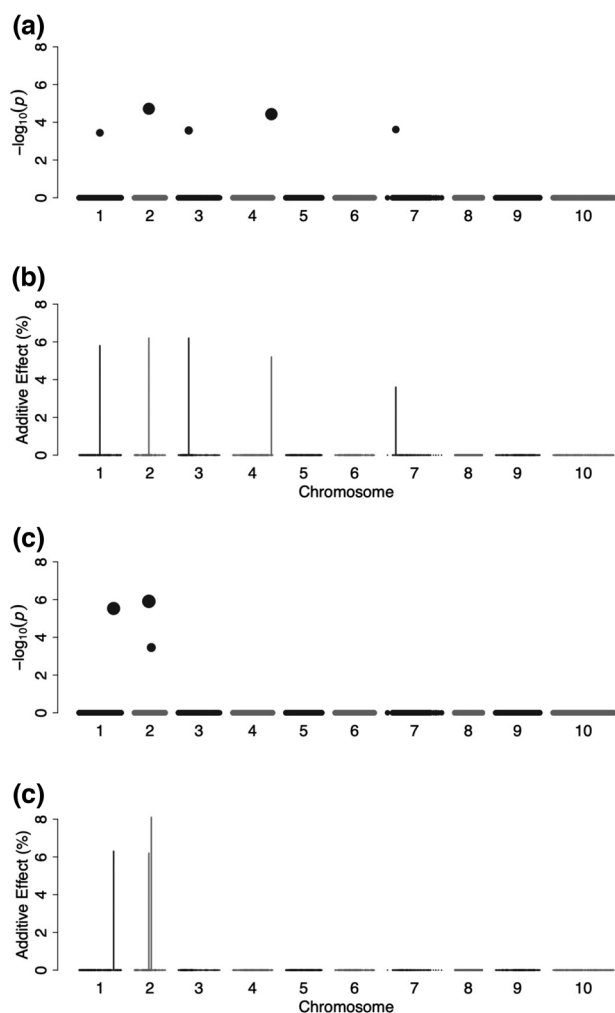


FIGURE 4 Stepwise regression identified loci associated with downy mildew resistance. Quantitative trait locus (QTL) plots showing loci detected using stepwise regression for (a) percent leaf area with sporulating lesions and (c) disease severity. Each solid circle represents each significant genetic locus identified, and their sizes represent the proportion of phenotypic variation each locus explained relative to other loci. Effect size plot for (b) percent leaf area with sporulating lesions and (d) disease severity. Each vertical bar shows the additive effect size of each locus in the QTL plot. The positions of the vertical bars in additive effect plots are symmetric to the positions of the same locus in the QTL plots

efficiently characterize DMR and other important complex agronomic traits in hop. However, establishing large mapping populations for increased power will be challenging in hop due to the space and infrastructure requirements of the species and the labor intensity associated with phenotyping. The development of high-throughput phenotyping platforms will be beneficial in hop to address challenges associated with phenotyping labor requirements.

A locus on Chromosome 2 on both genetic and physical maps was found to be associated with both disease severity and percent leaf area with sporulating lesions for both

CIM (S2_380501233; MAF = 0.30) and stepwise regression (S2_173203877; MAF = 0.26) mapping methods. This marker explained an average proportion of phenotypic variation of 5.5% for both traits. The association of this marker with the two traits corroborated their high phenotypic correlation with each other ($r = 0.74$). Further analysis using a statistical method for testing genetic pleiotropy developed by Schaid and Sinnwell (2018) for the marker associated with disease severity and percent sporulation showed evidence of pleiotropy (p value < 0.0001). Identification of such a pleiotropic marker will be beneficial for elucidating the genetic basis of DMR and contribute to better understanding of the host response to the pathogen.

In general, this study has cautiously shown that small effect and pleiotropic loci underscore the genetic architecture of DMR in hop in 64035M, a line that has played an important role in cultivar development in hop (e.g., Henning & Haunold, 1999a, 1999b, 2012; Henning et al., 2018, 2021). There are two major caveats associated with our study. First, genetic variation for DMR is limited to genetic diversity available between the two parents used to generate the pseudo F_2 population. Second, the extensive patterns of genome-wide LD that were observed suggest that our QTL span large chromosomal regions. Genome-wide association mapping populations and multiparental mapping populations offer increased genetic diversity, improved mapping resolution, and power for QTL identification (Altendorf et al., 2021; Bouchet et al., 2017; Myles et al., 2009; Olatoye et al., 2020). Thus, the development of larger diversity panels and multi-parental populations are needed for a comprehensive understanding of the genetic architecture and basis of DMR in hop. Given the polygenic nature of the genetic architecture of DMR, classical breeding and marker-assisted selection approaches will be challenging. Thus, studies examining the potential of GS for hop improvement will be needed to explore the benefits of the approach. Our findings in this study are based on DMR to leaf infection under greenhouse conditions. Further studies are essential to validate findings under field conditions where DMR can be in response to either leaf, shoot, or crown infection. Future work should also seek to evaluate the resistance in 64035M in different genetic backgrounds.

AUTHOR CONTRIBUTIONS

Michele Wiseman: Conceptualization; data curation; investigation; methodology; writing – review & editing. **David H. Gent:** Conceptualization; funding acquisition; investigation; methodology; project administration; resources; supervision; writing – review & editing. **John A. Henning:** Conceptualization; funding acquisition; investigation; methodology; project administration; resources; supervision; writing – review & editing. **Kayla R. Altendorf:** Conceptualization; investigation; resources; supervision; writing – original draft; writing – review & editing.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The dataset and scripts are available on github https://github.com/marcbios/Hops_DM_QTL_Mapping

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