IDENTIFICATION OF QUANTITATIVE TRAIT LOCI ASSOCIATED WITH POWDERY MILDEW AND PEPPER MOTTLE VIRUS RESISTANCE IN PEPPER

A Thesis

by

JORGE ALFREDO REYES LUCERO

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Chair of Committee,	Kevin M. Crosby
Co-Chair of Committee,	Patricia E. Klein
Committee Member,	Olufemi J. Alabi
Head of Department,	Patricia E. Klein

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ABSTRACT

Leveillula taurica, the causative agent of powdery mildew disease, and pepper mottle virus (PepMoV) are major pathogens of *Capsicum annuum* L. They cause damage to the foliage, leading to fruit quality reduction due to sunlight exposure of the fruits, stunted plant growth, reduced yields, and diminished economic returns in severe cases. This research aimed to identify quantitative trait loci (QTL) associated with disease resistance and develop powdery mildew and PepMoV resistant germplasm by evaluating an F_2 population derived from a cross between the Serrano pepper 'Hidalgo' and the bell pepper 'Bell 365'. Artificial inoculations and screening for powdery mildew was performed on 151 plants from the original F_2 population. A second clonal population consisting of 150 individuals was obtained from the original F_2 population and was used for artificial inoculation and screening for PepMoV. Phenotyping of the individuals was performed visually using newly designed symptom description scales as a reference. A double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) test was used for the PepMoV experiment to confirm the presence of PepMoV in the population. Genotyping-by-sequencing was performed on DNA extracts from the parents and progeny to discover single nucleotide polymorphisms (SNPs). A total of 1078 SNP markers were carefully inspected, and 597 markers were selected and used for genetic map construction and QTL analysis. The genetic map covered 1517.4 cM in total length with an average space of 2.6 cM between markers. A total of 37 highly and 67 moderately resistant individuals to powdery mildew were identified; likewise, PepMoV screening identified 30 resistant plants. Chi-square goodness of fit test and QTL analysis suggested that powdery mildew resistance might be controlled by a single dominant gene located at 31 cM on chromosome 1 responsible for ~49% of the phenotypic variance.

Resistance to PepMoV appears to be controlled by a recessive gene located at 4.0 cM on chromosome 6, explaining ~19% of the phenotypic variance.

DEDICATION

I dedicate this thesis to my family, who are always there to support and love me. Mainly this is dedicated to my mother Yolanda and father Héctor for supporting me emotionally and financially to achieve my goals; you mean a lot to me; to my brothers, Héctor, Marco, and José, for being my best friends and life companions, thank you for filling my life with happiness. To my grandparents Jorge and Yolanda for sharing their wisdom and advising me to grow positively as a person. Although my grandfather is no longer in this world, you will always be in my heart. Also, I want to thank all my uncles, aunts, and cousins for their support, especially for their unconditional love I hope you feel proud of this achievement. I love you very much, and God Bless you always. "Gracias familia los quiero mucho".

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This project was supervised by Professor Dr. Kevin Crosby (Advisor) and Professor Dr. Patricia Klein (Co-Advisor) of the Texas A&M Department of Horticultural Sciences and Associate Professor Dr. Olufemi Alabi (Committee member) of the Texas A&M Department of Plant Pathology and Microbiology.

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NOMENCLATURE

GBS	Genotyping-by-Sequencing
LOD	Logarithm of the odds
PepMoV	*Pepper mottle virus*
PVY	Potato Virus Y
CMV	*Cucumber mosaic virus*
FFN	First Flower Node
CP	Coat Protein
TEV	*Tobacco etch virus*
CM334	Criollo de Morelos 334
RH	Relative Humidity
P6	Pepper Chromosome 6
CTAB	Cetyltrimethylammonium bromide
QTL	Quantitative Trait Loci
PCR	Polymerase Chain Reaction
SNP	Single-nucleotide Polymorphism
cM	Centimorgans
HR	Hypersensitive response
NCBI	National Center for Biotechnology Information
Pvr	Potyvirus resistance locus
MLO	Mildew Locus O
DG	Digital Genotyping

HORTREC	Texas A&M University Horticulture Research Center
BLS	Bacterial Leaf Spot
MLE	Maximum Likelihood Estimator
ELISA	Enzyme-linked immunosorbent assay
DAS-ELISA	Double Antibody Sandwich ELISA
MW	Molecular Weight
CAB	Capture Antibody
GEB	General Extraction Buffer
PVP	Polyvinylpyrrolidone
ECA	Enzyme Conjugate
PNP	Para-Nitrophenyl phosphate
OD	Optical Density
RTM	Restricted Tobacco Etch Virus Movement
pLOD	Penalized LOD score
PepSMV	*Pepper severe mosaic virus*
SA	Salicylic acid
S-gene	Susceptibility genes
R-gene	Resistance genes
ICTV	International Committee on Taxonomy of Viruses
MAS	Marker Assisted Selection
HRM	High Resolution Melting
KASP	Kompetitive Allele Specific PCR

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CHAPTER I

INTRODUCTION

The genus *Capsicum* of the *Solanaceae* family is native to the Americas. Archeological records show that chili peppers have a lengthy history in several Latin American places (Andrews, 1984; Heiser, 1985; Long-Solis, 1986; Eshbaugh, 2012). Without the benefit of genetic insight, taxonomists of the 18th and 19th centuries named these species of any size, shape, and color as separate species, generating a plethora of plant names sorted into five domesticated species in the 20th century (Eshbaugh, 2012). The five species are *Capsicum annuum* var. *annuum* L., *C. chinense* Jacq., *C. baccatum* var. *pendulum*, *C. frutescens* L., and *C. pubescens* Ruiz and Pavon. *C. annuum* var. *annuum* L. includes jalapeño, poblano, ancho, bell, Anaheim, cayenne, and serrano. This species is prevalent across the Caribbean and south Colombia and north of Peru, throughout Mexico to the southern border of the U.S. (Eshbaugh, 2012). *C. chinense* Jacq. includes the cultivars in the habanero, rocotillo and Scotch bonnet types and it is the dominant domesticated pepper in the Amazonas (Eshbaugh 2012). *C. frutescens* L. contains tabasco, Piri-piri ,malagueta, and Thai pepper types. This wild species is prevalent from the south of Central America to the Amazonas (Eshbaugh, 2012). *C. baccatum* var. *pendulum* contains the Cuerno de Oro, aji Amarillo, and cumbia peppers. This taxon is widespread in Bolivia and northern Argentina and outlier populations are found in Peru and Paraguay (Eshbaugh, 2012). *C. pubescens* Ruiz & Pavon is known as chile Manzana. It is a domesticated species from the Andes, primarily Peru (Eshbaugh, 2012).

The United States is among the top five green peppers producers in the world, including chili and bell peppers, with California, Georgia, Florida, New Jersey, North Carolina, Ohio, and Michigan being the top bell pepper producing states and California, Texas, New Mexico, and Arizona being the top chili pepper producing states (WIFSS, 2016). The domestic bell and chili pepper production were approximately $ 642 million in 2019 (NASS, 2019).

1.1 Pepper breeding

Pepper *Capsicum annuum* L. is a diploid C3 dicotyledonous vegetable with a genome size of ~3.5 Gbps and encodes ~ 36,000 genes according to *C. annuum* Pepper 'Zunla' genome (Qin, et al., 2014. This taxon includes the most commercially essential peppers. Pepper breeding programs are aware about the introduction of disease resistance. They have introduced, for example, resistance to *Tobacco Mosaic Virus* (TMV) from *C. frutescens*, *Pepper Mild Mottle Virus* (PMMV) from *C. chinense* and *C. chacoense*, and *Cucumber Mosaic Virus* (CMV) and *Potato Virus Y* (PVY) from *C. baccatum*. (Negi et al., 2018). The research and development efforts are focused on creating valuable cultivars with high yield, high fruit quality, and organoleptic properties (e.g. flavor and smell) (Gicuta et al., 2016).

Pepper (*Capsicum annuum*) is considered a self-pollinated crop with a degree of cross-pollination of approximately 2% (Gicuta et al., 2016). The traits of interest in pepper improvement programs are high yield, earliness, fruit shape and size, fruit quality, disease resistance, resistance/tolerance to insects, and abiotic stress tolerance. Each of these pepper traits affects the marketability of the crop and, eventually, its economic value. The popularity of pepper is usually determined by features of growth performance in a specific environment,

handling, and market tastes. In the U.S. market, the type of peppers mostly preferred by consumers are bell peppers and jalapeño or chili peppers. Bell pepper is among the most popular sweet peppers mainly consumed as fresh; some of the most popular bell pepper varieties used for commercial production are ‘Aladdin’, ‘Vanguard’, and ‘Aristotle’ (WIFSS, 2016). Among chili peppers jalapeños are preferred over serrano and Habanero peppers (Lillywhite et al., 2013).

Pepper breeding programs in the U.S. have focused on developing new cultivars and hybrids resistant to biotic and abiotic stresses to provide the maximum commercial productivity and profitability of vegetable growing (Crosby, 2008). A primary objective of most breeding programs is the high and stable yield. Still, to achieve this, breeders must consider a cultivar's adaptability to a specific type of environment. Yield is a quantitative trait mediated by the heterosis in the following generations after the parental cross. An early maturing crop would also imply that farmers will get good yields when peppers are planted before the season gets too hot (Puozaa, 2010). Yield heterosis is a variable characteristic that depends strongly on the combinations of parents (Berke, 2000).

The development of a new elite inbred line should consider the following crop characteristics: 1) Fruit size and shape are complex traits regulated by various genes, representing yield and marketable quality. The first step in describing and classifying pepper germplasm for breeding purposes is the characterization of fruit. Using suitable statistical methods is helpful for description and genotype classification, as it allows plant breeders to select valuable germplasm and resources (Jankulovska et al., 2014). 2) A significant quality index in pepper production is the proportion of capsaicinoids in dried red capsicum powder and oleoresins. Pungency inheritance studies usually follow the hypothesis of the single dominant gene. However, some scientists also discovered a polygenic pungency inheritance

(Greenleaf, 1986). 3) The first flower node (FFN) is a significant feature for assessing pepper fruit earliness (Zhang et al., 2018). 4) Bacterial leaf spot caused by *Xanthomonas campestris* pv. *vesicatoria* is endemic in warm and humid environments (Hibberd et al., 1987; Crosby, 2008). Cook & Stall (1963) first identified resistance as a hypersensitive response in ´PI163192´, determined by a single dominant gene, *Bs1*. 5) The oomycete *Phytophthora capsici* (Leon.) is considered the most devastating pathogen for chili pepper cultivation. It causes stem, leaf, fruit blight, and root rot depending on the host, the point of infection, and environment (Barchenger et al., 2018). More than 45 physiological races were recognized for root rot and foliar blight disease syndromes (Da Costa Ribeiro & Bosland, 2012; Barchenger et al., 2018). Resistance to this pathogen was found in 'Criollo de Morelos 334', but it is associated to low fruit quality (Crosby, 2008). 6) One of the most common diseases of sweet peppers is powdery mildew, which is caused by the fungus *Leveillula taurica* (De Souza & Café-Filho, 2003). The first signs are yellow chlorotic patches on the oldest leaves. The disease begins with white sporulating regions on the lower surface of diseased leaves, then spreads to the younger leaves, culminating in plant defoliation (Daubeze et al., 1995). 7) Virus resistance breeding has led to significant advances in developing pepper cultivars with resistance to diseases. Many kinds of viruses infect pepper crops, some being carefully researched, and others yet to be characterized. Symptoms are generally leaf mosaic, mottling, severe deformation, and stunted plant growth (Crosby & Villalon, 2002). 8) Rhizoctonia root rot of pepper, caused by the fungus *Rhizoctonia solani*. Peppers are affected by *Rhizoctonia solani* in several states in the United States and other nations. The disease flourishes in heavy soils, and no commercially available resistant cultivars have been produced to combat it (Crosby, 2008).

1.2 *Pepper mottle virus* (genus Potyvirus; family *Potyviridae*)

The genus *Potyvirus* (family *Potyviridae*) is the second largest plant virus genera and it contains species with flexuous filamentous virions (virus particles) that range in length from 680 nm to 900 nm long and are 11–13 nm wide. Their virions contain a positive-sense, linear, single-stranded RNA molecule of approximately 9.7 kilobase pairs (Kb). This RNA molecule processed into a large polypeptide that is subsequently cleaved proteolytically by virus-encoded proteases into 10 mature proteins of different functions (Wylie et al., 2017). The genus contains some of the most economically important plant viruses in terms of their ability to infect diverse host plant species and cause significant economic damages. Members of the genus are traditionally distinguished by their host range and cytopathology, as well as coat protein serology, cytoplasmic body proteins, and molecular features (Vance et al., 1992).

Viruses in the genus *Potyvirus* are transmitted by aphids with different levels of effectiveness but some are non-aphid transmissible (Brunt, 1992). Potyviruses are also mechanically transmissible under experimental conditions. Several aphid vectors are often involved in the widespread occurrence of potyviruses in susceptible pepper cultivars (especially those belonging to the genera *Aphis, Myzus, and Macrosiphum*), which is probably due to their fertility and mobility in immature plants (Murant et al., 1988).

Transmission electron microscopy of infected tissues revealed filamentous particles (virion) with a length of 720-750 nm, which are typical of PepMoV (Kim et al., 2008). The PepMoV virion contains a linear, positive single stranded (ss) RNA genome of approximately 10 kb (Brunt, 1996). The coat protein (CP) sequence of PepMoV has a length comparable to that of PVY CP at 267 amino acids (Kim et al., 2008).

In Florida in the early 1970s, PepMoV was described based on symptoms induced by the virus in sweet peppers resistant to *Tobacco Etch Virus* (TEV) and PVY (Zitter, 1972). Resistance to 56 potyviruses has been discovered, while genetics and usefulness of l35 resistance genes have been clarified during the research of the last sixty years. Resistance to *Potyvirus* species has been acquired in the majority of plant species. In certain instances, two or more genes conferred resistance to potyviruses within a host genotype (Provvidenti & Hampton, 1992).

Symptoms induced by pepper infecting viruses may vary by species, including decreased development rates, when more than one virus infected pepper crops simultaneously. In many viral infections, PepMoV viral load does not increase considerably. When co-infected with CMV, PepMoV titers are slightly elevated compared to when it occurs alone in peppers. When testing various viruses, other viral symptoms did not mask PepMoV-induced symptoms. These findings indicate that PepMoV did not affect other viruses, although crops were pre-inoculated (Kim et al., 2010). PepMoV symptoms include mosaic, crinkled leaves, dark-green vein banding, and stunted plant growth (Kim et al., 2010; Venkatesh et al., 2018).

The *pvr1* gene found in *C. chinense* 'PI159236' and 'PI152225' provides resistance to PVY pathotype 0, TEV HAT isolate, and PepMoV (Kyle & Palloix, 1997; Yeam et al., 2005; Venkatesh et al., 2018). Gene *pvr3* reported in *C. annuum* 'Avelar' is responsible for PepMoV-resistance (Guerini & Murphy, 1999; Murphy & Kyle, 1995; Parrella et al., 2002; Venkatesh et al., 2018)

Capsicum chinense 'PI159236' gene *Pvr7* and *C. annuum* 'CM334' gene *Pvr4* conferred dominant resistance to PepMoV. Gene *Pvr7* locus was located on chromosome 10. Analysis of the '9093'-resistance spectrum against pepper potyvirus shows that '9093'

has resistance comparable to the 'CM334'. These findings suggest that Pvr4 and Pvr7 belong to the same locus. (Venkatesh et al., 2018).

