FERNANDA SOUZA LOPES

GENETIC MAPPING OF WHITE MOLD RELATED TRAITS USING PINTO BEAN RIL POPULATIONS DERIVED FROM THE USDA-ARS BEAN PROGRAM

LAVRAS – MG
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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Genética e Melhoramento de Plantas, área de concentração em Genética Molecular e de Fitopatógenos para a obtenção do título de Doutora.

Orientador
Dr. Welison Andrade Pereira– DBI/UFLA

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FERNANDA SOUZA LOPES

GENETIC MAPPING OF WHITE MOLD RELATED TRAITS USING PINTO BEAN RIL POPULATIONS DERIVED FROM THE USDA-ARS BEAN PROGRAM

MAPEAMENTO GENÉTICO DE CARACTERÍSTICAS RELACIONADAS AO MOFO BRANCO USANDO POPULAÇÕES RIL DE PINTO BEANS DERIVADAS DO PROGRAMA DE FEIJÃO DA USDA-ARS

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Genética e Melhoramento de Plantas, área de concentração em Genética Molecular e de Fitopatógenos para a obtenção do título de Doutora.

APROVADA em 18 de agosto de 2021.
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Orientador

LAVRAS-MG

2021

Aos meus pais e toda minha família.

DEDICO

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À minha família por todo amor, carinho e apoio em todos os momentos.

Ao Gustavo pelo companheirismo, paciência e muito amor durante todos esses anos que estamos juntos.

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RESUMO GERAL

O mofo branco, doença causada pelo fungo *Sclerotinia sclerotiorum*, tem limitado a produtividade do feijoeiro, causando perdas significativas. A melhor forma de controle dessa doença é a utilização de cultivares com algum nível de resistência genética. O primeiro trabalho foi conduzido com o objetivo de fenotipar e genotipar uma população de *pinto beans* ($F_{5:6}$) com 164 linhagens endogâmicas recombinantes (RIL) advindas do cruzamento de PT12-37xVCP-13 em casa de vegetação para reação fisiológica ao mofo branco. Um disco de ágar com micélio do fungo crescido foi inserido na haste principal da planta 21-28 dias após a semeadura com auxílio de ponteiras de micropipetas, após períodos de sete, doze e dezesseis dias, realizou-se a avaliação por meio de uma chave descritiva de notas. Simultaneamente à fenotipagem, o DNA genômico foi extraído e quantificado. As amostras de DNA foram adicionadas às placas e enviadas para serem genotipadas em um BeadChip (Illumina BARCBean6K) contendo ~5.398 SNPs. Neste mapeamento, o genoma de referência de *P. vulgaris* para G19833 v2.1 foi usado para alinhar os SNPs gerados. O teste do qui-quadrado para as taxas de segregação foi realizado e o desvio significativo das taxas de segregação Mendelianas esperadas ou marcadores SNP com >20% de dados ausentes foram removidos. Marcadores SNP redundantes mostrando ligação completa (100%) foram filtrados durante a construção do mapa de ligação. O mapa de ligação da população foi desenvolvido pelo programa *MapDisto* e analisado para QTL usando o R/QTL. A análise de QTL da população revelou um QTL principal em Pv05 (34%) e dois QTLs menores em Pv04 (6%) e Pv09 (5%) sete dias após a inoculação. O QTL WM5.4 encontrado no presente trabalho, corresponde ao meta-QTL WM5.4 previamente descrito na literatura. Dados de resequenciamento foram usados para encontrar SNPs presentes em *P. Coccineus* (G35346). Três marcadores (*C05_34901413*, *C05_34927709* e *C05_35044557*) foram desenvolvidos para o QTL WM5.4 e amplificados na população PT/VCP, e dois destes marcadores (*C05_34901413*, *C05_34927709*) podem ser utilizados para seleção assistida por marcadores (SAM). O segundo estudo foi conduzido com o objetivo de fenotipar e genotipar a população RIL de *pinto beans* ($F_{5:6}$) com 196 linhagens do cruzamento entre USPT-WM-12/PT9-5-6 em casa de vegetação e campo para reação ao mofo branco, acamamento e maturação. A mesma metodologia para a fenotipagem e genotipagem foi utilizada na realização deste segundo trabalho, a única diferença foi no BeadChip (Illumina BARCBean12K, com ~12.000 marcadores SNP) utilizado. Três QTLs para resistência fisiológica por *straw* test foram identificados: WM1.2 com 11% de variância fenotípica explicada, WM7.4 (19%) e WM11.1 (11%). O QTL WM11.1 apresentou interação epistática com WM1.2. Dois QTLs para resistência ao acamamento e dois para maturação foram observados. LDG7.1, LDG11.1, MAT2.1 e MAT3.1 apresentaram 40.69%, 12.15%, 46.94%, 10.32%, 12.40% e 9.71% da variância fenotípica explicada, respectivamente. Os novos (e alguns validados) QTLs, tanto para caracteres agronômicos (acamamento e maturidade), quanto para resistência ao mofo branco em casa de vegetação e ambientes de campo, fornecem aos melhoristas novas informações para gerar cultivares de feijão resistentes ao mofo branco com melhores características agronômicas.

