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# Precision QTL mapping of downy mildew resistance in hop (*Humulus lupulus* L.)

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**Abstract** Hop downy mildew (DM) is an obligate parasite causing severe losses in hop if not controlled. Resistance to this pathogen is a primary goal for hop breeding programs. The objective of this study was to identify QTLs linked to DM resistance. Next-generation-sequencing was performed on a mapping population segregating for DM resistance levels. Cloned plants were grown in a RCBD with three replicates under three environments: greenhouse (GH), field plots in Oregon (OR), Corvallis field plots in Washington (WA), Yakima). The linkage map of 3,341 SNP markers was determined with a four-stage process using Rqtl, TMAP, Joinmap v 4.0 and MERGEMAP. QTL analysis was performed using JMP Genomics and TASSEL 5.0. SNP markers were distributed across 11 linkage groups (LGs) with an average

distance between markers of 0.2 cM and total distance of 745.9 cM. QTLs for all three environments were identified using multiple interval mapping. Overall heritability across the three environments varied from  $h^2 = 0.38$  (GH) to 0.57 (OR). A total of 22 QTLs across 8 LG were identified for DM resistance: 5 identified from OR field data, 12 using WA data and five from GH DM data. No epistasis was observed. This study points out the complexity of genetic control of DM resistance in hop and identifies several markers that can be potentially be used to select for DM resistance in hop. It also provides the first linkage map suitable for genome sequencing due to the high density of SNP markers.

**Keywords** Downy mildew · Genetic map · Hop · *Humulus* · *Pseudoperonospora* · QTL mapping · SNPs

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## Introduction

Hop is a perennial dioecious plant species used principally in beer brewing as a flavoring and bittering agent. The female inflorescence is the harvested product for brewing. Production takes place in latitudes greater than 35° in both the Northern and Southern Hemispheres due to strong photoperiodism requirements for flowering. Regions of production in the Northern Hemisphere are also conducive for several plant diseases that have the potential for

significant effects upon harvest yields as well as quality (Gent et al. 2010). One such disease, hop downy mildew [DM; caused by: *Pseudoperonospora humuli* (Miy. et Tak.) Wils], causes both local and systemic infections resulting in significant yield losses if not controlled (Gent et al. 2010). Typical control measures include agronomic practices, such as stem pruning after initial spring growth, and chemical control throughout the growing season. The disease can overwinter in meristem tissue (local) as well as rhizomatous tissue (systemic) underground. Chemical control is effective for some hop varieties but others are so susceptible that they cannot be economically grown in regions with high incidence of the disease. The solution for these varieties is the development of replacement varieties with DM resistance (Beranek 1997).

Selection for resistance to DM is difficult (Beranek 1997; Parker 2007). There is speculation that DM resistance is quantitatively controlled (Parker 2007). If so, it reasons that QTL studies could potentially point out genomic regions that breeding studies should focus upon to increase accuracy of selection.

QTL studies have been reported for hop by several researchers (Koie et al. 2005; Cerenak et al. 2006, 2009; Henning et al. 2011; Patzak et al. 2012; McAdam et al. 2013; Jakse et al. 2013) but only a few (Henning et al. 2011; Jakse et al. 2013) have focused solely upon disease resistance. Henning reported on the identification of a QTL associated with susceptibility to hop powdery mildew (caused by: *Podosphaera macularis*). In this case, the presence of the susceptibility QTL overrode any QTLs conferring resistance. Inheritance studies on powdery mildew resistance (Henning unpublished data) as well as other reports (Neve 1991) suggested that powdery mildew resistance was primarily based upon single genic or qualitative resistance. Jakse et al. (2013) identified a single QTL on linkage group (LG) “3” associated with Verticillium wilt that explained more than 25 % of the phenotypic variance for this trait. There were approximately 200 markers used to create this linkage map.

In contrast, Parker (2007) identified 43 AFLP markers showing significant association with resistance to hop DM suggesting quantitative control over expression of resistance. In this study, 99 different hop varieties and experimental accessions were evaluated in a single environment for hop DM resistance based

upon percent leaf infection. Narrow sense heritability estimates for resistance to DM were reported as  $h^2 = 0.49$ . The author suggests that multiple regions of the genome, dominance, environment and/or epistatic effects may play a large role in the low heritability for this trait. Nevertheless, the genetic basis for resistance remains unsolved.

Next generation sequencing (NGS) technology and the identification of SNP markers remains the best means towards elucidating interactions between plant and pathogen. SNP markers have been shown to be the best markers for elucidating whole genome plant response to environmental triggers (Close et al. 2009). QTL studies based upon SNP markers have not been reported in hop. Previous QTL studies in hop have been limited in marker density due to use of older marker technology such as AFLP, DArT, RAPD or SSR (Seefelder et al. 2000; Koie et al. 2005; Cerenak et al. 2006; Patzak et al. 2002, 2012; Henning et al. 2011; Jakse et al. 2013; McAdam et al. 2013). NGS using massively parallel sequencing and multiplexing of mapping populations have enabled rapid and extensive identification of SNP markers for use in development of linkage maps. Unfortunately, errors in genotyping and missing data have resulted in incorrect marker order and expansion of distances between markers (Hackett and Broadfoot 2003; Close et al. 2009). In these cases, use of multiple linkage mapping programs have been utilized to arrive at a consensus map (Close et al. 2009).

This study was undertaken to determine the genetic control of resistance to hop DM. The objectives were to develop a high-density genetic map using SNP markers and to identify QTLs linked to resistance to hop DM.