1.3 Powdery Mildew (*Leveillula taurica*)

Leveillula taurica is categorized as an obligate fungal plant pathogen that belongs to the ascomycetes (Cerkauskas et al., 2011). *Leveillula taurica* causes powdery mildew disease on peppers, which can result in economic losses in over 500 species, including different vegetable crops (Zheng et al., 2013). Experiments performed in commercial greenhouses demonstrated that infected pepper leaves are shed when powdery mildew is present. This translates to reduced photosynthetic area in the plant, affecting the flowers and fruit development. The loss of leaves also results in more sunburn damage on fruits exposed to irradiation (Elad et al., 2007).

Powdery mildew is a growing issue in pepper-producing areas, especially in coastal climes or greenhouse production (Lefebvre et al., 2003). Salmon in 1906 was the first to report powdery mildew caused by *L. taurica* in the United States on *Diplacus aurantiacus* (Correll et al, 1986). The pathogen was subsequently reported on several economically significant plant hosts in the US, including pepper (*Capsicum annuum L.*) (Correll et al., 1986; Mihail, 1984). While most powdery mildew pathogens are ectoparasites, the mycelia of *L. taurica* develops in host tissue intercellularly, restricting chemical control effectiveness (Lefebvre et al., 2003). This endoparasitic lifestyle differentiates *L. taurica* from other powdery mildew fungi that are classically ectoparasites. Some of the life cycle of *L. taurica* takes place within the leaf while other stages develop on the leaf surface; the fungus forms haustoria through which it penetrates the host tissues and absorbs nutrients from it. (Elad et al., 2007).

Premature leaf shed due to the disease significantly impacts pepper production and makes fruits unmarketable (Lefebvre et al., 2003). Massive powdery mildew epidemics may lead to a substantial loss of up to 2 to 4kg/m^2 of yield in greenhouse production (Cerkauskas & Buonassisi, 2003; Zheng et al., 2013). According to Homma et al. (1980), 70% of the conidia germinate within 3 hours after artificial inoculation. After host tissue penetration, the mycelium develops in the leaf intercellularly for up to 4 weeks. Mycelium emerges from the leaf via the stomata, and conidiophores with conidia develop on the leaf surface (Homma et al., 1981). This conidiation is visible as white colonies. The majority of the visible symptoms are found on the lower leaf surface, either because of the higher stomata density or because the microclimate is more conducive to disease growth (Elad et al., 2007).

Elad et al. (2007) found that conidial germination temperature range is from 10 to 37°C, with 20°C being the optimum; its viability declines as temperatures rose to 40°C. The highest germination rates were noted at 75 to 85% relative humidity (RH). In plants subjected to long temperature periods between 10 and 15°C and daily RH of between 85 and 95%, foliar symptoms of powdery mildew disease was high in severity, and this was related favorably to nighttime RH (Elad et al., 2007).

Several accessions to pepper have different levels of powdery mildew resistance under natural infections, although most are not *C. annuum*, which has low resistance levels (Daubeze et al., 1995). Accessions with varying levels of powdery mildew have been identified in *C. annuum*, *C. baccatum*, and *C. chinense* (Ullasa et al., 1980; Lefebvre et al., 2003).

Powdery mildew resistance in pepper has been reported to be a dominant and polygenic trait (Murthy & Deshpande, 1997; Blat et al., 2005; Jo et al., 2017). A genetic analysis has also revealed that a few genetic factors with significant additive and epistatic

effects confer powdery mildew resistance to different pepper genetic backgrounds. (Daubeze et al., 1995; Blat et al., 2005, 2006; Jo et al., 2017). Three incompletely dominant genes in the *C. frutescens* line 'IHR703' are believed to provide resistance to powdery mildew (Jo et al., 2017). Small-fruited pungent C. annuum 'H3' from Ethiopia is the most significant source of reported powdery mildew resistance. (Daubeze et al., 1995; Lefebvre et al., 2003). At least three genes appear to regulate *L. taurica* resistance in 'H3'. The QTL that could explain most of the discovered variance (26%) was at the P6 (Pepper chromosome 6) locus (Lefebvre et al., 2003).

A QTL discovered by Eggink et al. (2016) could explain 56.8% of the observed variance in *L. taurica* resistance alone, located on LG1/8, which created extremely resistant plants. The resistance is inherited as a polygenic recessive trait; achievable resistance is noted only in homozygous plants, while heterozygous plants are not resistant (Eggink et al., 2016).

Fungicide efficacy depends on early identification of symptoms and broad plant coverage, which may be difficult to achieve (Goldberg, 2004; McCoy & Bosland, 2019). Powdery mildew can be controlled in a most economically and environmentally sustainable manner by growing resistant cultivars (McCoy & Bosland, 2019). So, in this study, a family of 150 F_2 progeny were screened for powdery mildew resistance.

The inheritance mode of powdery mildew resistance is complex (Jo et al., 2017). Results obtained by virus-induced gene silencing indicate that pepper's susceptibility to *L. taurica* may involve both *CaMlo1* and *CaMlo2* genes (Zheng et al., 2013). According to Zheng et al. 2013, the *CaMlo2* gene is transcriptionally responsive to *L. taurica* host tissue penetration, and either *CaMlo1* or *CaMlo2* silencing decreases *L. taurica* susceptibility of pepper. These results provided evidence that the susceptibility to *L. taurica* is controlled by

at least one pepper *Mlo* homolog. Identified by Kim & Hwang (2012), the *CaMlo2* allele may explain population variation, but other genetic mechanisms are involved in powdery mildew resistance (McCoy & Bosland, 2019). PMR1, a newly discovered gene, has been found to confer L taurica resistance in C. annuum (Jo et al., 2017; McCoy & Bosland, 2019). Through the mapping of two pepper populations, Jo et al. (2017) identified the single dominant gene, *PMR1* located on chromosome 4.

CHAPTER II

PEPPER MOTTLE VIRUS RESISTANCE TRAIT

2.1 Introduction

The majority of commercially cultivated pepper varieties are relatively susceptible to PepMoV, which can cause significant losses in yield and fruit quality leading to substantial economic losses to growers. Hence the importance of searching for disease resistance sources that can be used in the development of PepMoV resistant germplasm.

Diseases caused by potyviruses have been one of the most dangerous diseases in pepper plants in the United States. A 100% disease incidence has been estimated to cause a yield loss of up to 70% in severe cases (Kenyon et al., 2014; Bayer Group, 2019). Due to the lack of chemical control measures for viruses, PepMoV is primarily managed using disease-resistant pepper varieties. Potyvirus-resistance loci are named with the nomenclature *Pvr*. Several alleles are known, including *pvr3* and *Pvr4*, which convey PVY and PepMoV resistance (Kenyon et al., 2014). Analysis of a 6,338 bp fragment of the PepMoV genome of the Texas isolate, spanning the partial helper component protease (HC-Pro) through a portion of the RNA-dependent RNA polymerase (NIb) cistron of the virus, revealed that it is phylogenetically distinct from PepMoV isolates from California, Florida and other pepper growing regions of the world (O. J. Alabi, personal communication, June 1, 2021). The *Pvr4* gene is comprised by seven exons encoding a coiled-coil NB-LRR family protein (Venkatesh et al., 2018).

When conducting genetic diversity studies, QTL analysis, genetic map construction, pedigree analysis, association studies, and marker-assisted breeding, the obtainment of high-quality genotyping information is crucial (Morishige et al., 2013). Nevertheless, the

acquisition of whole genome sequences in large and highly repetitive genome species such as pepper is inefficient and too costly for genotyping purposes (Morishige et al., 2013).

However, in most genotyping implementations, only a subset of genetic diversity among individuals is required, and some of the methods for obtaining these subsets of the genome for genotyping include the use of restriction enzymes (Morishige et al., 2013). Since the pepper genome contains a large portion of highly methylated repetitive DNA, technologies like GBS utilizing a methylation-sensitive restriction enzyme such as *Ngo*MIV (G^CCGGC) is recommended (Morishige et al., 2013).

For this experiment, two pepper inbred lines were used for *Pepper mottle virus* resistance screening in the resulting F_2 population. The first parent is the 'Hidalgo' pepper. "Hidalgo is a mildly pungent, serrano pepper with multiple virus resistance against TMV, TEV, PVY, and PepMoV" (Villalon, 1985). "The plants are more compact than other serranoes but will grow between 50 to 60 cm high with a strong central stem suitable for mechanical harvesting" (Villalon, 1985). The second parent is the 'Bell 365' pepper, a Bacterial Leaf Spot Resistant (BLS) breeding line developed by Dr. Kevin Crosby.

This research will identify regions in the pepper genome (QTLs) that are associated with PepMoV resistance through the phenotyping of an F_2 population derived from the cross of 'Hidalgo' parent by the 'Bell 365' parent and its comparison with genetic data from the construction of a genetic map using SNPs discovered through Genotyping-by-Sequencing (GBS).

2.2 Materials and Methods

The experiment was carried out at the Texas A&M University Horticulture Research Center (HORTREC) facilities, Sommerville, Texas. Seeds were collected from the pepper germplasm collection of Dr. Kevin Crosby's Breeding Program. For this experiment, the Serrano pepper 'Hidalgo' and the Bell pepper 'Bell 365', both *C. annuum* species, were used as parents to analyze the inheritance of resistance to PepMoV in 150 F_2 progeny. Only150 plants were screened, out of the 167 plants initially planted, due to the loss of some seedlings before the study was performed.

2.2.1 Genotyping

The PepMoV highly resistant cultivar 'Hidalgo' and the susceptible breeding line ´Bell 365´ were used to generate a diploid (2n=2x=24) F_2 population for mapping. The F_2 population of 167 plants was grown in 10 L pots with Pro-Mix in a greenhouse with an average temperature of 80°F (26.6°C); irrigation and chemical applications were applied as required.

The parental types and 167 plants from an F_2 population derived from the 'Hidalgo' x 'Bell 365' cross were genotyped. DNA was extracted from unexpanded young leaves (0.5-1 g) collected into 2 mL extraction tubes containing six grinding beads using a modified cetyltrimethylammonium bromide (CTAB) protocol (Doyle, 1991). The OneStep™ PCR Inhibitors Removal Kit (Zymo Research Corporation, Irvine, CA, USA) was used to purify and clean DNA. Fluorometry with the Qubit® Fluorometer 3.0 (Thermo Fisher Scientific, Rochester, NY, USA) was used to measure DNA concentration and assess its quality. All DNA samples were stored at -20°C.

GBS was performed utilizing the restriction enzyme *Ngo*MIV following the protocol of Morishige et al. (2013). Briefly, genomic DNA from the parents and each F_2 progeny was organized into 96-well plates and 250 ng of DNA was digested with the restriction enzyme in a 20 µl solution for 2 to 4 hours at 37°C. Heat inactivation was performed at 65°C for 15 min. Individual 12 bp barcode in-line adapters were added to each sample by adding 5 pmol index adapter and 1.5 units T4 DNA ligase to the digested DNA followed by incubation for 4 to 16 hrs. at 20°C. Up to 48 individual reactions were then pooled. The pooled DNA were randomly sheared to a target size of 250 bp. The fragments were then size-selected on a 2% agarose gel to a range of 250 +/- 50 bp. The pools were ligated to an Illumina-specific adaptor and purified using magnetic beads after overhang fill-in, blunting, and adenylation (Agencourt AMPure XP, Beckman Coulter). The derived pool of DNA fragments comprised a population of molecules with two adapters ligated to opposite ends of the genomic DNA. Twenty PCR cycles were performed to enrich the fragments using Phusion DNA polymerase (New England Biolabs) with primers integrated into the two adapter sequences. According to Ilumina's protocol for final PCR (14 cycles), single-stranded DNA products were used with primers having sequences complementary to the flow cell. Using the QIAquick PCR Purification Kit (Qiagen, Inc.), amplified products were purified, and quantified utilizing UV spectroscopy, then diluted to 10nM. The Illumina template pools were submitted to the AgriLife Genomics and Bioinformatics Services facility for 150 bp single-end sequencing on the NovaSeq S1 flowcell. Following sequencing, the fastq files were demultiplexed based on the unique barcode sequences into individual parent or progeny read files. The resulting read files were processed with a series of custom perl and python scripts. This processing pipeline included removing the 12 bp barcode from each read and compression of duplicate reads. The parental reads that were sequenced at least 3 times were

then mapped to the pepper genome (Zunla-1; Qin et al. 2014) using BLAST analysis. Polymorphisms between the two parents were scored using a series of perl scripts and mapped through the progeny as described by Morishige et al. (2013).

2.2.2 Genetic Map Construction

The linkage map construction was done in RStudio® with the "qtl" package developed by Broman (2003). The 1078 SNP marker data obtained from GBS was filtered to choose a subset of high-quality markers. Individuals and markers with more than 10% missing data were omitted using the "sub-set" and "drop markers" functions due to their negative effect on the quality of the Linkage Map. It is also useful to look for duplicate individuals and markers to notice unusual similar genotypes, the function "findDupMarkers" was used to identify any possible duplicate markers, but none were found. In the case of the identification of duplicate or unusually similar individuals the "comparegeno" was used to compare the genotypes of all pairs, and one individual of each pair with more than 90% genotypic similarity were omitted. A pair of F_2 siblings have about 40% matching genotypes (Figure 1) (Broman, 2010).

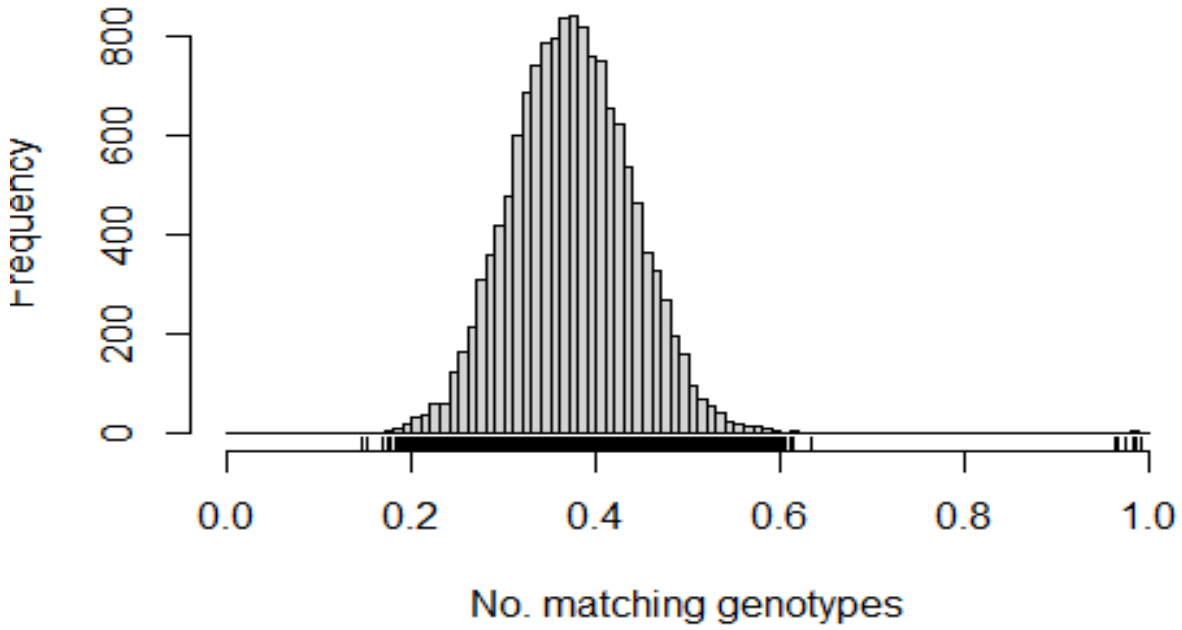

Figure 1. Histogram of the matching genotypes of all pairs of the 'Hidalgo' x 'Bell 365' F_2 individuals.