Palavras-chave: Mofo branco. QTL. Mapa de ligação. *Phaseolus vulgaris*.

GENERAL ABSTRACT

White mold disease caused by the fungus *Sclerotinia sclerotiorum* has limited the high productivity of the bean, causing significant losses. The best way to control this disease is to use cultivars with some level of genetic resistance. The first work was carried out with the objective of phenotyping and genotyping an $F_{5:6}$ pinto bean RIL population with 164 lines from the crossing of PT12-37xVCP-13 in the greenhouse for physiological reaction to white mold using the straw test method. In this method, an agar disk with mycelium of the grown fungus was inserted into the main stem of the plant 21-28 days after sowing with the aid of micropipette tips, and seven, twelve, and sixteen dpi, the evaluation was carried out by a diagrammatic scoring scale. Simultaneously with phenotyping, genomic DNA was extracted and quantified. DNA samples were added to the plates and sent to be genotyped on a BeadChip (Illumina BARCBean6K) containing ~5,398 SNPs. In this mapping, the *P. vulgaris* reference genome G19833 v2.1 was used to align the generated SNPs. A Chi-square test for segregation ratio was performed and significant deviation from expected Mendelian segregation ratio or SNP markers with >20% missing data were removed. Redundant SNP markers showing complete linkage (100%) were filtered out during linkage map construction. The population linkage map was developed by the MapDisto program and analyzed for QTL using R/QTL. The QTL analysis revealed one major QTL on Pv05 (34%) and two smaller QTLs on Pv04 (6%) and Pv09 (5%) seven dpi. The WM5.4 QTL found in this study corresponds to the WM5.4 meta-QTL previously described. Resequencing data were used to find SNPs present in *P. coccineus* (G35346). Three markers (C05_34901413, C05_34927709, and C05_35044557) were developed for WM5.4 QTL and amplified in the P3/VCP population, and two of these markers (C05_34901413, C05_34927709) can be used for marker-assisted selection (MAS). The second study was carried out with the objective of phenotyping and genotyping the F5:6 pinto bean RIL population with 196 lines from the cross between PT9-5-6/USPT-WM-12 in greenhouse and field for reaction to white mold, and for lodging and maturity in the field. The same methodology for phenotyping and genotyping was used in this second population except the Illumina BARCBean12K BeadChip with ~12,000 SNP markers was used. Three QTL for physiological resistance were identified by the straw test: WM1.2 with 11% phenotypic variance explained, WM7.4 (19%), and WM11.1 (11%). QTL WM11.1 showed epistatic interaction with WM1.2. Two QTL for lodging resistance and two for maturity were observed. LDG7.1, LDG11.1, MAT2.1 and MAT3.1 presented 40.69%, 12.15%, 46.94%, 10.32%, of the phenotypic variance explained, respectively. The new (and some validated) QTL, both for agronomic traits (lodging and maturity) and for white mold resistance in greenhouse and field, provide new information for breeders to generate white mold resistant bean cultivars with better agronomic traits.