## Materials and methods

### Plant material

An initial mapping population of 125 genotypes was developed for the study. The mapping population resulted from the cross between the DM-resistant ‘Teamaker’ (Henning et al. 2008) and the DM susceptible ‘USDA 21422M’. Losses due to disease and sequencing costs resulted in the use of data from 91 genotypes. Poor or incomplete sequencing results

reduced the final number of genotypes used in QTL studies to 83. Offspring and parents were cloned and planted into fields in Oregon (OR; Corvallis) and Washington (WA; Yakima). Field experiments were arranged in randomized complete block designs with three replications. Plants were not treated with fungicides after planting to allow natural spread of the disease. Supplemental inoculation of plants was conducted to standardize inoculum pressure and ensure disease development. Plants were inoculated by spraying the foliage with a suspension of sporangia (approximately 50,000/mL of water) derived from an OR or WA population of the pathogen growing on the hop variety ‘Pacific Gem’ under optimum growth chamber conditions. In WA, inoculations were conducted in September 2010, a total of three times during May and June 2011, and once during May 2012. In OR, inoculations were conducted a total of three times during April and May 2011 and once during May 2012. In WA, overhead irrigation was provided immediately after inoculation and at least weekly thereafter to promote DM. Overhead irrigation was unnecessary to ensure adequate levels of DM in OR. Normal production practices were followed for fertilization, weed control, stringing and training except, watering was increased to encourage disease intensity in WA.

Disease scoring consisted of counting the number of diseased shoots per plant and the total number of shoots per hill plant to calculate the percentage of shoots with DM. Dead or missing genotypes were not included in the final analysis if uniformly missing across all replications within a location. Previous research (Parker 2007) demonstrated that number of diseased shoots per plant was correlated with percent diseased leaf area and could be used for estimating resistance levels. Disease scores for 2011 at the WA plots were inconsistent due to difficulties in establishing disease. As a result, only data from 2012 were used in the analysis. Significant loss of plants under continuous high disease pressure (caused by either *P. macularis* or *P. humuli*) in OR field trials during 2012 necessitated use of data from 2011. Scores were averaged over sampling dates within a year.

The same mapping population was grown in a replicated block design in a greenhouse (GH) located in Corvallis, OR during 2010 and 2011. For each genotype, three independent inoculations (replications) were conducted over time. In each inoculation,

at least three plants of each genotype were inoculated using an OR population of the pathogen and a similar titer of sporangia. The percent leaf area with sporulating DM lesions were estimated on the four youngest, fully expanded leaves from each plant. Disease severity was estimated visually with the aid of a standard area diagram using a 0–10 scale based on equal intervals, where a rating of 10 corresponds to 100 % of leaf area diseased and 0 corresponds to no lesions. The highly susceptible cultivar Pacific Gem was included as a positive control in every experiment to ensure the inoculum was infective and conditions were permissible for infection.

#### DNA extraction and library preparation

Plant samples representing the mapping population were grown in a GH in Corvallis, OR during the summer of 2012. Plant leaf tissue consisting of small juvenile leaves approximately 3–4 cm in diameter were collected in the morning and immediately placed on ice. Tissue samples were kept on ice for the majority of treatment. Fresh tissue was subsequently weighed to obtain approximately 120 mg of leaf tissue per sample. After weighing, leaf tissue was ground in liquid nitrogen using a mortar and pestle. This process was repeated three times to ensure complete breakage of cell wall material and subsequent release of nuclear contents. The frozen plant material was scraped into collection vials for DNA extraction using a modification of the Qiagen Plant DNAeasy Kit (Qiagen, Inc., USA) procedure. Higher levels of RNAase (140 % of the recommended volume) were added to the process to ensure complete breakdown and removal of RNA. The DNA shearing tube step was eliminated as this process tended to create excessive amounts of small fragment DNA. Enzymatic breakdown of cell walls and RNA during the first step was allowed to stay longer under heated conditions (300 % longer) to ensure complete cell lysis as well as RNA destruction.

Sample DNA concentration was determined using a Qubit fluorometer (Life Technologies, Inc., USA). DNA samples were subsequently run on an agarose gel to determine the level of shearing as well as potential RNA impurity. Subsets of DNA samples were cut with *EcoR1* to determine if DNA samples would be sufficiently cut for library preparation. Samples were considered ready for library preparation for NGS once a predominant high molecular weight band and little to

no sheared DNA were observed on the agarose gel during sample screening.

Library preparation for sequencing on an Illumina HiSeq 2000 was performed according to procedures reported by Elshire et al. (2011). Cornell University's Institute for Genomic Diversity performed all library preparation and sequencing. Ninety-five samples on a single plate were utilized for the study. Parents of the bi-parental mapping population were duplicated to obtain higher depth of sequencing. Six of the 91 offspring performed poorly in library preparation and were subsequently dropped from further consideration. Thus, 85 offspring along with the two parents were included in the study. Library preparation consisted of restriction enzyme cutting (*ApeK1*) of high molecular weight DNA, ligation of adapters and bar-codes, followed by bridge PCR amplification of short fragments for pyrosequencing on an Illumina HiSeq 2000 (Elshire et al. 2011). Samples were run on a single lane.

#### Assembly and SNP calling

Raw data were input into TASSEL UNEAK pipeline (Lu et al. 2013) for separation of bar-coded reads into different genotypes followed by the removal of barcodes and adapter sequences as well as restriction enzyme cut regions on the tail ends to produce 64-mer reads for data analyses. Default settings in TASSEL UNEAK pipeline were used for processing raw reads and SNP calls. Filtering of SNPs were based first upon elimination of SNP markers with more than 20 % missing data, then SNP markers being present in 80 % of the offspring, followed by removal of any markers not found in both parents. Using these criteria, 9,081 SNP markers were filtered out from 120,435 total SNP markers. The resulting data were then formatted for direct input into TASSEL as a hapmap flat text file.