Next, segregation patterns of the markers were analyzed and compared with the expected mendelian frequencies of 1:2:1. Departures from these frequencies are not uncommon and often indicate partial lethal alleles or problematic markers (Broman, 2010). The "geno.table" function was used to calculate the genotypic frequencies and the P-value for a test of departure from the expected frequencies (Bonferroni correlation). Markers with a P-value < 0.001 were omitted because it may be indicators of genotyping. Also, the individuals are expected to have genotypic frequencies around the 1:2:1 ratio (Figure 2). Problematic individuals with unusual observed number of crossovers were omitted, most of the individuals have 5-60 crossovers and only those within this range were used.

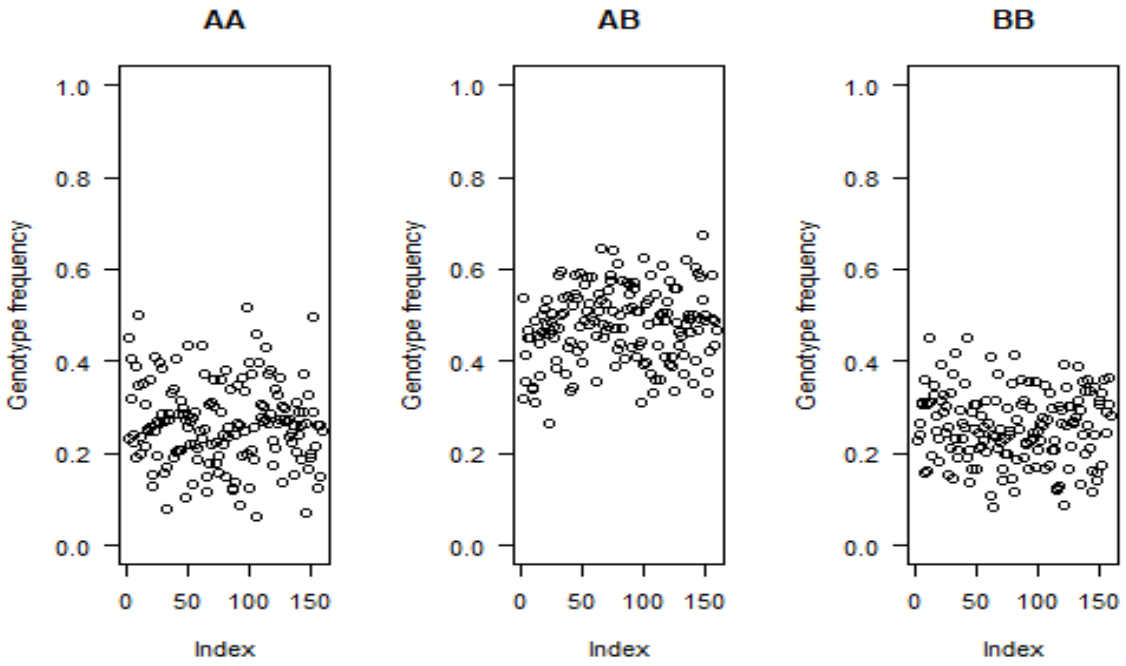

Figure 2. Genotypic frequencies by individuals derived from the 'Hidalgo' x 'Bell 365' F_2 population.

A logarithm of odds (LOD) score of 6 and a maximum recombination fraction of 0.35 were used to designate linkage groups. For each linkage group, markers were ordered using the "orderMarkers" function, the best order of markers was then confirmed using the "ripple" command with an error probability of 0.0075 equivalent to the Maximum Likelihood estimator (MLE) (Figure 3). The linkage map was generated with the "plotMap" command in Kosambi distance.

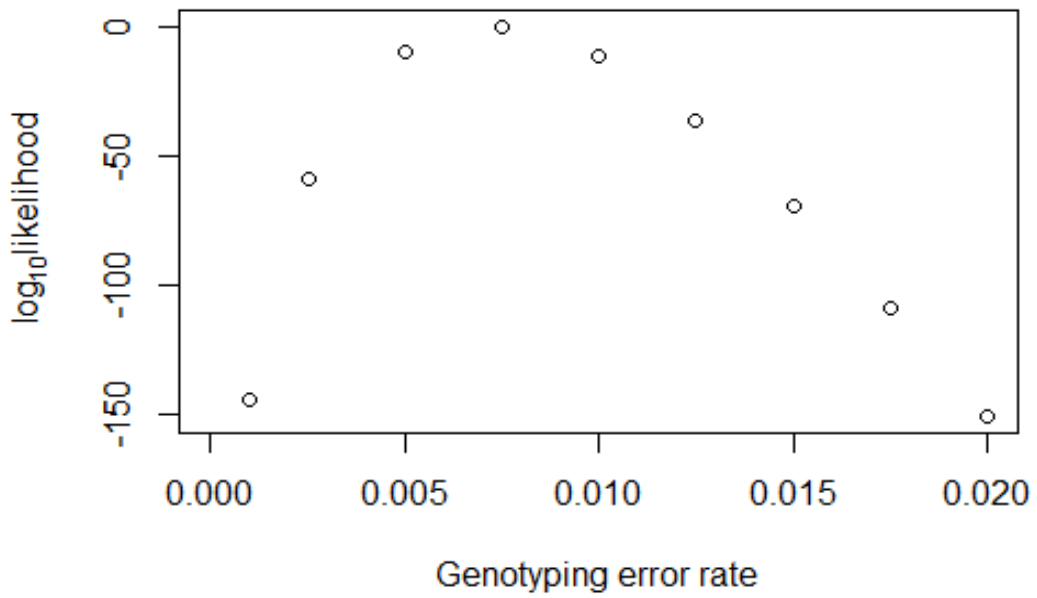

Figure 3. The log10 likelihood for the genotyping error rate estimates of the data set.

2.2.3 *Pepper mottle virus* phenotyping

The F_1 seeds were sown in 2-gallon plastic pots using peat-based growing mix (Promix ®) with perlite at a 2:1 ratio. The F_1 hybrids were selfed to obtain the F_2 population. Since phenotyping was performed for two diseases in the same population, stem cuttings were created from the original F_2 population to generate a genetically identical population set. The population developed from cuttings was used for PepMoV resistance experiment. The evaluation was carried out on 6 weeks old plants. A total of 150 plants were screened out of the 167 F_2 plants that were planted initially since 16 plants were lost due to seedling mortality and one plant because it was not successfully propagated asexually.

The F_2 population was evaluated for resistance to PepMoV under greenhouse conditions in Somerville, Texas. All plants under evaluation were mechanically inoculated to ensure uniform disease pressure. The original inoculum was obtained from naturally infected plants from Texas A&M AgriLife Research and Extension Center at Weslaco.

Several plants of PepMoV susceptible lines ‘Trinidad Scorpion’ and ‘Habanero 51’ were inoculated with the virus isolate and then used as an inoculum source for maintaining the virus. To confirm PepMoV presence and to exclude the presence of other common pepper infecting viruses, leaf tissue samples from 5 plants were submitted to Agdia Inc. (Elkhart, IN) for diagnosis by DAS-ELISA test. The Agdia pepper screen test includes assays for 15 commonly reported pepper-infecting viruses (https://www.agdia.com/testing-services/pepper). The results demonstrated that 4 out of 5 samples contained a virus coinfection, mainly PepMoV+ TEV, except for an individual of ´Trinidad Scorpion´ that was positive for PepMoV alone (data not shown). This singly infected plant was separated from the rest and appropriately fertilized for promoting new infected vegetative growth and maintained in an insect-proof greenhouse. Newly developed leaves from the PepMoV-positive ‘Trinidad Scorpion’ were resampled for confirmation of virus presence by DAS-ELISA at Agdia Inc. (data not shown). Subsequently, this plant served as an inoculum source for mechanical inoculation of the F_2 population for screening purposes. The TX-PepMoV isolate used for the resistance screening was also molecularly analyzed via analysis of a 6,338 bp fragment of its genomic RNA, spanning the partial HC-Pro through a portion of the NIb gene. The analyses revealed that the TX-PepMoV isolate is a genetic variant that is phylogenetically distinct from PepMoV isolates from California, Florida and other pepper growing regions of the world (O. J. Alabi, personal communication, June 1, 2021). The source and recipient plants were uniformly maintained using standard fertilization practices, irrigation, and insect management.

The F_2 population was exposed to three inoculation regimes to ensure that all the plants were exposed to the virus and that the plants showing few, or no symptoms were not

due to escapes during the inoculation process. A new set of leaves were inoculated on each inoculation regime.

The mechanical inoculation process involved the following steps: firstly, the determination of the number of leaves that were inoculated from every F_2 plant (5 leaves/plant) mainly selecting young leaves because they are more likely to respond better using this inoculation method due to their softer leaf surfaces. Once the leaves to be inoculated were picked, the necessary amount of inoculum was determined (0.5-1 mL liquid inoculum per leaf), then leaf tissue from the naturally singly infected ´Trinidad Scorpion´ plant source of PepMoV was collected at a ratio of 0.5 g of leaf tissue per ml of inoculum solution (Table 1). Next, the collected tissue samples were ground using a mortar and pestle previously placed in a freezer at -20°C before starting. Wearing gloves, the mortar and pestle along with a tiny amount of 0.1M phosphate buffer (Table 1) was used to grind up the leaf material into a watery 'green juice' paste at a ratio of 2 ml of buffer for every 1.0 g of leaf tissue. Using cheesecloth, the paste of leaf material was filtered, and the liquid put into a 50 ml conical tube; the carborundum powder (Silicon carbide) was added to the solution before inoculation. The conical tubes containing the inoculum were placed on ice and transported to the greenhouse; the inoculum was gently rubbed onto each leaf to be inoculated using a cotton swab (a new swab per leaf or every other leaf was used as needed). Once the leaves were inoculated, they were gently rinsed with tap water 5 minutes after the inoculation to prevent further damage of chemicals and additives to the leaves. The plants were observed weekly for symptoms.

Table 1. *Pepper mottle virus* inoculation buffer recipe.

***PEPPER MOTTLE VIRUS* inoculation buffer (pH 7)**	
Step 1. 0.1M (potassium) phosphate buffer	
Item	**Quantity**
Potassium phosphate monobasic (MW: 136.09)	6.309 g
Potassium phosphate dibasic (MW: 174.18)	9.344 g
Distilled water	1 l
Step 2. Inoculation buffer	
Item	**Quantity**
Phosphate buffer	500 mL
2-mercaptoethanol	350 µL
Carborundum powder	1% w/v concentration (1 gr per 100 ml)

Adapted from: Viral Inoculation of Pepper Plant for Tomato spotted wilt virus (TSWV) protocol of Dr. Crosby lab at Texas A&M University.

After the third inoculation, data collection began, precisely 40 days after the final inoculation was performed. The assessment of the recipient plants for disease response was carried out using the parameters specified in Table 2 and Figure 4.

2.2.3.1 Disease Scoring

Table 2. Symptom description and severity rating for PepMoV screening.

Rating scale	Severity range (%)	Response
0	No symptoms (0%)	Immune
1	Mild mosaic chlorosis observed up to 10% of the plant (10%)	Highly Resistant
2	Mosaic and chlorosis up to 25 % of the plant (11-25%)	Moderately Resistant
3	Mosaic, mottle, changes in leaf texture up to 50 % of the plant (26-50%)	Moderately Susceptible
4	Mosaic, mottle, reduced leaf size with deformation up to 70% (51-70%)	Susceptible
5	Severe mosaic, mottle, deformation up to 100% (71-100%)	Highly susceptible

Adapted from: *Potato virus Y* disease rating scale by Ahmad et al. 2017.

Figure 4. Visual representation of the Disease Score (DS) for PepMoV resistance evaluation.

Along with the visual phenotypic evaluation, an ELISA test was performed to confirm the presence of the pathogen as described by the Agdia, Inc. protocol. The ELISA test detects the target analyte via DAS-ELISA. A set of 96-well microtiter plates were coated with Capture Antibody (CAB) diluted in 1X Carbonate coating buffer (0.015 M sodium carbonate (anhydrous), 0.035 M sodium bicarbonate, pH 9.6, containing 0.02% of NaN3) at a ratio of 9.6µl of capture antibody/ ml of Coating buffer. After thoroughly mixing both components, 100µl of diluted CAB were pipetted into each test-well of the provided microtiter plate and then the plates were incubated overnight at 2-8 °C and then emptied and washed three times with 1X PBST wash buffer (0.14 M sodium chloride, 0.008 M Sodium phosphate dibasic (anhydrous), 0.001 M Potassium phosphate monobasic (anhydrous), 0.003 M Potassium chloride, pH 7.4, containing 0.05% Tween® 20). About 0.5 g of symptomatic young tissue per plant was collected, ground, and diluted at a 1:10 ratio with 1X General Extraction Buffer (GEB) (0.01 M sodium sulfite anhydrous, 0.0005 M

polyvinylpyrrolidone (PVP) MW 24-40,000, containing 5% of a powdered egg (chicken) albumin, grade II and 46% of Tween® 20). A 100µl aliquot of the extracted samples and GEB hydrated fresh positive and negative controls were dispensed into their respective test-wells. The plates were incubated in a humid box for 2 hours at room temperature. After the incubation period, the plates were washed eight times with 1X PBST. The alkaline phosphate enzyme conjugate (ECA) was diluted in 1X ECI buffer (0.0005 M Polyvinylpyrrolidone PVP MW 24-40,000, containing 9% Bovine serum albumin BSA) at a ratio of 9.6µl of enzyme conjugate/ ml of ECI Buffer. A100µl aliquot of diluted ECA was pipetted into each test-well, then the plates were incubated for 2 hours at room temperature in a humid box followed by eight times wash with 1X PBST. Then, Para-Nitrophenyl phosphate (PNP) substrate tablets were diluted in 1X PNP substrate buffer (0.0005 M magnesium chloride hexahydrate and 1 M Diethanolamine) at a ratio of 1 tablet per 5 ml PNP buffer) and kept in the dark until use (15 minutes before use to ensure that the tablets were well dissolved). An amount of 100µl of diluted PNP solution was dispensed per test-well, and the plates were incubated and protected from light for 1 hour at room temperature. Finally, the ready to read plates were placed in Epoch™ 2 Microplate Spectrophotometer at 405 nm for obtaining the absorbance value OD values of each test-well with its corresponding sample for further interpretation of results to evaluate the presence of the target pathogen. Tests were considered positive when the OD_{405} of the sample was at least two times greater than the value of healthy control (negative control) supplied in the Agdia kit.