Keywords: White mold. QTL. Linkage map. *Phaseolus vulgaris*.

SUMÁRIO

CHAPTER 1

1 GENERAL INTRODUCTION

The fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, is the cause of the disease known as white mold. It is a soil pathogen that affects many crops, including *Phaseolus vulgaris* L. This disease can lead to losses of 30% or more in bean crops, and in rainy periods this loss can reach 100% when using susceptible cultivars and preventive measures are not taken (Schwartz and Singh, 2013). The use of integrated measures, including fungicides, crop rotation, greater spacing between rows, low population, less fertilizer, reduced irrigation, and upright cultivars can reduce the incidence of the disease (Kolkman and Kelly, 2003). However, the use of such integrated measures can reduce productivity and economic return (Schwartz and Singh, 2013).

Even with these disease control mechanisms, the use of resistant cultivars is the least costly measure that causes the least environmental impact. One of the main difficulties in using resistance to control this pathogen is the occurrence of few Mesoamerican and Andean genotypes with moderate levels of resistance for potential use in breeding programs. The highest levels of resistance are found in secondary gene pools such as *P. coccineus* L. (Singh et al., 2014). Thus, understanding pathogen-host relationships, identifying stable phenotypes in plants, using biotechnological tools such as DNA markers, can help breeders to identify superior genotypes.

The first common bean genetic map based on molecular DNA markers was published by Vallejos et al. (1992). Today with the common bean genome sequenced, each QTL detected can be physically located in the genome. One of the important steps to analyzing QTL is constructing a linkage map that provides detail about QTL location and its relationship with other markers (Doerge, 2002). For genetic mapping in plants, different population types with specific characteristics can be used. However, the parents of the mapping population must be genetically contrasting, to allow the identification of polymorphic markers and construction of the genetic map.

Soule et al. (2011) developed a comparative linkage map with 35 QTL and 4 of these QTL were found in partially resistant lines to white mold, VA19 and I9365-31, with *P. coccineus* in their pedigrees. Miklas et al. (2013) expanded the comparative linkage map with 79 QTL, of which 27 were considered for physiological resistance to white mold, 36 for disease avoidance traits, and 16 for root traits.

The identification of QTL can present reproducibility problems due to QTL x environment interaction. The identification of Meta-QTL arose to overcome this difficulty, representing a great advance in the study of QTL, as it seeks genomic regions that have effects in different locations, different populations, or different environmental conditions (Goffinet and Gerber, 2000; Vasconcellos et al., 2017). Meta-QTL analysis compiles information from multiple studies and improves QTL location by comparing individual experiments and refinement of QTL intervals obtained from individual analyses (Goffinet and Gerber 2000).

One of the most important applications of linkage maps is the location of genes that control traits of economic importance such as grain production, disease resistance, and plant architecture, which are the result of the cumulative action of genes. This project aimed to identify QTL, in two RIL populations, with major effects for resistance to white mold disease and traits such as lodging and days to maturity. And from this information, generate white mold resistant pinto beans cultivars with better agronomic traits.

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CHAPTER 2

ARTICLE 1 - SCARLET RUNNER BEAN CONTRIBUTES A MAJOR QTL FOR RESISTANCE TO *SCLEROTINIA SCLEROTIORUM* IN A PINTO BEAN RIL POPULATION

The preliminary version of the article was written according to the standard of the scientific journal Crop Science. The editorial board of the journal can recommend changes to adjust it to its style.

SCARLET RUNNER BEAN CONTRIBUTES A MAJOR QTL FOR RESISTANCE TO *SCLEROTINIA SCLEROTIORUM* IN A PINTO BEAN RIL POPULATION

Core ideas

- The identified QTL can assist generate pinto beans cultivars with better agronomic traits.
- The new (and some validated) QTL can assist breeders and geneticists to improve resistance against white mold.