#### Linkage mapping

Genetic mapping was done following the pseudo-testcross mapping strategy outlined by Grattapaglia and Sederoff (1994) on an initial group of filtered SNPs consisting of 9,081 SNPs. SNP markers were separated into male and female groups using parental allelic make-up as guides. Markers were subsequently separated out into three segregation groups ( $ab \times aa$ ,  $ab \times ab$  and  $bb \times ab$ ) based upon parental genotype.

These six groups of markers (male and female groups of the three segregation types) were subsequently filtered individually for unusual segregation, extreme genetic disequilibrium and initial assignment into LGs ( $r = 0.12$ ;  $LOD = 10$ ) using the R v 2.15.1 (R Core Team 2012) package 'qtl' v 1.31-9 (Broman et al. 2003). After filtering, checking for correct allele assignment and initial assignment into individual LGs, the data were saved as MapMaker (Lander et al. 1987) formatted files for use in TMAP. Initial ordering of the resulting markers within each of the six groups was performed using TMAP (Cartwright et al. 2007; default settings). Markers not placed into an initial order on a LG were discarded. The three groups of initially ordered markers, within either male or female groups, were then bulked together to form male and female preliminary maps. The marker sets making up the male and female maps were then reintroduced into Rqtl for a new round of marker filtering, LG assignment and allele switching. Resulting LGs for male and female groups were re-run using TMAP at default settings. For both the male and female linkage maps, individual LGs containing more than 200 markers were broken up into multiple overlapping sets of markers based upon the marker order obtained from TMAP. LGs containing less than 200 markers were mapped as a single linkage set using Joinmap v 4.0 (Van Ooijen 2011). Joinmap v 4.0 was also used on the smaller overlapping subsets of the LGs containing more than 200 markers to obtain precise small overlapping maps of individual subsets. Regression mapping using default settings was used on all Joinmap v 4.0 mapping actions due to its un-inflated marker distances as compared to maximum likelihood (option). For LGs with more than 200 markers, final marker order and distances for both male and female maps was performed using MERGEMAP based upon the overlapping maps obtained from Joinmap v 4.0. Finally, the male and female maps (Supplementary Data) were merged into a consensus map using the same process outlined above. The resulting map (Fig. 1, Supplementary Data) consists of 3,341 markers aligned along 11 LGs.

#### QTL mapping

The resulting consensus map was incorporated into a hapmap containing haplotype data along with a flat file and both imported into JMP Genomics v 11.0 (JMP<sup>®</sup>,

Version 11. SAS Institute, Inc., Cary, NC, 1989–2007). Multiple interval mapping (MIM) was performed using default settings upon averaged phenotypic data (averaged across reps and screening events) from OR and WA field plots and GH samples. Initial QTL models were formulated using interval mapping (IM). Forward search followed by backwards elimination (again with default settings) was subsequently used to identify main effects QTL. Tests for QTL by QTL epistatic effects were then tested.

## Results and discussion

### Disease phenotype

There was no significant interaction between genotype and environment but significant differences were observed between environments ( $p = 0.002$ ) and genotypes ( $p < 0.0001$ ). Average disease score for OR ( $0.105 \pm 0.012$ ) was significantly higher than disease scores for WA ( $0.073 \pm 0.011$ ). Because there were no significant differences between replicates, data were averaged across replicates for use in QTL estimation.

### Linkage mapping

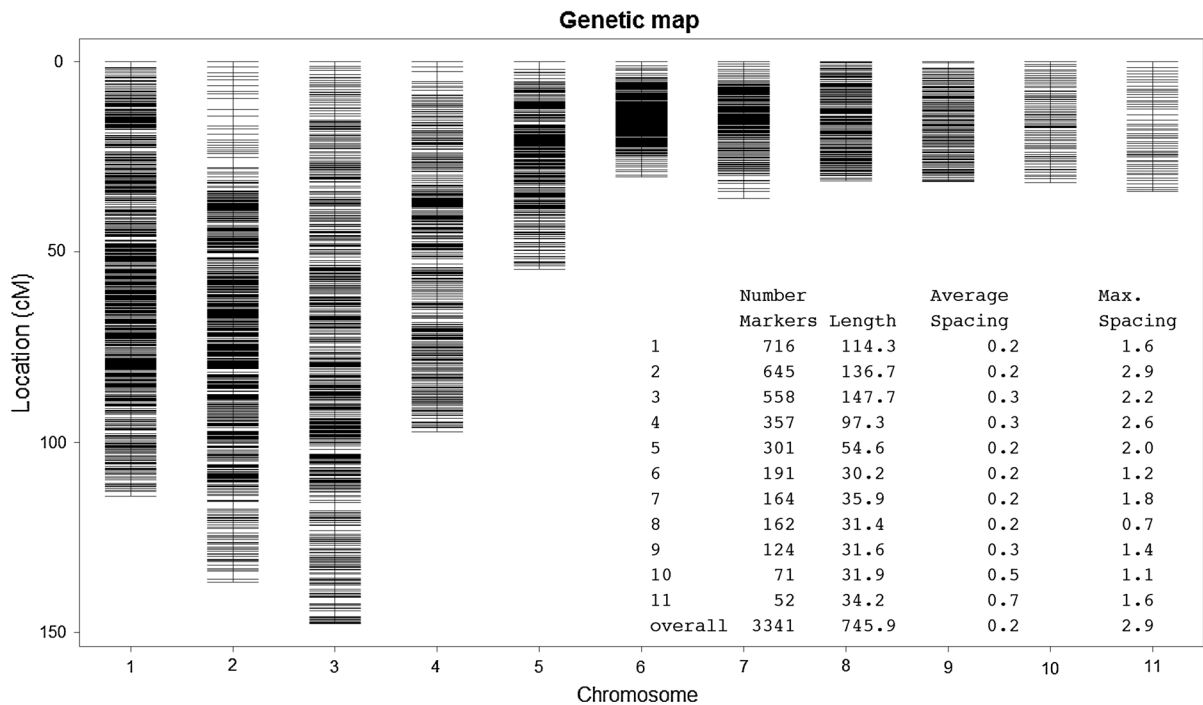
The final linkage map for 21422M (male parent) consisted of 2,420 markers on 11 LGs that spanned 619.55 cM with an average distance between markers of 0.257 cM (Supplementary Data). The final linkage map for ‘Teamaker’ consisted of 2,537 markers arranged on 11 LGs (9 autosomes + X and Y chromosome) covering 545.25 cM with an average distance between markers of 0.22 cM (Supplementary Data). Consensus mapping of the two parental maps resulted in a genetic map consisting of 3,341 markers with an average distance between markers of 0.2 cM (Fig. 1). Average marker distance for each LG varied from 0.2 to 0.7 cM. The longest distance between two markers was 2.9 cM (LG 2); the range of maximum distances for each LG between 0.7 and 2.9 cM. Total genomic distance across all 11 LGs was 745.9 cM. The number of markers per LGs ranged from 52 markers (LG 11) to 716 markers (LG 1).