The negative threshold was equivalent to 0.32 of OD_{405} value. The final evaluation of the test plants for PepMoV resistance was based on combined results from the visual phenotyping and ELISA. And the plants were categorized into two groups: 1) plants with a disease score equal or less than 1 and that tested negative to the virus by DAS-ELISA were

considered resistant and rescored as 0; 2) plants that did not meet these thresholds were regarded as susceptible and rescored as 1. Disease scores were analyzed using a binary phenotype model in R Studio®.

2.2.4 QTL Analysis

The "qtl" package from RStudio® developed by Broman (2003) was used to identify the QTLs for the datasets. The "scanone" function with 1,000 permutations was used to calculate the logarithm of odds (LOD) score. The Haley-Knott regression was implemented to calculate the QTL model. The PepMoV trait was analyzed as a binary model due to the resistant (DS=0) or susceptible (DS=1) responses obtained from visual phenotyping plus ELISA results. The QTL model selection was performed using the function "stepwiseqtl" with a maximum QTL=3. The proportion of the phenotypic variance and LOD score of the QTLs were obtained using the functions "makeqtl" and "fitqtl" (Broman & Sen, 2009). The QTL location was determined using the linkage map derived from the SNPs physical order on the reference genome.

2.3 Results

2.3.1 Genotyping-by-Sequencing and Genetic Map Construction

A total of 1078 SNP markers were aligned to the 'Zunla-1' pepper reference genome. After omitting SNPs with more than 10% missing data and those with too much segregation distortion a total of 597 markers were selected and used for genetic map construction and QTL analysis. The SNPs obtained from the GBS data were named based on their physical position on the pepper reference genome and the chromosome where they were located. For example, the first marker of chromosome 1 was called C1M1 and so on with the rest of the

SNPs on each chromosome. The genetic map covered 1517.4 cM in total length with an average space of 2.6 cM between markers. (Figure 6).

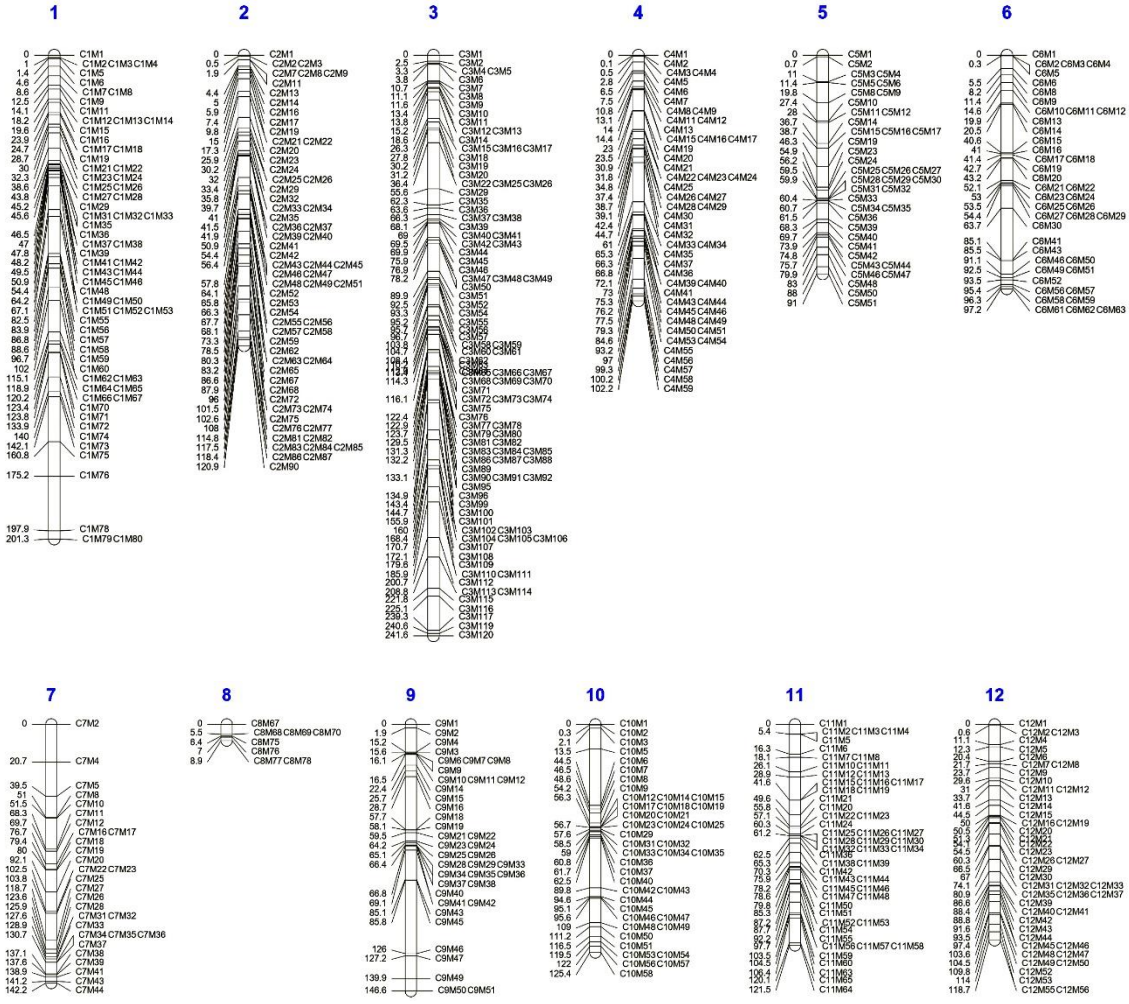

Figure 5. 'Hidalgo' x 'Bell 365' F_2 population linkage map constructed in the QTL package of RStudio®. The visual representation was obtained using the LinkagemapView package by Ouellette et al. (2018) of RStudio®.

A heatmap was used to visually evaluate the quality of the genetic linkage map (Figure 6) and it shows strong linkage between nearby markers, indicating a good quality genetic map.

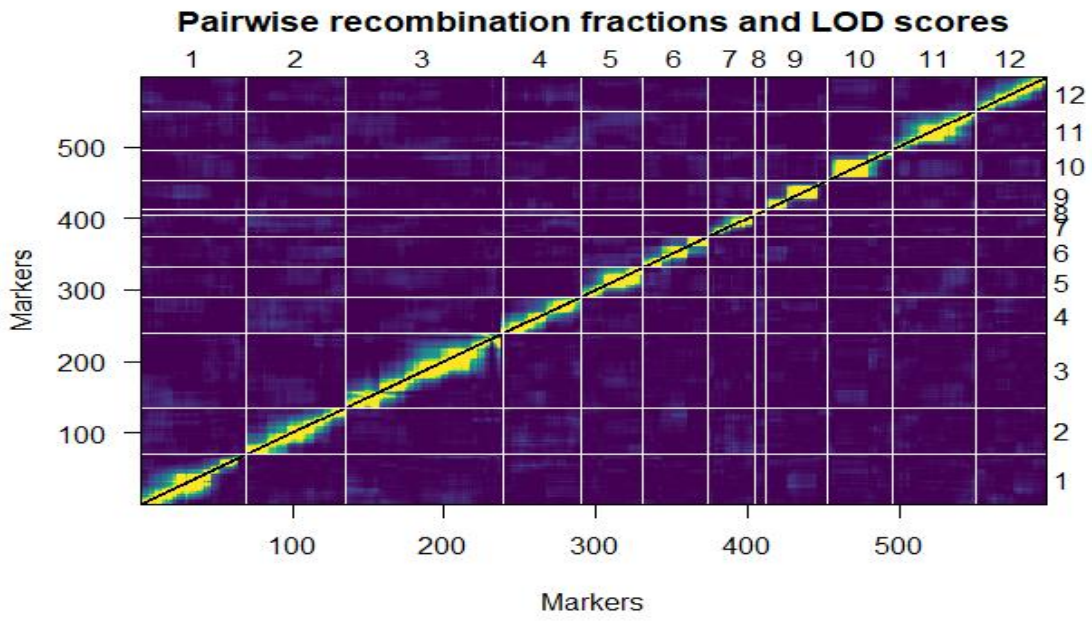

Figure 6. Heatmap of estimated recombination fractions (upper-left) and LOD scores (lower-right).

2.3.2 PepMoV phenotypic data

A total of 150 F_2 individual plants were screened for PepMoV, 40 days after the third and final inoculation, phenotypic data was taken, and a Disease Score (DS) was assigned to each plant, then individuals with the same DS were grouped to observe the F_2 population symptomatic response to PepMoV (Figure 7). Most of the F_2 plants (76 out of 150) received a DS of 1. In other words, they showed mild mosaic chlorosis observed on up to 10% of the plant, indicating that they were resistant or slightly susceptible to PepMoV.

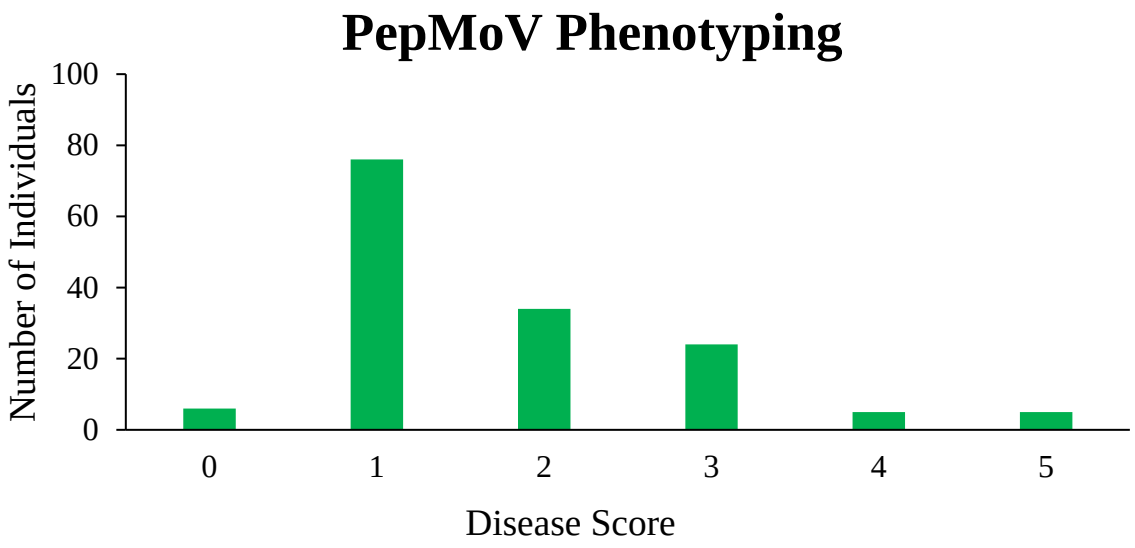

Figure 7. Number of individuals grouped by their respective PepMoV Disease Score.

After the visual scoring of the test plants, they were assayed by DAS-ELISA to ensure that the symptoms observed on the plants were due to PepMoV infection. A difference in color between positive (yellow) and negative (colorless) samples, due to the Alkaline Phosphate test used by this ELISA kit, was noticed when the target analyte was present or absent, respectively. The results interpretation and relative virus quantification was performed by measuring the Optical Density values (OD) with an Epoch ™ 2 Microplate Spectrophotometer at 405 nm wavelength. The positive and negative controls supplied by Agdia, Inc. were similarly processed as the test samples to to ensure the validity of the results. The positive and negative thresholds were determined using two times the OD_{405} value of the negative control or healthy average. Samples with an OD_{405} value higher than the negative point were scored as positive. In this experiment, we obtained a negative control OD_{405} average value equal to 0.16, resulting in a threshold of 0.32 (Figure 8).

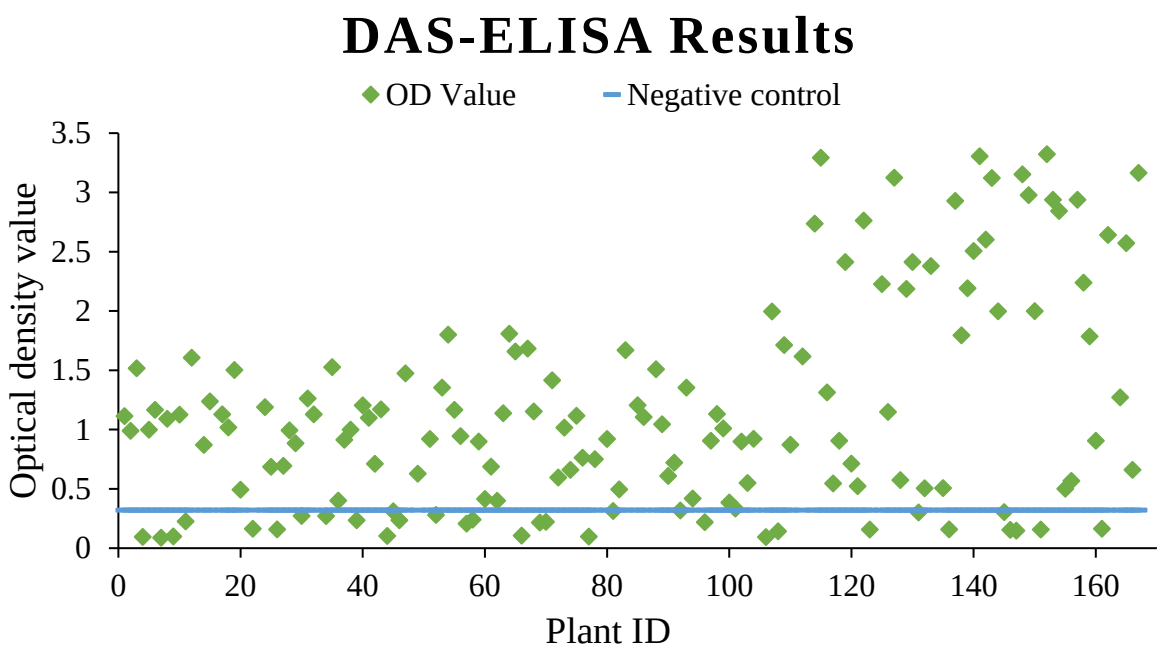

Figure 8. The distribution of optical density values at 405 nm wavelength (OD_{405}) obtained during diagnosis of mechanically inoculated pepper test plants for PepMoV by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). The blue horizontal line depicts a threshold of $OD_{405} \leq 0.32$ for negative samples.

After the visual evaluation of each plant and the performance of an ELISA test, we obtained information about the symptoms that each individual showed and confirmed that the symptoms were consistent with the presence of PepMoV. All the phenotypic data was integrated into two categories (Binary model), resistant and susceptible. Only 30 out of 150 plants were categorized as Resistant using the visual phenotyping plus DAS-ELISA results (Figure 9). The PepMoV resistance trait's phenotypic information fit a 1:3 ratio (α=0.05), indicating that the trait seems to be controlled by a single recessive gene (p-value=0.157) (Table 3).

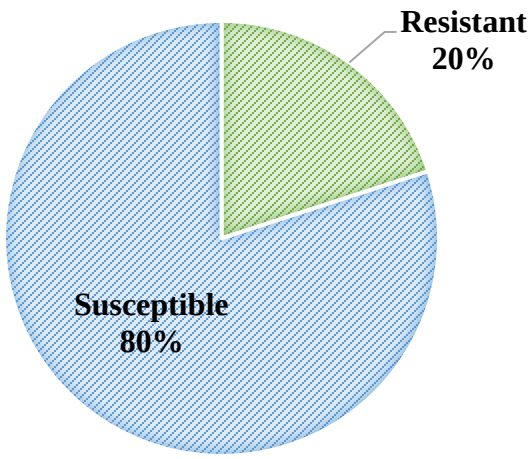

Figure 9. PepMoV screening phenotypic distribution.