The most recent linkage maps for hop were reported by McAdam et al. (2013). This map utilized an initial

set of 834 DArT markers along with up to 250 additional markers (AFLP, RAPD, microsatellites). Map construction only utilized a portion of the total available markers for three different linkage maps (337, 286, 189 markers). One observation from these maps is that DArT markers tend to cluster in specific regions with large distances between clusters. This observation was somewhat corroborated by Henning et al. (2011) with use of DArT and AFLP markers. Henning et al. (2011) constructed a consensus map of 326 markers from a mapping population designed to identify QTLs for powdery mildew resistance. The total length of the consensus map was 703 cM for an average distance between markers of 2.2 cM. Unfortunately, most markers were clustered in multiple small regions leaving large regions of the genome without marker placement. The genetic map developed for this study has a similar total length and indeed, would be quite equivalent if LG 11 is not included (711.7 cM).

SNP markers on the other hand appear to not have extensive problems with clustering in widely separated regions of the genome. With an average distance between markers of 0.2 cM in the consensus map it may be possible to use this map for ordering or genomic scaffold orientation as suggested by Ren et al. (2012). In both the male and female maps, average distance between markers was also approximately 0.2 cM. Thus, regardless of the map (male, female or consensus), SNP markers obtained from NGS massively parallel sequencing—given sufficient genome coverage rates—can provide a highly dense genetic map. The development of this consensus map also provides a high-density genetic map that should enable the identification of QTL regions linked to DM with great precision.

This study resulted in the development of a high-density, SNP-based genetic map that will enable several genomic opportunities to proceed. The consensus map reported herein may act as a basic structure from which other markers can be added via bin-mapping protocols (Van Os et al. 2006). It can also be used in conjunction with QTL identification for other traits in other populations when used with the original fasta-formatted file and hapmap flat file used to construct and identify SNPs. Finally, it may be possible to utilize this genetic map as a scaffold alignment for the development of pseudo-chromosomes built from genome assembly scaffolds.



**Fig. 1** Consensus genetic map of the female  $\times$  male ('Teamaker'  $\times$  21422M) cross along with pertinent mapping information

### QTL mapping of DM resistance

QTL mapping using single marker analysis identified numerous markers that were associated ( $p \leq 0.01$ ) with DM (Fig. 1). Marker association with field data from OR identified three markers on LG 1 and an additional marker on LG 2 (Fig. 1). Another marker on LG 3 was significant at  $p \leq 0.05$ . Markers significantly associated with DM resistance ( $p < 0.01$ ) based upon data from WA showed the presence of eight markers on LG 1 and one marker for LG 2. Significantly associated markers appear to be clustered in two regions on LG 1 (Fig. 1) based upon phenotypic data from both OR and WA. Single marker analysis based upon data from the GH paint a different picture than field-based estimates of QTLs (Fig. 1). Over 60 markers from LG 1 appear to be significantly associated with DM with an additional 16 markers spread across 5 LG (LGs 2, 4, 7, 8 and 10).

It's unclear why almost 10-fold number of markers were linked to DM resistance based upon GH data. Several possibilities for this observation exist. Disease screening under GH conditions may increase the accuracy of phenotyping by reducing screening errors

and increasing the power of linkage analysis. Alternatively, the form of phenotype screened under GH conditions differed from field-based observations in that localized infection was observed rather than systemic infection (number of infected shoots). The phenotype observed under field-based observations almost certainly involves systemic resistance (Kinkema et al. 2000), while phenotype in the GH is speculated as being a localized response that perhaps differs in scope. Regardless of actual causative effects for the differential response due to environment, it remains that GH screening of DM resistance appears to be linked to multiple QTLs across several LG when analyzed with single marker analysis (Fig. 2).

Single marker analysis is rarely used in plant breeding settings due to the potential for false positives in analyses based upon a simple t test. Recommendations are that stringent F-tests be utilized with p-levels set below  $p < 0.0001$  (Kim et al. 2009). Furthermore, such tests do not provide information concerning the influence of other QTLs upon the marker in question. Additive, dominance or epistatic effects are also not discernable using these analyses. As a result, most investigators utilize QTL mapping

analyses such as IM (Lander and Botstein 1989), composite IM (Jansen 1994; Zeng 1994) or MIM (Kao et al. 1999). Of the three methods, MIM is the most informative method as it not only provides accurate mapping of single QTL markers, it also provides information on both the additive or dominance effect of each marker as well as the epistatic effects between QTLs.

MIM analysis of markers based upon field data obtained from OR identified five significant QTLs that were located across three LG (Table 1). All major effects (additive and dominance) were significant for all five QTLs with the exception of the additive effect for QTL 2 located on LG 1 at 106.83 cM. There were no epistatic interactions among QTLs that were significant. The SNPs located at the QTLs are TP63560, TP24529, TP68535, TP69853 and TP71637. Most t-tests had probability levels lower than  $p < 0.0001$  suggesting strong QTL effects. The  $R^2$  for this model was 0.64.

MIM results using phenotypic data collected in WA identified 12 QTLs distributed along seven linkage groups: LGs 1, 2, 5–8 and 10. No significant epistatic interactions were identified (Table 2). Several QTLs had large LOD values for the test  $H_0 = \text{dominance} = \text{additive} = 0$ ; these were located on LGs 2 and 5. QTL 2 (LOD = 26.87) is located on LG 2 at 48.78 cM and the marker located at this QTL is TP17336. The other QTL with a highly significant LOD (31.38) is QTL 6 located on LG 5 at 14.1 cM. The marker located at this QTL is TP58641. Four of the QTLs had non-significant effects: QTL 1 (TP7178) had non-significant dominance, QTL 3 (TP78316) had non-significant additive effects, QTL 9 (TP61259) had non-significant additive effects and QTL 11 (TP59891) also had non-significant additive effects. All other QTL effects were highly significant ( $p \leq 0.01$ ). Interestingly, the  $R^2$  for this analysis was significantly higher than either the experiment in OR, or the experiment conducted in the GH ( $R^2 = 0.93$ ).

The experiment conducted in the GH resulted in identifying five QTLs spread across three LG (Table 3). QTLs 1 and 2 were located on LG 1, QTLs 3 and 4 located on LG 2 while QTL 5 was located on LG 10. All QTL effects were highly significant ( $p < 0.01$ ) with the exception of QTL 1 (additive effects) and QTL 2 (dominance effects). Like the field-

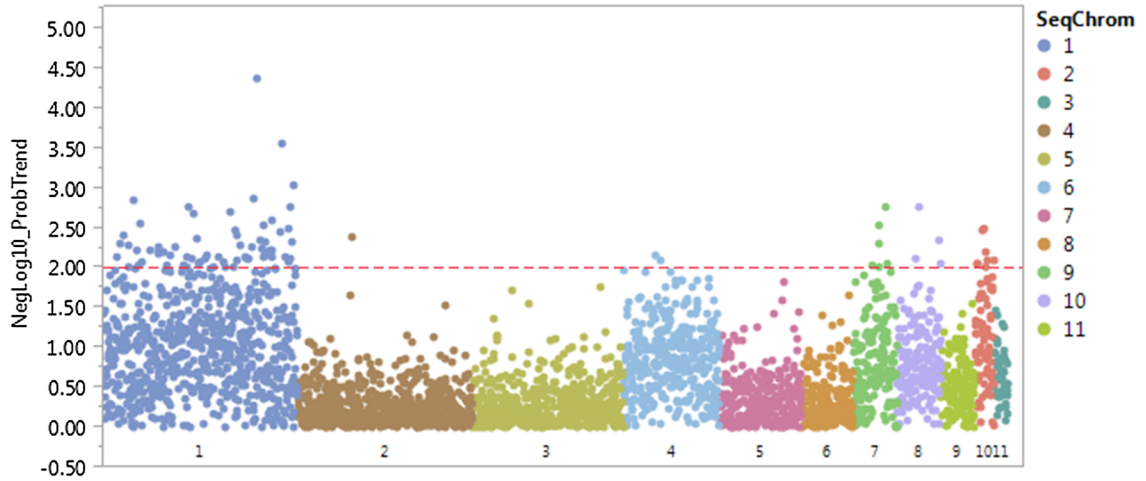
based studies, no epistasis was identified among QTLs. The  $R^2$  for this analysis was equal to 0.65.

Previous research on hop DM suggested that heritability of resistance was based on a quantitative response rather than a qualitative or single gene control (Parker 2007; Henning unpublished research). Similar findings have been reported for the closely related DM pathogen in cucumber (Kozik et al. 2013). Phenotypic expression of a trait that appears to be under quantitative genetic control can be the result of multiple loci (G), environmental influences (E) or some combination thereof ( $G \times E$ ). Investigations on phenotypic expression of DM resistance in multiple environments had not been reported prior to this study. We identified significant differences between environment and genotypes but no significant  $G \times E$  interactions. This suggests that because no  $G \times E$  was observed, it would be possible to utilize the environment that exhibited the highest degree of phenotypic variability for breeding purposes. The two environments showing high degree of variability were OR field plots and GH. Because GH screening is based upon different criteria (localized—leaf area infected) from field plots (systemic—percent infected shoots) it would be wise to screen under both conditions to increase the likelihood of selection success.