Table 3. Chi-Square test for PepMoV phenotypic data.

Chi-Square Goodness of fit Test

Category	**Observed**	**Expected**	**Residual (Obs-Exp)**	**(Obs-Exp)^2**	**Contribution to X^2**
Resistant	30	37.5	-7.5	56.25	1.5
Susceptible	120	112.5	7.5	56.25	0.5
			X^2 value		2 ^{ns}
			P value		0.157 ^{ns}
			Degrees of Freedom		1

^{ns}= No significant.

2.3.3 PepMoV Quantitative Trait Loci analysis

The PepMoV QTL analysis was performed using the Haley-Knott regression method and binary phenotype model. A single QTL was discovered to be associated with PepMoV disease resistance located on Chromosome 6 (α=0.05) (Figure 10). Specifically, the PepMoV resistance-associated QTL was identified at 4.0 cM on Chromosome 6 (Figure 11). The

Confidence Interval shows that with 95% certainty, the QTL mentioned above can be located from 0 cM to 20.55 cM on Chromosome 6 (Table 4).

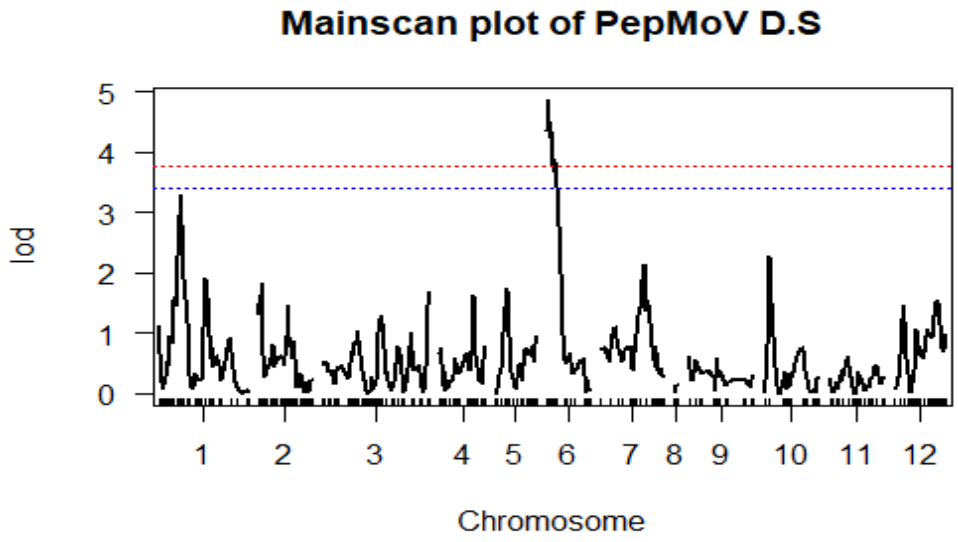

Figure 10. LOD scores by Haley-Knott regression of PepMoV resistance. The Blue dotted line represents a 0.90 confidence level (threshold=3.5), and the red dotted line is equivalent to a 0.95 level of confidence (threshold=3.75).

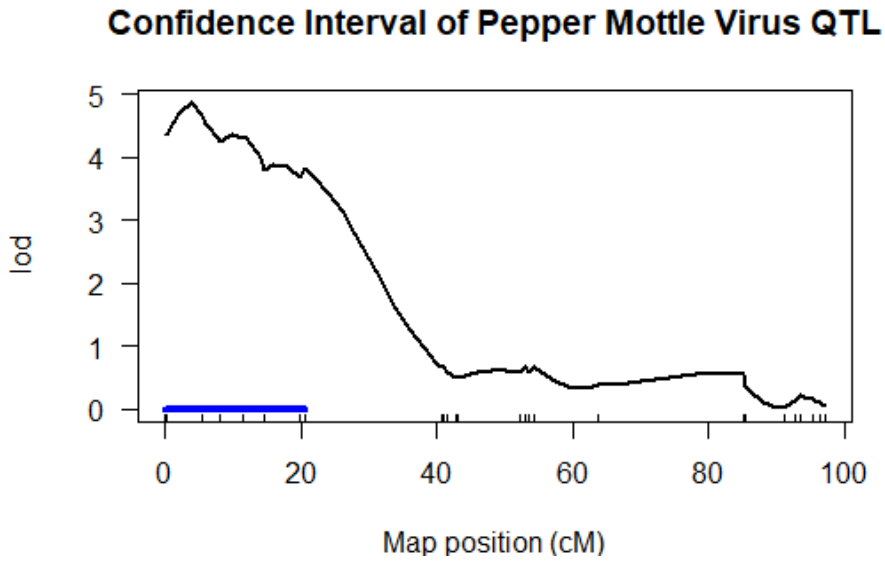

Figure 11. Confidence Interval (a=0.05) of the position of PepMoV resistance QTL on chromosome 6.

Table 4. Position (cM) and LOD score interval of PepMoV resistance QTL.

Marker	Position (cM)	LOD
Lower Marker C6M1	0.000000	4.34691302652796
Upper Marker C6M14	20.54699	3.81025016326891

A binary phenotype model was used for performing the QTL analysis, where the value 0 denoted that the individual's phenotype was resistant or unaffected to PepMoV, and Disease Score equal to 1 represented susceptibility to PepMoV. A significant QTL was identified to be associated with PepMoV resistance in pepper at 4.0 cM near marker six on Chromosome 6 (2.86Mb), responsible for explaining about 19% of the phenotypic variation (Table 5); the trait seems to be controlled by a single recessive gene inherited from the ´Hidalgo´ Parent (AA) (Table 4). The AA Genotypic mean is significantly lower than the Heterozygous AB and Homozygous genotype BB, meaning that plants that inherited both alleles from the resistant parent 'Hidalgo' had a better disease resistance response compared with the rest of the plants (Figure 14).

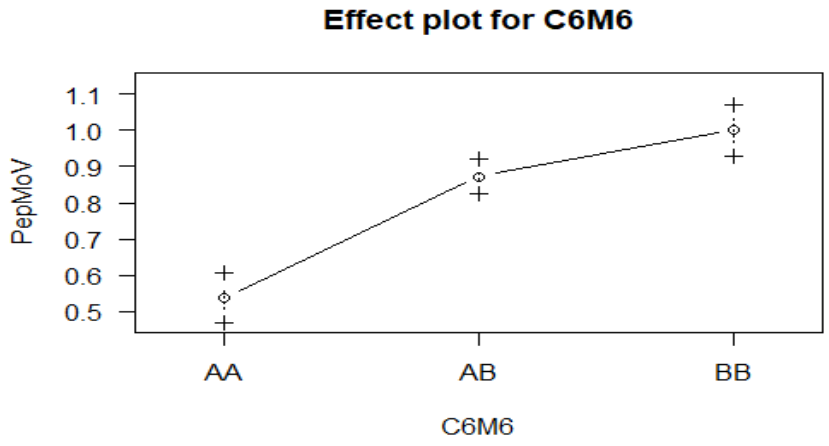

Figure 12. Disease Score Means of each Genotype at PepMoV resistance QTL location (C6M6).

Table 5. QTL effect in the Phenotypic variance of PepMoV resistance trait.

Summary				
Method		Haley-Knott regression		
Model		Binary Phenotype		
	Df	LOD	%var	P value (Chi^2)
Model	2	4.85642	19.18411	1.39181e-05

2.4 Discussion

Results obtained from the F_2 population derived from the cross of 'Hidalgo' x 'Bell 365' showed that 1078 SNP markers were obtained from GBS and subsequently filtered out for linkage map construction. Only individuals and markers with less than 10% of missing data were used. Then, the genotypes of all pairs of individuals were compared to identify unusually similar genotypes. One individual of each pair with more than 90% similarity was removed. The marker segregation distortion patterns were analyzed; the markers were expected to segregate in a 1:2:1 ratio or approximate proportion. Markers with a significant departure from the ratio mentioned above were omitted, because according to Broman, 2010 moderate deviations from these frequencies may indicate the presence of partially lethal alleles, and flagrant departures often are indicators of problematic markers like monomorphic markers, markers difficult to call (AA could be called as AB), or markers with rare genotypes that have difficulties in genotyping. Departures from segregation patterns were especially noticeable on Chromosome 8, where some of the markers were omitted due to its small P-value for a test of deviation from the expected ratio after the use of the function "geno.table" ($P<0.05$). The segregation distortion clusters noticed in Chromosome 8 could happen due to an unplanned selection in the breeding process (Taylor & Butler, 2017).

Typically, markers with segregation distortion are distributed in clusters and unbalanced in the same direction as we observed in several markers in Chromosome 8, these clusters are called segregation distortion regions (SDRs) (Dai, et al., 2017). The inadvertent selection of an allele at a specific locus results in the deviation of nearby markers from the expected mendelian ratio, this phenomenon is defined as the genetic hitchhiking theory (Dai, et. al., 2017). After filtering and omitting undesirable markers, 597 markers were used to form the linkage groups. A total of 12 linkage groups were created using a maximum recombination fraction (max.rf) of 0.35 and a minimum LOD score (min.lod) of 6 to group the markers. The markers were structured, prioritizing the order that resulted in the shortest chromosomal length and the LOD (log_{10} likelihood) value possible relative to the initial order. Finally, the average space between markers was 2.6 cM, with a total linkage map coverage of 1517.4 cM.

The PepMoV phenotyping was based on the combined results of the visual evaluation of the plants and the DAS-ELISA test. The visual analysis of the plants demonstrated that many of the plants (76 out of 150) had mild PepMoV symptoms. After performing the DAS-ELISA test, a difference was noticed among individuals with a DS of 1. Approximately 29% of these 76 individuals (n = 22) tested negative for PepMoV, while the other 54 individuals tested positive for the virus. Hence, the criteria were established to categorize only plants with a DS≤1 and with a negative PepMoV DAS-ELISA test as Resistant, resulting in a binary phenotype where the unaffected or resistant individuals got a score of 0, and the susceptible a score of 1. The comparison between the visual data and the ELISA results showed a moderate positive correlation (ρ=0.31) (data not shown), suggesting that the OD_{405} values from ELISA tend to increase with the DS value increment. But the relationship

between these two types of results significantly decreases among the susceptible individuals (ρ=0.06), where the two variables seem not to be associated (data not shown).

The movement of PepMoV could explain the lack of correlation among the susceptible individuals through the vascular tissue of the plant that directly impacts the virus's capacity to infect new tissue. A PepMoV-FL infection analysis in Avelar pepper plants showed that the virus has a restricted movement to invade young tissue (Guerini & Murphy, 1999; Murphy & Kyle, 1995). The slow movement of the virus inside an infected plant directly affects the amount of symptomatic tissue, which at the same time will impact the visual phenotyping of individuals (DS) and its relationship with the amount of antigen that could be identified in an ELISA test (OD_{405} value). This is because a high absorbance value is an indicator of the virus accumulation in a specific foliar section in the plant, not necessarily of the plant's distribution of symptoms. That is why some individuals observed with relatively low DS=2 (symptoms up to 25% of the plant) scored high OD_{405} values and vice versa. Another possible explanation could be that the PepMoV resistance gene involved modifies the resistance by explicitly restricting the long-distance movement of PepMoV without triggering a hypersensitive response (HR). A similar response was found by Whitham et al. (2000) in a study with *Arabidopsis*, where two dominant genes, *RTM1* and *RTM2*, mediated a specific movement restriction for TEV. The *RTM* gene system could recognize TEV factors and induce a TEV-specific defense response and stimulate anti-TEV in inoculated leaves (Whitham et al., 2000).

The phenotypic distribution of the PepMoV resistance trait fit a 1:3 ratio (α=0.05), suggesting that the trait might be recessive. Also, a single gene model seems to be the best explanation for the results obtained (P-value=0.157). These results are similar to the findings

reported by Zitter & Cook (1973), where they showed that PepMoV resistance was a monogenic recessive factor. This monogenic recessive factor was named *pvr3*, with the resistance locus identified in *C. annuum* cv. 'Avelar' (Murphy et al., 1998). Besides, an evaluation in *C. annuum* 'CM334' also reported that the resistance is controlled by a single gene located at the *Pvr4* locus, but with the difference that the *Pvr4* conferred dominant resistance to the virus (Venkatesh et al., 2018).

The QTL analysis was performed using the Genotypic information from GBS and the phenotypic information obtained after screening each F_2 plant under greenhouse conditions, using reference tables and visual representation of symptoms to give a specific DS. The log10likelihood (LOD) score was calculated by comparing the hypothesis that there is a QTL at the marker location (Ha) against the hypothesis that there is no QTL elsewhere in the genome (Ho).

The Haley Knott regression method was used to identify the QTLs associated with the PepMoV resistance trait. The "hk' regression was used mainly because this method provides a fast approximation of standard interval mapping; the Haley-Knott method is more robust than standard interval mapping when missing genotype data is not appreciable (Broman & Sen. 2009). Also, the Haley-Knott method was selected because the comparison of this method with standard interval mapping and multiple imputations did not show differences in the LOD curves while saving computing time (see Figure 13 and 14).

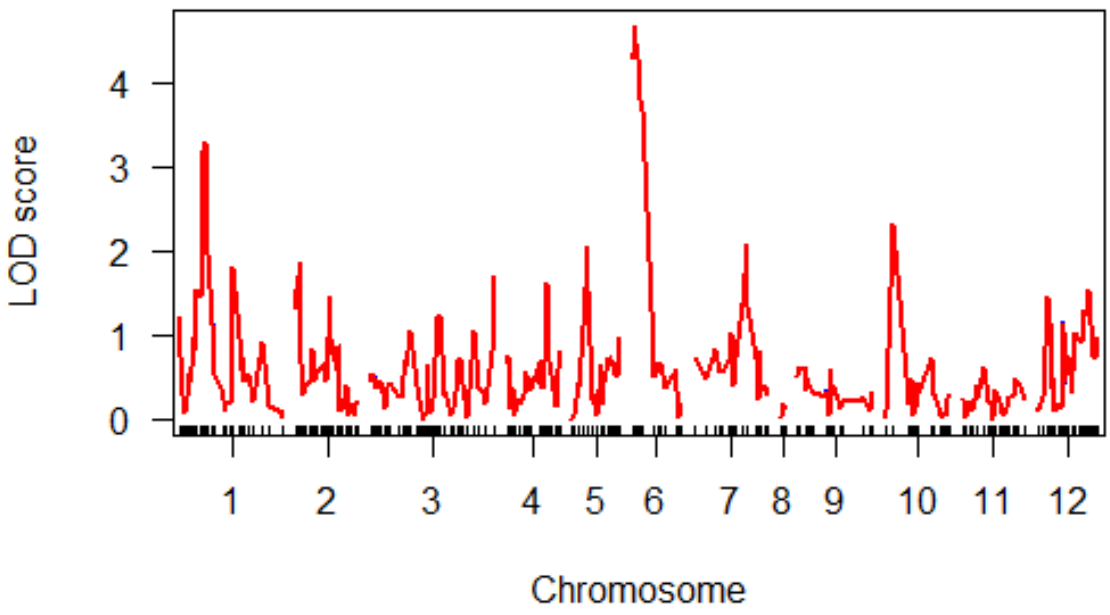

Figure 13. LOD scores for the PepMoV data by Haley-Knott regression (blue) and standard interval mapping (red).