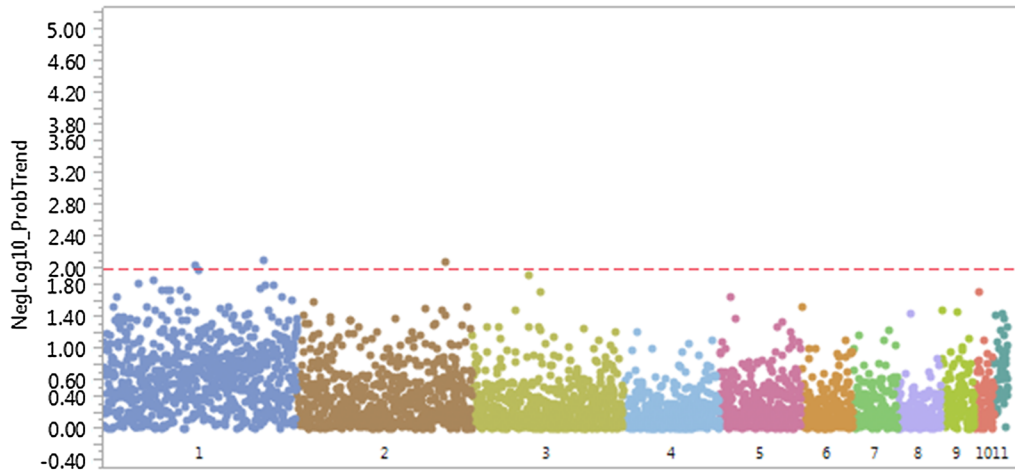
A total of 22 SNP markers linked to DM resistance across 3 different environments were identified. These markers were located on all LG with the exception of LGs 3, 4 and 11. The QTLs associated with field-based DM resistance are located primarily on LGs 1, 2 and 5: LG 1 has QTLs located at three locations (12.77, 100.93 and 106.83 cM), LG 2 has QTLs located five regions (48.78, 66.07, 84.27, 113.24 and 128.19 cM) and LG 5 has QTLs located in three regions (14.1, 33.31 and 46.5 cM). The highest LOD values for QTLs identified based upon data from WA were for QTL 2 (LOD = 26.87) and QTL 6 (LOD = 31.38), while the highest LOD values for OR were for QTL 3 (LOD = 12.03). We suggest that one model for breeding would be to use the three QTLs with highest LOD values (markers TP17336, TP23600, TP68535) as primary markers for selection. Another model could include all significant QTLs from both OR and WA field trials in a mini-array. This latter method would probably have the highest chance of breeding success as all QTL-associated genomic regions would be selected for and genetic response would be applicable



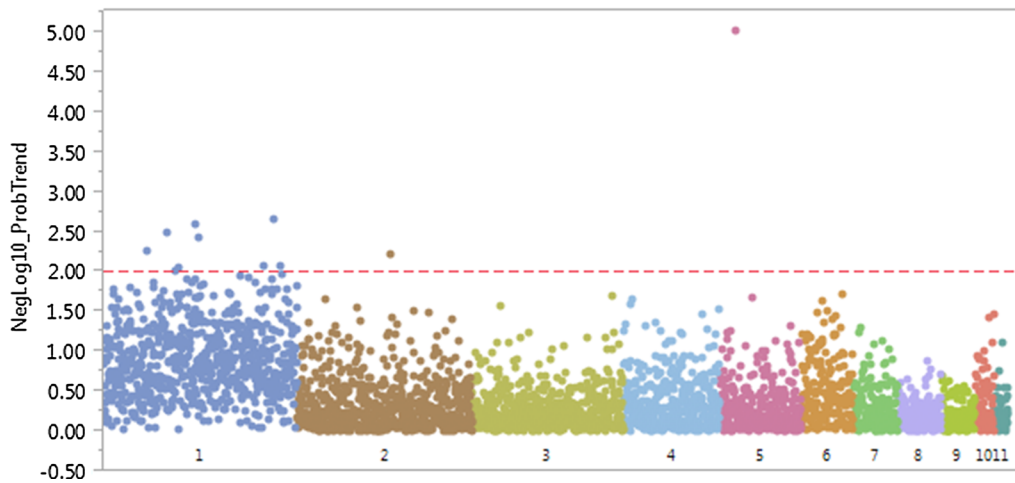
**Trait=GH\_AVG**



**Trait=OR\_AVG**



**Trait=WA\_AVG**



◀ **Fig. 2** Manhattan plot of single marker analysis across three different environments: greenhouse (*GH\_AVG*), Oregon field plots (*OR\_AVG*) and Washington field plots (*WA\_AVG*)

to both environments. Being that this information is based upon a bi-parental mapping population, it is also imperative to check these markers against other genetic lines to validate their effectiveness in identifying superior lines with DM resistance.

The  $R^2$  of the models representing QTLs for OR ( $R^2 = 0.64$ ) and for WA ( $R^2 = 0.93$ ) are relatively high in comparison to the  $R^2$  reported for the four SNP markers identified using association mapping as suggested by Henning et al. (unpublished research) as a potential marker-assisted-selection (MAS) protocol. Depending upon marker system ultimately used for MAS, the 12 markers identified as linked to DM resistance in WA may prove too unwieldy for routine selection using routine PCR methodologies unless all markers were included in a mini-array or other means of multiplexing were available. It may be necessary to identify and validate the top markers required to provide sufficient genetic progress due to selection, prior to utilizing a MAS protocol.

While Parker (2007) showed that leaf area infected and percent shoot infection was correlated ( $p < 0.05$ ), the possibility remains that markers for field-based studies differ from those identified from GH-based scoring. Whether or not selection, using markers

identified by GH-based scoring, will translate into resistance under field conditions is unknown at this time. It is possible that a combination of GH-based and field-based markers will be necessary to reduce the impact of both localized and systemic infection. This would be desirable from the standpoint of reducing overall disease impact upon production.

Previous research using association mapping techniques on the same population, but with up to  $3\times$  the number of SNP markers resulted in the identification of four SNP markers that were ultimately validated with high resolution melting curve analysis (Henning et al. unpublished research). All four markers were not mapped to any LGs in this QTL study due to either unusual segregation ratio's or strong disequilibrium. This is not unusual according to Kim et al. (2009). Association mapping is less sensitive to minor aberrations in data when compared to linkage mapping and can handle far greater numbers of markers. Furthermore, linkage mapping only utilizes markers identified in both parents. All markers used in our QTL mapping had genotypes present in both parents while in Henning et al. (unpublished research) the filtering of loci only required the presence of loci in one or both parents. Furthermore, in Henning et al., unusual marker segregations were not eliminated, as was the case for QTL analysis. It is possible that unusual segregations in the association mapping study resulted in strong associations with DM resistance that might not be detected in QTL analysis. Ultimately, markers

**Table 1** Statistical summary of main effects for downy mildew resistance under field conditions in Corvallis, OR using multiple interval mapping (MIM) showing five QTLs located across three linkage groups (LG)

QTLs	LGs	cM	Markers	LODs	Effects	Estimates	Std Err	DF	t Value	Pr >  t
1	1	12.77	TP63560	6.669	Additive	0.0630	0.016	72	3.91	0.0002*
1	1	12.77	TP63560	6.669	Dominance	-0.0963	0.022	72	-4.45	<0.0001*
2	1	106.83	TP24529	3.667	Additive	0.0049	0.016	72	0.3	0.7635
2	1	106.83	TP24529	3.667	Dominance	0.0982	0.023	72	4.31	<0.0001*
3	2	84.27	TP68535	12.029	Additive	-0.1092	0.016	72	-6.65	<0.0001*
3	2	84.27	TP68535	12.029	Dominance	-0.1924	0.022	72	-8.76	<0.0001*
4	9	4.33	TP69853	7.868	Additive	-0.0512	0.012	72	-4.15	<0.0001*
4	9	4.33	TP69853	7.868	Dominance	-0.0967	0.017	72	-5.7	<0.0001*
5	9	26.45	TP71637	4.306	Additive	0.0482	0.012	72	3.92	0.0002*
5	9	26.45	TP71637	4.306	Dominance	0.0487	0.017	72	2.8	0.0065*

LOD test (H02) = additive = dominance = 0; t-value test (H01) = additive = 0, dominance = 0; Model  $R^2 = 0.64$

\* Statistically significant

**Table 2** Statistical summary of main effects for downy mildew resistance under field conditions in Yakima, WA using multiple interval mapping (MIM) showing 12 QTLs located across 7 linkage groups (LG)

QTLs	LGs	cM	Markers	LODs	Effects	Estimates	Std Err	DF	t Value	Pr >  t
1	1	100.93	TP7178	6.784	Additive	0.035269	0.006133	58	5.75	<0.0001*
1	1	100.93	TP7178	6.784	Dominance	0.007687	0.007964	58	0.97	0.3384
2	2	48.78	TP17336	26.870	Additive	-0.23887	0.015039	58	-15.88	<0.0001*
2	2	48.78	TP17336	26.870	Dominance	-0.24375	0.015632	58	-15.59	<0.0001*
3	2	66.07	TP78316	3.277	Additive	0.020486	0.010524	58	1.95	0.0564
3	2	66.07	TP78316	3.277	Dominance	0.054851	0.013511	58	4.06	0.0001*
4	2	113.24	TP120345	12.787	Additive	-0.03062	0.007639	58	-4.01	0.0002*
4	2	113.24	TP120345	12.787	Dominance	-0.08087	0.011254	58	-7.19	<0.0001*
5	2	128.19	TP47437	17.886	Additive	0.213034	0.020493	58	10.4	<0.0001*
5	2	128.19	TP47437	17.886	Dominance	0.157196	0.020299	58	7.74	<0.0001*
6	5	14.1	TP23600	31.377	Additive	-0.11652	0.006112	58	-19.06	<0.0001*
6	5	14.1	TP23600	31.377	Dominance	-0.09053	0.010567	58	-8.57	<0.0001*
7	5	33.31	TP58641	6.275	Additive	-0.02727	0.007969	58	-3.42	0.0011*
7	5	33.31	TP58641	6.275	Dominance	0.032465	0.010142	58	3.2	0.0022*
8	5	46.5	TP49746	17.398	Additive	0.028572	0.009002	58	3.17	0.0024*
8	5	46.5	TP49746	17.398	Dominance	-0.09407	0.012296	58	-7.65	<0.0001*
9	6	20.11	TP61259	6.781	Additive	-0.01283	0.006415	58	-2	0.0502
9	6	20.11	TP61259	6.781	Dominance	0.03193	0.009413	58	3.39	0.0013*
10	7	25.17	TP592	17.477	Additive	-0.07893	0.007083	58	-11.14	<0.0001*
10	7	25.17	TP592	17.477	Dominance	-0.0888	0.00948	58	-9.37	<0.0001*
11	8	22.24	TP59891	13.177	Additive	-0.00902	0.004851	58	-1.86	0.0681
11	8	22.24	TP59891	13.177	Dominance	0.061463	0.006673	58	9.21	<0.0001*
12	10	13.46	TP116029	5.688	Additive	-0.01815	0.005805	58	-3.13	0.0028*
12	10	13.46	TP116029	5.688	Dominance	0.028971	0.007022	58	4.13	0.0001*

LOD test (H02) = additive = dominance = 0; t-value test (H01) = additive = 0, dominance = 0; Model  $R^2 = 0.93$

\* Statistically significant

**Table 3** Statistical summary of main effects for downy mildew resistance under greenhouse conditions in Corvallis, OR using multiple interval mapping (MIM) showing five QTLs located across three linkage groups (LG)