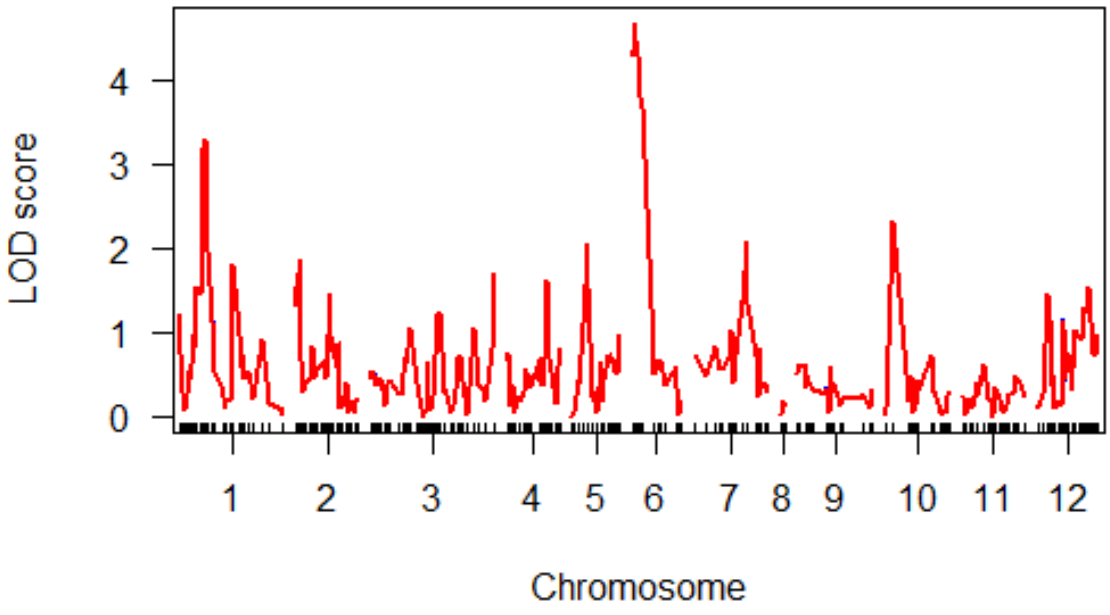

Figure 14. LOD scores for the PepMoV data by Haley-Knott regression (blue) and multiple imputations (red).

Table 6. Relative advantages and disadvantages of different interval mapping methods. Source: Broman & Sen., 2009. A guide to QTL mapping with R/qtl.

	Use of genotype information	Robustness	Selective genotyping	Speed
Standard interval mapping	++	-	+	-
Haley-Knott	-	+	-	+
Extended Haley-Knott	+	+	+	-
Multiple imputation	++	+	+	--

After comparing the different interval mapping methods and selecting "hk" regression due to its reduced computing time and similarity of the results to other methods (Table 6), the selection of the QTL model was performed using the "stepwiseqtl" to identify the QTL by optimizing the penalized LOD score (pLOD). This function is an automated stepwise algorithm that optimizes the model's penalized scores (Manichaikul et al., 2009; Broman & Sen, 2009). The penalized LOD score for a model compares the null model LOD score (no QTL), with a penalty subtracted for each main effect and separate penalties subtracted for each pairwise interactions among QTLs. The QTL model with the highest pLOD was selected (Table 7). The single QTL model had the highest pLOD (pLOD=1.89). A major QTL was detected for PepMoV resistance at 4.0 cM (Figure 15) of the distal region (p) on chromosome 6, explaining about 19% of the phenotypic variation.

Table 7. QTL models searched by the "stepwiseqtl" function with the PepMoV data.

Formula	Location		pLOD
	Chr	Pos	
y ~ Q1	6	6@4	1.891518
y ~ Q1 + Q2	6,1	6@4, 1@46.5	1.153147
y ~ Q1 + Q2 + Q3	6,1,3	6@4, 1@46.5, 3@14	-0.2654249

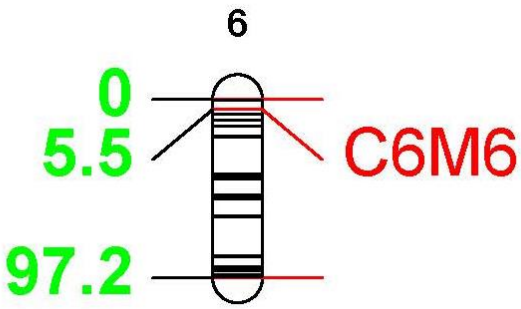

Figure 15. PepMoV QTL location on chromosome 6.

Viruses are obligate parasites that required a living cell to survive. A few R-genes have been reported that confer resistance to potyviruses like PepMoV in pepper hosts. These R-genes are known as *Pvr* (*Potyvirus* resistance) genes, which typically confer a broad-spectrum resistance, like *Pvr4* that confers resistance against all known strains of PepMoV, pepper severe mosaic virus (PepSMV), and PVY (Kim et al., 2015). Also, it has been reported that *Pvr* genes like *Pvr4*-mediated resistance in pepper plants show a hypersensitive response (HR), where the plant rapidly restricts the spread of the pathogen by triggering the death of the infected cells (Kim et al., 2015). Unlike Pvr*4*, the results obtained in this project indicate that the trait seems to be controlled by a single recessive gene. The results observed in the F_2 population suggest that the *pvr3* locus might be controlling the resistance found in

our study since the ‘Hidalgo’ parent is the result of the hybridization of *C. annuum* cv. ‘Avelar’ and ‘PI 342947’. Currently, there are two proposed hypotheses to explain how resistance genes confer resistance to pathogens. The first hypothesis suggests that the resistance results from a passive mechanism, in which the virus cycle is compromised due to the lack of a specific host factor or due to a mutated version of that specific factor (Díaz-Pendon et al., 2004). The second hypothesis proposes that the resistant plants produce inhibitors that interrupt the virus cycle, or the plant can recognize virus encoded molecules and therefore trigger a resistance response (an active mechanism) (Díaz-Pendon et al., 2004).

SNPs are proven to be universal, the most abundant forms of genetic variation among individuals, and amenable to high throughput automation (Mammadov et al., 2012). The QTL mapping results obtained on this experiment can be valuable for the establishment of Marker Assisted Selection (MAS) to develop germplasm with resistance to PepMoV. MAS has advantages over conventional phenotypic selection in that it helps to reduce the time required for selection of plants carrying the desirable trait since with MAS, selection can be performed at the seedling stage; it also reduces resource and effort needs compared to phenotypic (Collard & Mackill, 2008). However, the results obtained from the QTL analysis about the position and effect of the QTL identified on chromosome 6 must be validated by testing the QTL in several locations, years, and different genetic backgrounds to confirm the QTL position, effect, and effectiveness (Mammadov et al., 2012). Marker validation, which is the process of assessing markers in relevant breeding material and may include the development of a "toolbox" of polymorphic markers within a certain genetic distance window (e.g., 10cM) that may be used for MAS, is also crucial. (Collard & Mackill, 2008). For the detection of the presence/absence of the PepMoV resistance locus that can be

identified using the QTL information provided in this experiment, a post-PCR analysis method like the high-resolution melting (HRM) analysis combined with a rapid DNA extraction system can be used to perform marker assisted selection. According to Noh et al. (2017), HRM-based assays require instruments that cost slightly more than a regular quantitative real time PCR system for SNP genotyping. But, the cost of each probe using a regular quantitative real time PCR system is approximately $200 and it requires two probes per marker, while HRM analysis does not need advance sequencing of the target region and HRM markers cost about $6, which can detect any SNP presence in a targeted region (Noh et al., 2017). SNP genotyping can also be done using a platform such as KASP (Kompetitive Allele Specific PCR). KASP is a fluorescence-based genotyping method, in which the DNA is amplified using a thermal cycler and allele-specific primers to interrogate samples for a specific locus (Yang, et al., 2019). According to Zhao et al. (2017), KASP is frequently used for SNP genotyping studies because a genotype is easy to detect by reading a fluorescent signal and due to its low cost, high throughout, specificity and sensitivity.

Recently, resistance to other potyviruses like the *Chili Veinal Mottle Virus* (ChiVMV) has been identified on the short arm (p) of chromosome 6 of the pepper genome, the source of the resistance is known as 'NW4' pepper cultivar, and it is reported as a single dominant gene mapped at 3 cM away from the C2At2g39690 CAPS marker (Lee et al., 2013). Despite the fact that there are a few pepper resistance genes against potyviruses such as PepMoV, none of them have been described at the molecular level, with the exception of the recently revealed Pvr9 gene. Tran et al. (2015) studied the molecular properties of the Pvr9 gene, which is situated on the short arm of pepper chromosome 6, approximately 2.1 Mb away from the C2At3g25120 and 5.4 Mb from the C2Atg39690 COSII markers. In

pepper, Pvr9 gene did not respond to PepMoV, possibly due to an unknown mechanism that facilitates Pvr9 protein recognition of PepMoV NIb and Tran et al. (2015) suggested that a third factor is present in *Nicotiana benthamiana* that is absent in peppers. It is not known if the QTL identified in this study is allelic to the *pvr3* gene, but this should be examined further based on the similar recessive PepMoV resistance response and the genetic background of the 'Hidalgo' parent derived from 'Avelar'. Several genes have been identified and located between the PepMoV resistance QTL confidence interval (α=0.05), but the genes CA06g03140 (Lactoylglutathione lyase/glyoxalase I family protein), CA06g03150 (putative methyltransferase family protein), CA06g03170 (FRIGIDA-like protein 3-like), CA06g03180 (putative transposon MUDR mudra-like protein), CA06g03190 (Glycosyltransferase), CA06g03200 (mediator of RNA polymerase II transcription subunit 19a-like), CA06g03210 (ribosomal protein L5), CA06g03220 (P-type H+-ATPase), and CA06g03230 (probable LRR receptor-like serine/threonine-protein kinase At1g74360-like) seem to be the closest to the QTL location on chromosome 6 (Fernandez-Pozo et al., 2015).

2.5 Conclusion

The primary objectives of this research study were to identify QTLs associated with PepMoV resistance and observe the pattern of inheritance of the resistance in an F_2 population derived from the cross of the serrano pepper 'Hidalgo' by the bell pepper 'Bell 365'.

The results obtained from the PepMoV screening showed that the resistance might be monogenic and recessive. A major QTL was identified on chromosome 6, explaining ~20% of the phenotypic variation. According to Hulbert et al., (2001) plant resistance genes

are frequently found in clusters of closely related genes. The QTL associated with PepMoV resistance identified on this research project might be linked to another resistance genes like the ChiVMV gene or the *Pvr9* gene mapped on chromosome 6.

Unlike gene-for-gene resistance, which depends on the quick activation of defense responses when the host recognizes the pathogen and habitually results in the development of an HR conferred by single dominant resistance gene (R-genes), recessive resistance seems to be due to mutations in the genes encoding host factors required for infection and can restrict viral propagation in the host or it may be due to an active mechanism based on the activation of plant defenses (Díaz-Pendon et al., 2004). According to Lellis et al. (2002), recessive resistance genes are more frequently seen for plant pathogenic viruses than other pathogens, especially more frequently for potyviruses (Días-Pendon et al., 2004).

CHAPTER III

POWDERY MILDEW RESISTANCE TRAIT

3.1 Introduction

Powdery mildew caused by *Leveillula taurica* (Lev.) Arn. (anamorphic stage*:* *Oidiopsis taurica* (Lev.) Salmon) is a disease that affects pepper leaves in greenhouses and fields (mainly coastal regions) (Brand et al., 2009; Karkanis et al., 2012). The disease's main sign is white powdery, fungal growth covering the abaxial surface of the leaf. The adaxial leaf surface may be yellow or brownish, and the fungus may sporulate on this surface in some instances. Infected leaves fall prematurely from the plant, exposing the fruit to the sun, resulting in sunscald (Goldberg, 2004). A severe outbreak of powdery mildew has been reported to cause up to 80 percent loss in yield (Karkanis et al., 2012).

Due to the productivity and monetary damage caused by diseases like powdery mildew, it is of utmost importance to continue developing resistant germplasm adapted to the needs of farmers and consumers. This research would provide new data and information about pepper disease-resistant germplasm that could be used to develop new commercial lines in the future. Also, it would identify regions in the pepper genome (QTLs) that are associated with powdery mildew resistance through the phenotyping of 151 F_2 population derived from the cross of ‘Hidalgo’ parent by the ‘Bell 365’ parent and the use of SNP's data for the construction of a genetic map that is a prerequisite for the QTL analysis.

3.2 Materials and Methods

3.2.1 Genotyping

The same data obtain from GBS described in section 2.2.1 was used for this second experiment. The SNP´s data was prepared with the appropriate format to be used in Rstudio®.

3.2.2 Genetic Map Construction

The linkage map construction was done using the methodology described on section 2.2.2. The genetic map construction was carried out using the "qtl" package from Rstudio®.

3.2.3 Powdery mildew phenotyping

Powdery mildew screening for pepper resistance was performed on the original F_2 population obtained from the cross of 'Hidalgo' x 'Bell 365' (from which a second clonal population was obtained through stem cuttings to conduct *Pepper mottle virus* screening, as previously described). The original F_2 population was selected because they had more growth time than the clonal population to be exposed for more days to the infection of *L. taurica*, which occurs naturally in the greenhouse, where the project was conducted if fungicide application is not practiced.

Powdery mildew screening was performed during Spring 2020 because the symptoms and signs of the disease are most frequently observed in peppers during this period, likely due to cooler (10 to 20°C) temperatures that favor the pathogen. The optimal temperature for L. taurica development in pepper, according to Elad et al. (2007), is approximately 20°C, while leaf loss is more severe at temps between 10 and 20°C.

In addition to the natural inocula present in the experimental greenhouse, five artificial inoculations were carried out for each test plant to ensure a significant disease pressure. The main objective of these artificial inoculations was to select the most resistant genotypes in the F_2 population. The first and the final inoculations were done using white powdery mildew spores collected from leaves of naturally infected plants grown in the same location via the dropping method, which consists of collecting leaves with fungal spores and shaking them generously on the foliage of the plants to be inoculated. The three intermediate inoculations were performed via preparation of a solution obtained by washing the fungal spores from ~10 g of the infected material with distilled water, followed by atomizing the solution onto the foliage of the F_2 population. The plants were spaced approximately 25 cm apart within a row in 1-meter-wide benches and irrigated and fertilized as required.

3.2.3.1 Disease Scoring

The ‘Hidalgo’ x ‘Bell 365’ F_2 plants were evaluated for powdery mildew disease resistance 60 days after the final inoculation using the disease scale shown in Table 8 and Figure 16. Each F_2 plant was divided into two parts, the top, and the bottom portion, and a score was assigned according to the general appearance of each section (at least 75% of whole leaves with similar symptoms). The total score of the plant was obtained by averaging the two scores.

Table 8. Symptom description and severity rating for a pepper F_2 population infected with powdery mildew fungus *Leveillula taurica*.

Disease score	Severity range (%)	Response
0	No visible sporulation	Immune
1	Local chlorotic spots on the adaxial leaf surface with weak or no sporulation on the corresponding abaxial leaf areas.	Highly Resistant
2	Several isolated sporulation spots on the abaxial leaf surface.	Moderately Resistant
3	Numerous sporulation spots covering up to 40% to 50% of the abaxial leaf area.	Moderately Susceptible
4	Numerous coalescent sporulation spots covering up to 75% of the abaxial leaf area.	Susceptible
5	The complete abaxial surface of the leaf, and parts of the adaxial surface, covered with dense sporulation.	Highly susceptible

Adapted from: Zheng et al. (2013) and McCoy & Bosland (2019).