QTLs	LGs	cM	Markers	LOD	Effects	Estimates	Std Err	DF	t Value	Pr >  t
1	1	26.1	TP57325	5.292	Additive	0.237676	0.211186	72	1.13	0.2641
1	1	26.1	TP57325	5.292	Dominance	1.280061	0.244555	72	5.23	<0.0001*
2	1	80.73	TP114231	4.844	Additive	0.863676	0.183918	72	4.7	<0.0001*
2	1	80.73	TP114231	4.844	Dominance	-0.13439	0.230942	72	-0.58	0.5624
3	2	66.76	TP2869	6.498	Additive	-0.72315	0.159966	72	-4.52	<0.0001*
3	2	66.76	TP2869	6.498	Dominance	-1.12815	0.214717	72	-5.25	<0.0001*
4	2	104.08	TP109884	7.248	Additive	-1.27438	0.204946	72	-6.22	<0.0001*
4	2	104.08	TP109884	7.248	Dominance	-1.27425	0.26507	72	-4.81	<0.0001*
5	10	28.51	TP10533	3.266	Additive	0.537274	0.134792	72	3.99	0.0002*
5	10	28.51	TP10533	3.266	Dominance	-0.08613	0.180566	72	-0.48	0.6348

LOD test (H02) = additive = dominance = 0; t-value test (H01) = additive = 0, dominance = 0; Model  $R^2 = 0.65$

\*Statistically significant

**Table 4** SNP markers showing significant linkage with resistance to DM in plots grown in Corvallis, OR

TP24529: CAGCCAGGTGGAGTGGCGAAGACCCAGTYGAGTCCAGCTCGGATGCCATCTCCGGCTTGC GGCG  
 TP63560: CAGCTTGAAAAGCATACTGAAGATATGCTTGATGGACTGGATTATACTTGTGGTCRAGCC  
 TP68535: CTGCAAATGAGGCTGRITTTGTGTGAGAGCAAGTTCACACCACCAGGCTCTTCTCCTATTACTCA  
 TP69853: CTGCAACTGAMATGAGCTAGTAATAGTAATAAGCCATACATACAAGAATTTTATCCCTTCTCT  
 TP71637: CTGCAATATAAATGGCCTTTGAGATACATAAATTCATTGGTAGATTGACYTATTTCAAGACCA

**Table 5** SNP markers showing significant linkage with resistance to DM in plots grown in Yakima, WA

TP116029: CTGCTCCCGGYTTCTGTTCGCCGGCGCCGGCATTGACTGTAGCTGTAGTAGTAGCAGAAAAA  
 TP120345: CTGCTCTTATCCTGCAATTCATCTTCRATTGGATTTTCATCTTTCTCTTGTGGTAGTTTCAT  
 TP17336: CAGCATGAGGAGTAAATATTGMCAATATATAGTTCATTATTGTTGAGAATTCTGTCTTTTTT  
 TP23600: CAGCCAGAAGGGCTGTAATTATCCAAGTGTGATTAGCTTCCAAGACATTTAATTCACCTGTCAK  
 TP47437: CAGCGGGTCTTCTTGTGGCAGTTCTCTTTGTCGYAACACCTCCGTCAGGGCTCACCGGACAAC  
 TP49746: CAGCTAATAACACCCTATTGAATAGATGTTTGTCTACTCAATCTTGTGGCCCTCATGCYAGT  
 TP58641: CAGCTGACYATATACTTATCTAAGCTCCTCCAAAAGCAAACATCTCTGTACGTTCTTCTTCT  
 TP592: CAGCAAAAGCTTYTGAAAAGCCCCAACTGGAGAGTCTCGACAATGAGACTCTTTTCTTGATCA  
 TP59891: CAGCTGGGATAGCCATTTACTGGGGCCAAAACGGCAATGAAGGCACCTTARCCGATACCTGCGC  
 TP61259: CAGCTGTTGATAAATTAAGRAATCAACTTGAAGTTTTTTTAGGAACCAAAAAGAAGCAAAGAGAA  
 TP7178: CAGCAATCCACATCTCGGCTTGCAAGAGTAGCATACATAGCTATGAATCCTGTTTCACTAAAAAR  
 TP78316: CTGCAGTCAGGTTGCGGGTGMATACCGTCCATCAACAGGGTACCTCCTTATTCCAGGAATCCT

**Table 6** SNP markers showing significant linkage with resistance to DM in plots grown under greenhouse conditions in Corvallis, OR

TP10533: CAGCACTAACAAAAAATCTAACYAATTGTTGAGTGTGTGTGAAAGACAAAAAGGGCAATGCAT  
 TP109884: CTGCTAACTTGACTTTTCCCAGATTGAGGATCTTATGTTGAGGTATCACTATCAGAAWTATA  
 TP114231: CTGCTCACGGTGGTGGATTYCTGCTCTTTATGGTCGACGACGGAGACTTCTCTGCTTTCCTTCA  
 TP2869: CAGCAACACAAATCTTCTTTCATCATACTCRITTTCAATTTTCCCTAAATGGTCCTATAGAAT  
 TP57325: CAGCTCTATTGTCCATATAGTTTACCTGAAAACAAATAAACTATWAGAAGCAACCGATGCTTCT

from both studies will require validation on different populations and or genotypes than those used in this study.

The next step towards developing a MAS (Francia et al. 2005; Gupta et al. 2010) is to validate significant markers across other populations or germplasm pools. The SNP sequences for all significant markers are listed in Tables 4, 5 and 6. These, coupled with the four markers identified in Henning et al. (unpublished research), could form an effective MAS protocol that may reduce screening costs and genetic gain from selection.

This research reports the first high-density genetic map of hop for use in identifying SNP markers

associated with DM-resistant hop lines. The consensus map resulting from this study will act as a ladder to which genome assembly scaffolds will be aligned in future research by the authors. In addition, we identified 22 QTLs located at SNP sites that will provide future marker possibilities to aid selection efforts for DM resistance. We also showed that resistance to DM is quantitatively controlled by multiple loci across several LG, providing evidence for what has been hypothesized for many years (Neve 1991). Finally, we present a means to develop high-density genetic maps by combining results from commonly used software programs that, by themselves, have limitations in what can be accomplished.

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