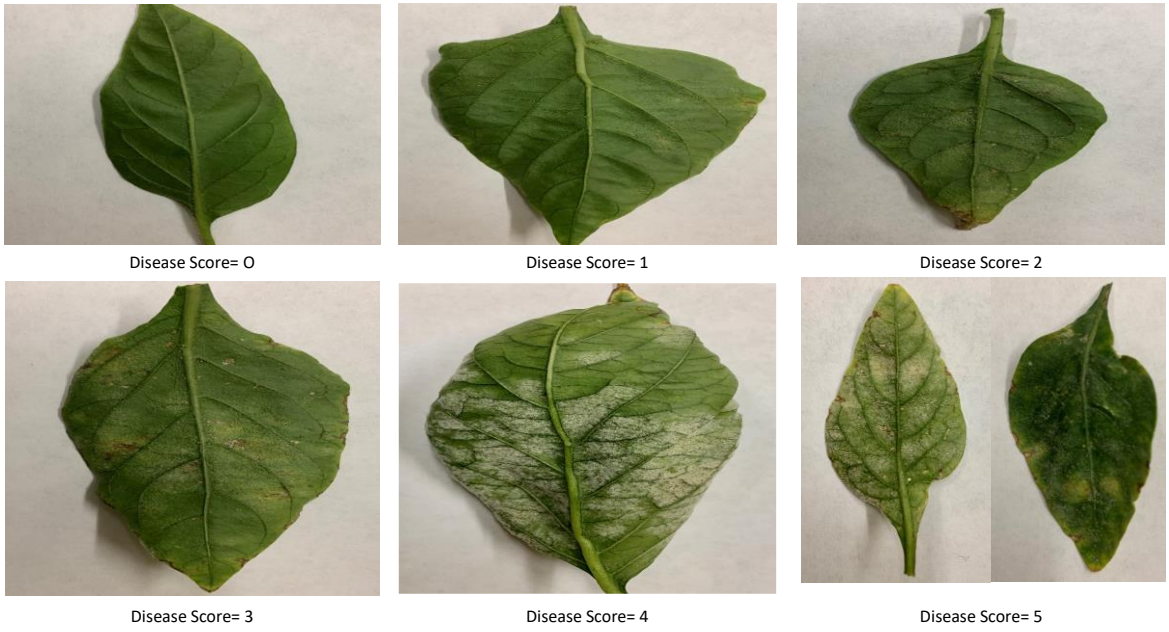

Figure 16. Visual representation of Disease Scores (DS) for powdery mildew based on Zheng et al. (2013) and McCoy & Bosland (2019).

Plants with a total average score of DS≤1 were considered resistant. Moderately resistant plants were defined as plants with an average score of $1.5 \leq DS \leq 2.5$, and plants with an average score of DS >2.5 were considered susceptible.

3.2.4 QTL Analysis

The QTL package from RStudio® developed by Broman (2003) was used to identify the dataset's QTLs. The "scanone" function with 1,000 permutations was used to calculate the logarithm of odds (LOD) score. The Haley-Knott regression was implemented to calculate the QTL models. The powdery mildew data was analyzed as a normal model. The QTL model selection was performed using the function "stepwiseqtl" with a maximum QTL=5. The proportion of the phenotypic variance and LOD score of the QTLs were obtained using the functions "makeqtl" and "fitqtl" (Broman & Sen, 2009). The QTL location was determined using the linkage map derived from the physical order on the reference genome.

3.3 Results

3.3.1 Genotyping-by-Sequencing and Genetic Map Construction

A total of 597 SNP markers were selected and used for the construction of the linkage map. The genetic map covered 1517.4 cM in total length with an average space of 2.6 cM between markers. For more detailed information refer to section 2.3.1.

3.3.2 Powdery mildew phenotypic data

A total of 151 F_2 individual plants out of the 167 initially planted (16 seedling died before the evaluation) were screened for powdery mildew resistance 60 days after the last inoculation. Phenotypic data were taken where every individual was visually evaluated and then grouped by its corresponding Disease Score to observe the F_2 population symptomatic

response. (Figure 17). Most (~69% or 104/151) of the F_2 plants received a Disease Score that ranged from 0 to 2.5 on average. The severity of symptoms scale ranged from non-visible sporulation to several isolated sporulation spots on the abaxial leaf surface. Most plants had a response of moderately resistant to resistant (Figure 18).

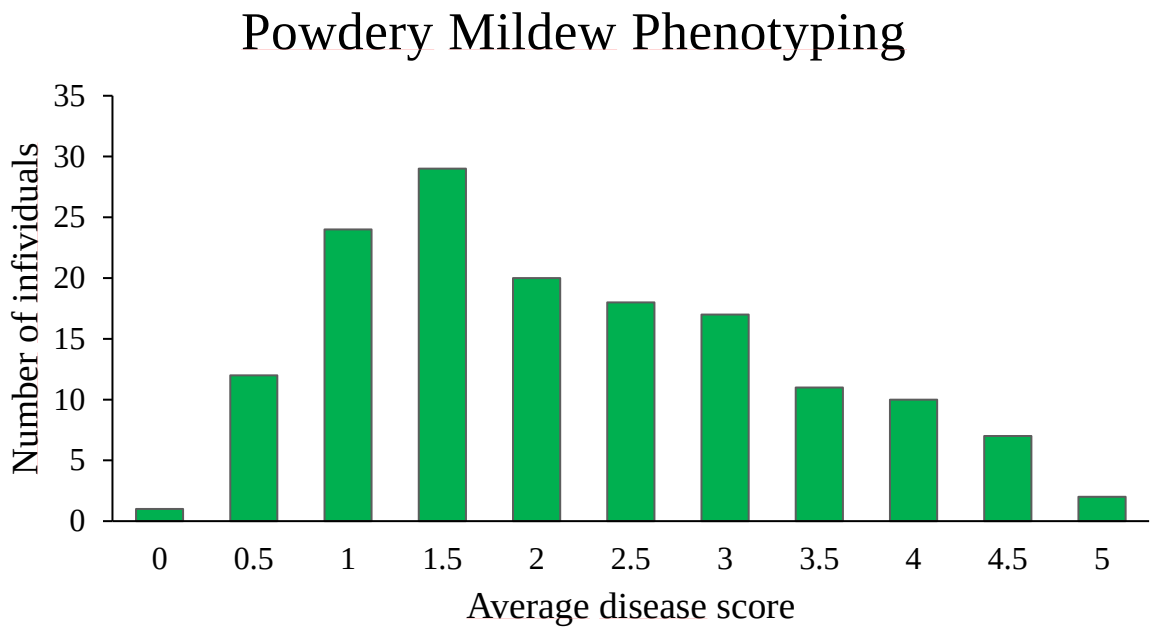

Figure 17. Number of individuals grouped by their respective powdery mildew disease score.

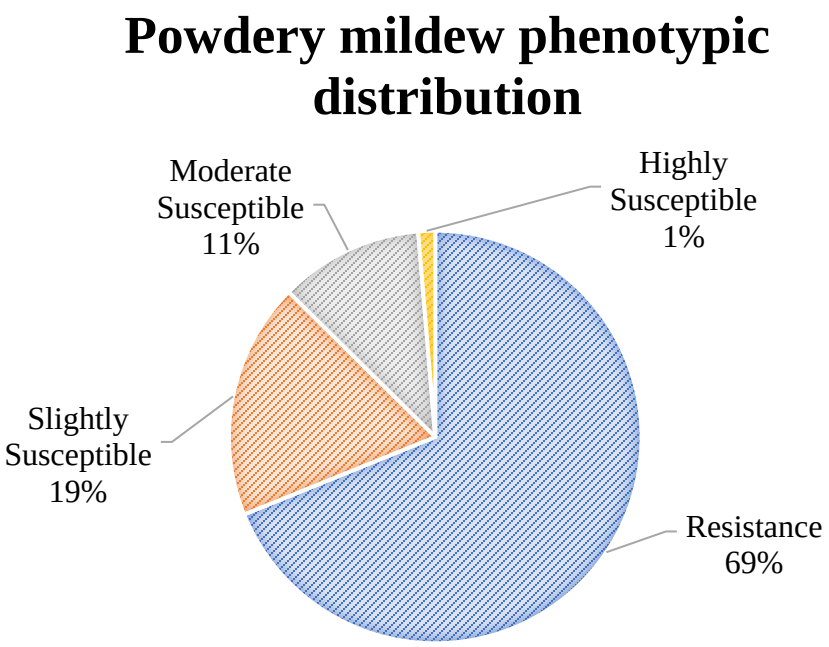

Figure 18. Powdery mildew screening phenotypic distribution.

The phenotypic data of powdery mildew screening fits a 3:1 ratio (α=0.05), indicating that the trait seems to be controlled by a single dominant gene, which means that both Homozygous dominant and Heterozygous individual will show a similar level of resistance to the pathogen (p-value=0.0821) (see Table 9).

Table 9. Chi-Square test for the powdery mildew phenotypic data.

Chi-Square Goodness of fit Test

Category	**Observed**	**Expected**	**Residual (Obs-Exp)**	**(Obs-Exp)^2**	**Contribution to X^2**
Resistant	104	113.25	-9.25	85.5625	0.7555187
Susceptible	47	37.75	9.25	85.5625	2.2665562
			X^2 value		3.0221 ^{ns}
			P value		0.0821^{ns}
			Degrees of Freedom		1

$^{ns=}$No significant.

3.3.3 Powdery Mildew Quantitative Trait Loci Analysis

The powdery mildew QTL analysis was performed using the Haley-Knott regression method and normal phenotype model. A large effect QTL was discovered to be associated with powdery mildew disease resistance located on chromosome 1 (Figure 19). Specifically, the powdery mildew resistance-associated QTL was identified at 31 cM on Chromosome 1 (Figure 20). The Confidence Interval shows that with 95% certainty, the QTL, as mentioned earlier, can be located from 30 cM to 32 cM on chromosome 1 (Table 10).

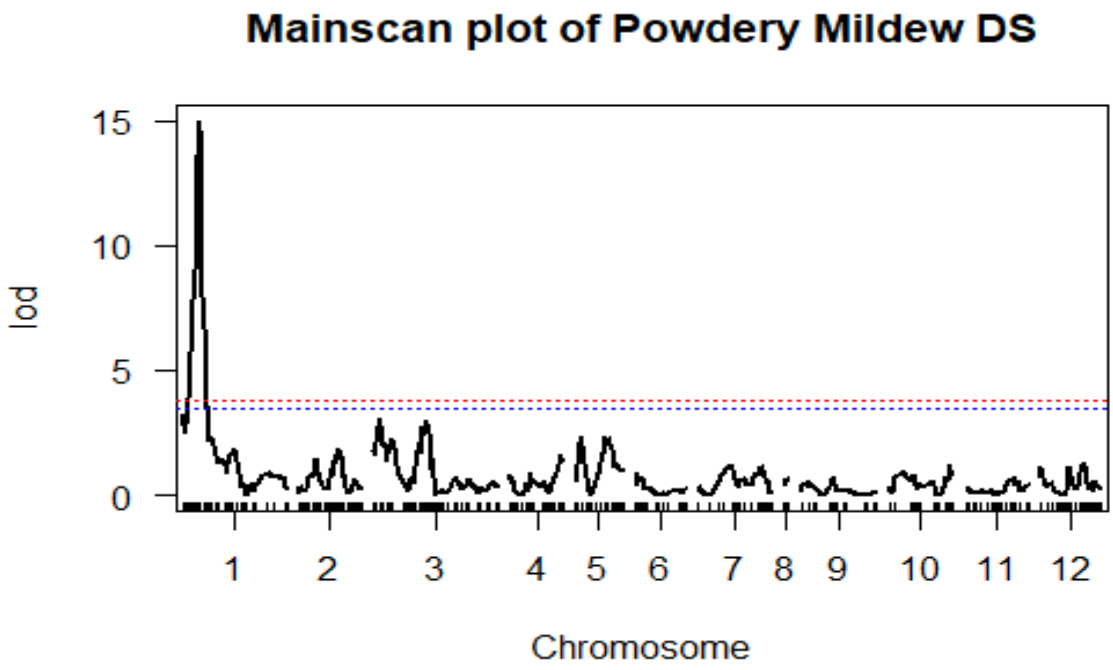

Figure 19. LOD scores by Haley-Knott regression of powdery mildew resistance. The blue dotted line represents a 0.90 confidence level, and the red dotted line is equivalent to a 0.95 level of confidence.

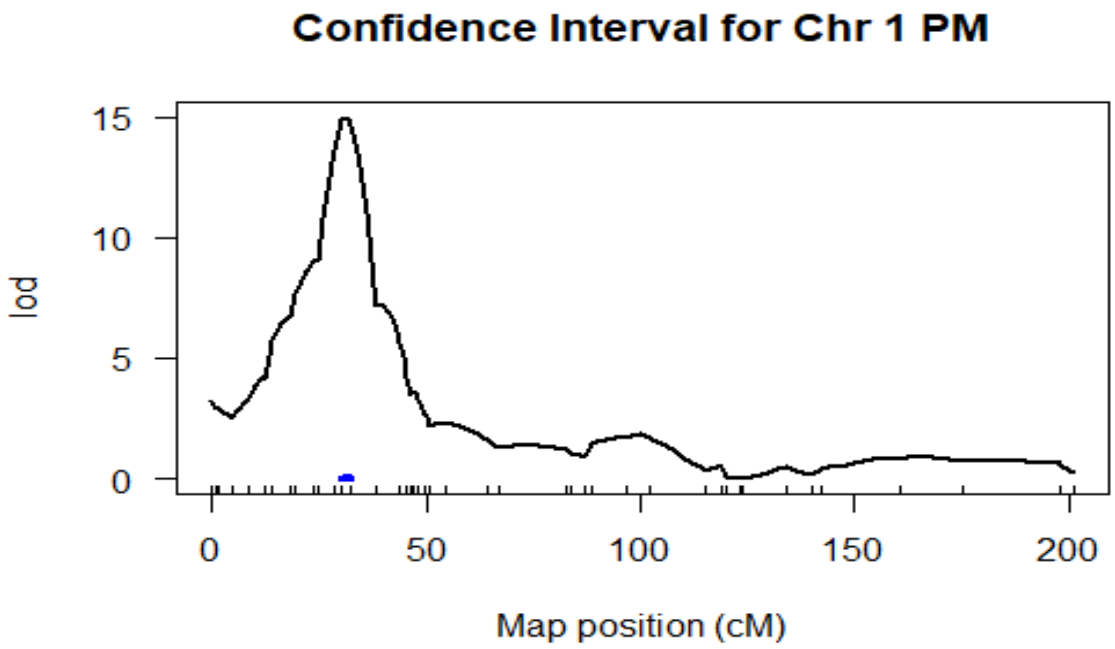

Figure 20. Confidence Interval (a=0.05) of the position of powdery mildew resistance QTL on chromosome 1.

Table 10. Position (cM) and LOD score interval of powdery mildew resistance QTL.

Marker	***Position (cM)***	***LOD***
Lower Marker C1M21	30.0321674	14.8543654325403
Upper Marker C1M24	32.3040423	14.6669207781536

A normal phenotype model was used for performing the QTL analysis, where the disease values, denoted as the individual's phenotype score, was equal to or less than DS=2.5. A plant with this score is classified as an individual of interest due to its moderate to a high degree of resistance to powdery mildew, while a DS greater than 2.5 represented susceptibility to the fungus. A significant QTL was identified to be associated with powdery mildew resistance on pepper at 31 cM near marker 23 on Chromosome 1 (14.88 Mb),

responsible for explaining 49% of the phenotypic variation (Table 11). The trait seems to be controlled by one gene inherited from the Hidalgo Parent (AA) because the genotypic mean of AA is significantly lower than the Homozygous genotype BB and like the Heterozygous genotype (AB) (Figure 21).

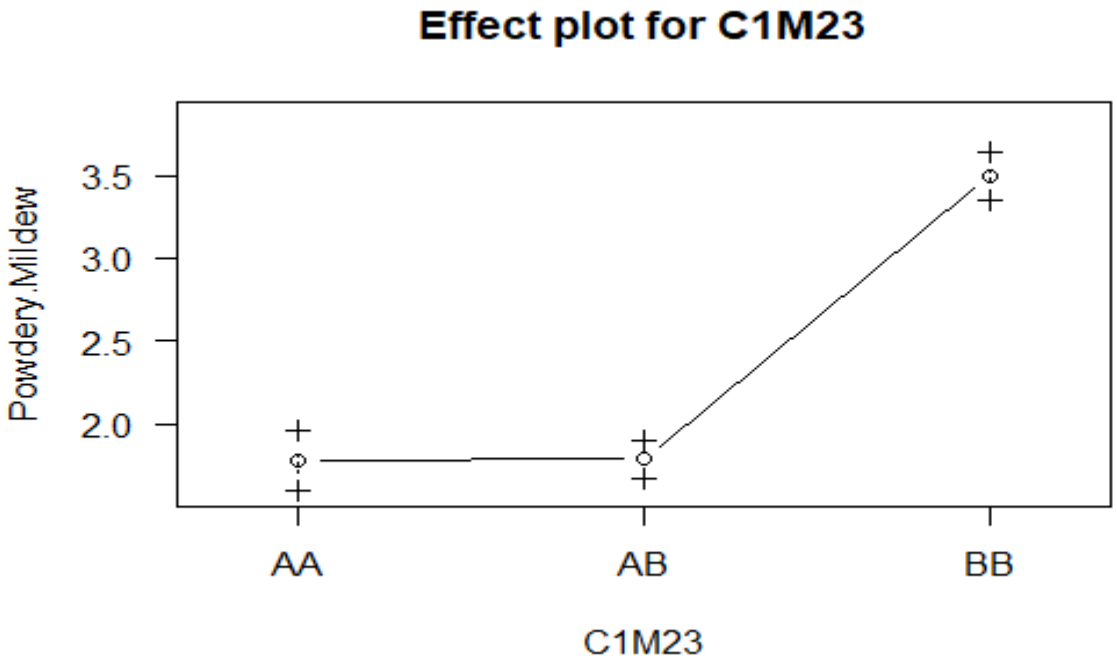

Figure 21. Disease score means of each genotype at powdery mildew resistance QTL location (C1M23).

Table 11. QTL effect in the phenotypic variance of powdery mildew resistance trait.

Summary							
Method				Haley-Knott regression			
Model				Normal Phenotype			
	Df	SS	MS	LOD	%var	P value (Chi^2)	P value (F)
Model	2	64.73457	32.3672852	15.00028	49.19847	9.992007e-16	2.775558e-15
Error	99	66.84386	0.6751905				
Total	101	131.57843					

3.4 Discussion

The powdery mildew phenotyping was based on a visual evaluation of signs and symptoms of the inoculated plants. Firstly, a severity and symptoms rating scale and its visual representation were created as a reference to reduce human error while visually inspecting the plants in the greenhouse. Five different DS categories were established to score the severity and the area covered by the symptoms in percentage. Low DS was desired since they relate to a favorable response of the host to the pathogen infection. The results demonstrated that most of the plants screened for powdery mildew were categorized as resistant due to the low virulence rate of the fungus in the 104 resistant plants. Susceptibility to the pathogen was observed in ~31% of the individuals.

The powdery mildew resistance trait phenotypic data fits a 3:1 ratio (α=0.05), suggesting that the trait might be controlled by a single dominant gene (P-value=0.0821). A similar finding was reported by Jo et al. (2017) and McCoy & Bosland (2019). They reported that a single dominant gene as a source of powdery mildew resistance in *C. annuum* is called the *PMR1* gene. The *PMR1* region is in a 4-Mb region of chromosome 4 (Jo et al., 2017). *PMR1*, ZL1-1826 SCAR marker, and five SNP markers (HPGV_1313, HPGV_1344, HPGV_ 1412, KS16052G01, and HRM2_A4) were identified to confer powdery mildew resistance mapped on pepper chromosome 6 (Barka & Lee, 2020). On the other hand, some reports mentioned that powdery mildew resistance in pepper is a dominant and polygenic trait, like the resistance source found in the *C. annuum* 'H3' that seems to be controlled by at least three genes (Lefebvre et al., 2003).

The same criteria described in section 2.4 was used to select the Haley-Knott method for powdery mildew data (Figures 22 and 23), but with the difference that a normal model was used instead of a binary model.

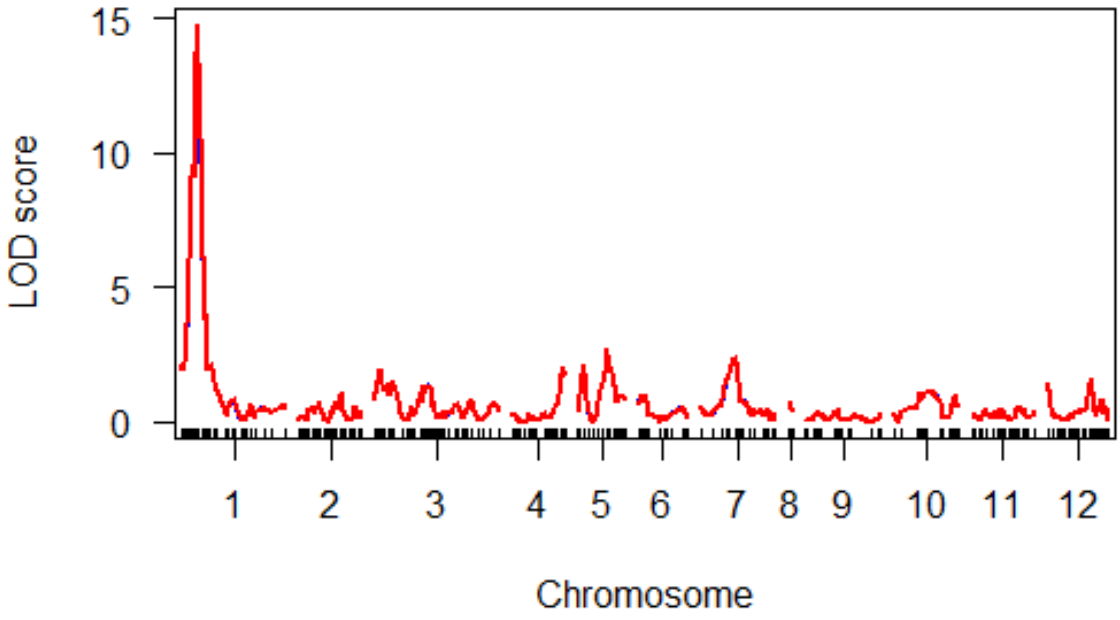

Figure 22. LOD scores for the powdery mildew data by Haley-Knott regression (blue) and standard interval mapping (red).

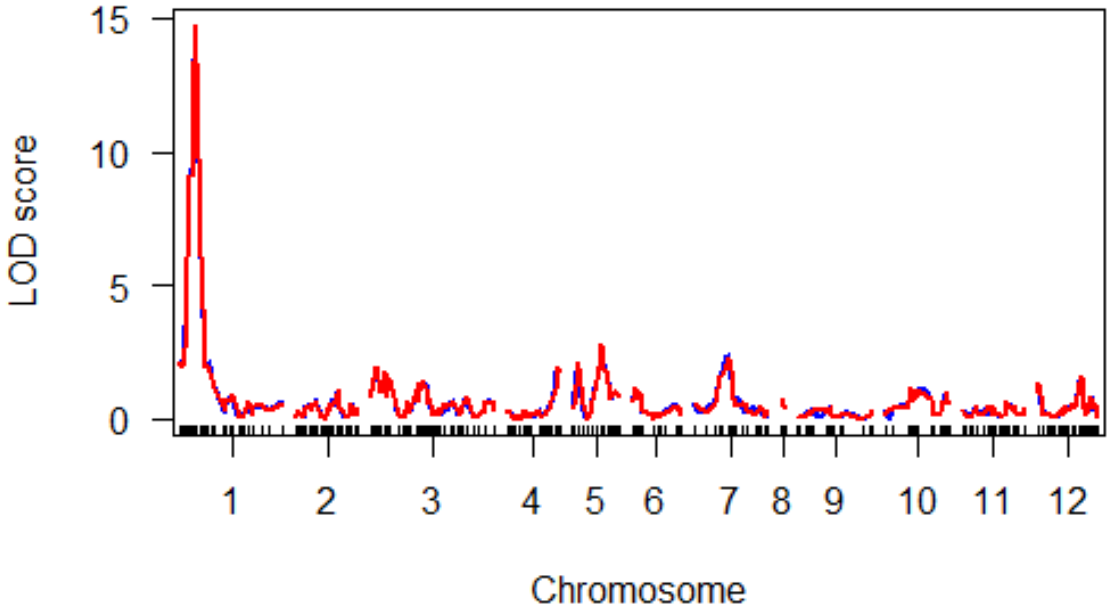

Figure 23. LOD scores for the powdery mildew data by Haley-Knott regression (blue) and multiple imputations (red).

The same procedure showed in section 2.4 was used for the QTL model selection. The single QTL model had the highest pLOD (pLOD=10.91) (Table 12). A major QTL was detected for powdery mildew resistance at 31.0 cM (Figure 24) on the short arm (p) of Chromosome 1, explaining about 49% of the phenotypic variation.

Table 12. QTL models searched by the "stepwiseqtl" function with the powdery mildew data.

Formula	Location		pLOD
	Chr	Pos	
y ~ Q1	1	1@31	10.91355
y ~ Q1 + Q2	1,3	1@31, 3@185.9	9.350932
y ~ Q1 + Q2 + Q3	1,3	1@30, 3@184, 3@177	7.763261
y ~ Q1 + Q2 + Q3 + Q2:Q3	1,3	1@30, 3@185.9, 3@172.1,	7.664063
y ~ Q1 + Q2 + Q3 + Q2:Q3 + Q4	1,3	1@30, 3@186, 3@172.1, 3@129	6.192964
y ~ Q1 + Q2 + Q3 + Q2:Q3 + Q4 + Q5	1,3	1@30, 3@186, 3@172.1, 3@129, 1@24.7	4.915214

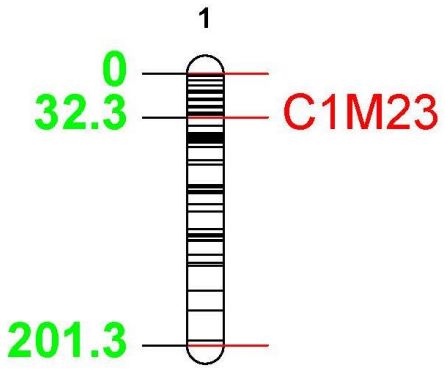

Figure 24. Powdery mildew QTL location on chromosome 1.

The powdery mildew disease causal agent, *Leveillula taurica,* is a biotroph pathogen. In peppers, *L. taurica* attaches to the leaf surface by adhesion bodies (no appressoria formation like other mildew pathogens), penetrates the leaf through stomata, and forms endophytic mycelium mainly in the abaxial leaf surface and endotrophic haustoria in the mesophyll cells (Zheng et al., 2013). According to the literature, host defense against biotrophic infections is caused by programmed cell death (hypersensitive response) and the activation of mechanisms controlled by salicylic acid(SA) (Glazebrook, 2005). Studies have been performed to analyze powdery mildew resistance, and some of them suggested that loss of function alleles of *MLO* (Mildew Locus O) conferred resistance to *L. taurica* (Jo et al., 2017). According to Pessina et al. (2014) the *MLO* genes have a recessive inheritance (Jo et al., 2017). The results obtained in this study deviate from the theory that Susceptibility genes (S-genes), like *MLO* genes, determine a recessive powdery mildew resistance, in that it revealed a lack of involvement of S-genes in powdery mildew resistance. Unlike the *MLO* genes, it was observed that the 'Hidalgo' x 'Bell 365' F_2 powdery mildew resistance might be dominant. Jo et al. (2017) discovered a similar finding when they identified the PMR1 (Powdery mildew resistance) locus on chromosome 4, suggesting that resistance was regulated by a single dominant locus in C. annuum 'VK515R' and 'PM Singang'. Resistance to powdery mildew is thought to be primarily based on the limitation of pathogen invasion, colonization, and leaf defoliation (Shifriss et al., 1992). In general, several previous findings indicate that powdery mildew resistance in pepper is highly variable, mainly due to the different degrees of resistance of the host, with most of the resistance sources found among *Capsicum* species outside the *C. annuum* taxon.

The QTL information provided in this study can be useful for the establishment of MAS in peppers using tools and methods as described in section 2.4. The genes CA01g06680 (RAB6-interacting protein), CA01g06690 (Gypsy/Ty-3 retroelement polyprotein), CA01g06700 (DNA repair and recombination protein RAD54-like), CA01g06710 (Uridylate kinase plant), CA01g06720 (RNA-binding protein 5-A-likeisoform X5) and CA01g06730 (Pentatricopeptide repeat-containing protein) have been identified and located near the genomic region on pepper chromosome 1 where the QTL associated with powdery mildew resistance was discovered (Fernandez-Pozo et al., 2015).

3.5 Conclusion

The results of powdery mildew resistance trait phenotyping showed that a single gene might be controlling the resistance because the data fit the 3:1 ratio, which means that it is also a dominant gene. The QTL analysis demonstrated that a large-effect QTL on Chromosome 1 is responsible for ~49% of the phenotypic variation. These findings suggest that a Resistance gene (R-gene) might be involved, enabling plants to recognize pathogen molecules and rapidly activate defense responses. Defense mechanisms against biotrophs like *Leveillula taurica* suggest that R-gene products can detect alterations in the host proteins caused by pathogen effectors (Guard Model) (Glazebrook, 2005). Among the different plant defenses against biotrophs, it is likely that the powdery mildew resistance documented in this research project might be explained by a gene-for-gene resistance because of two factors that were observed in the evaluated plants. Firstly, some sort of hypersensitive response was observed in some plants, which responded by dropping leaves (defoliation). Also, different degrees of resistance were observed among the individuals ranging from immune to highly susceptible, supporting the gene-for-gene resistance that suggests that plants with defense

defects can show reduced resistance to the pathogen, resulting in a continuum (not binary) of possible interactions of resistance and susceptibility (Glazebrook, 2005). Currently there are no other known sources of resistance to powdery mildew identified on pepper chromosome 1 and this should be examined further.

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