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ABSTRACT

White mold is a serious disease of common bean under wet and cool environmental conditions worldwide. Beans with physiological resistance and avoidance to white mold contribute to disease control. Our objective was to phenotype and genotype a pinto RIL population with 164 $F_{5:6}$ lines from the cross PT12-37xVCP-13 for physiological reaction to white mold using the straw test in the greenhouse. The P3/VCP population was genotyped with ~5398 SNPs from the Illumina BARCBean6K BeadChip. 509 SNPs were used to construct the linkage map with 550.2 cM length. At 7 days post inoculation (dpi), three QTL (WM4.3, WM5.4, and WM9.4) influenced physiological resistance to white mold in the straw test were detected. The WM5.4 QTL on chromosome Pv05 had major effect (34% of phenotypic variation) compared to WM4.3 (6%) and WM9.4 (5%). WM5.4 was identified in

previous populations with *P. coccineus* derived resistance to white mold. Given the major and validated effect for WM5.4, assays for tightly linked SNP markers (*C05_34901413*, *C05_34927709*, and *C05_35044557*) were developed for marker-assisted selection. Putative candidate genes influencing plant defense to diseases underly the WM5.4 QTL were discussed. The knowledge and tools gained from this study will assist breeders and geneticists to improve resistance against white mold.

INTRODUCTION

White mold, caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, is a disease which can cause considerable losses to common bean production in certain regions and periods of the year. *Sclerotinia sclerotiorum* has structures called sclerotia that vary in shape and size and ensure its survival in the soil for many years, even in harsh environments.

The partial resistance of common bean to white mold is quantitative, with low to moderate heritability, and significant environmental influence (Miklas et al., 2001; Soule et al., 2011). Genetic resistance is restricted to some Mesoamerican bean cultivars, such as Ex-Rico 23 (Tu and Beversdorf, 1982) and Andean cultivars such as A 195 (Singh et al., 2007), G 122 (Miklas et al., 2001; Maxwell et al., 2007), and Cornell 605 (Griffiths, 2009). The highest levels of resistance are found only in secondary gene pools such as *P. coccineus L.* (Singh et al., 2014).

Plants with higher levels of resistance may have different mechanisms that allow them to defend themselves from the aggression of this fungus. Two of these mechanisms are physiological resistance and escape mechanisms. Physiological resistance is determined by the plant's ability to defend itself by preventing, restricting, or delaying the penetration of the pathogen into its tissues and reducing potential damage. And escape mechanisms are determined by plant morphological characteristics that provide an unfavorable environment

for the proliferation of the pathogen, such as upright plant architecture, open canopy, and early maturity (Kolkman and Kelly, 2003).

The expression of phenotypic characters with continuous distribution, such as resistance to white mold, is most often multigenic and quantitatively inherited. With the emergence of molecular markers, it became possible to map chromosomal regions that affect quantitative characters [quantitative trait loci (QTL)]. Therefore, genetic mapping is very useful for plant breeding as it enables the mapping of QTL which leads to candidate genes, gene cloning, interpretation of evolutionary mechanisms and synteny, and comparative mapping studies (Shirasawa et al., 2012). Furthermore, from the QTL mapping, it is possible to study the genetic basis of complex characters (Paterson, 1991; Bhering et al., 2008).

Some biochemical responses have been identified and induced by the presence of the pathogen. The activation of genes encoding enzymes such as superoxide dismutases, polyphenol oxidases, and peroxidases, as well as the accumulation of phenolic compounds and lignin in the presence of *S. sclerotiorum*, has been reported in soybean (Malencic et al., 2010) and other crops. These genes can link important plant defense response pathways to pathogen infection. Yang et al. (2007), studying canola, showed that *S. sclerotiorum* induced expression changes in genes encoding peroxidase, ascorbate peroxidase, glutathione peroxidase, and others related to the metabolism of reactive oxygen species and the structure and function of the cell wall. These works show that there are genes that can link important plant defense response pathways to pathogen infection. The main objective of this study was to identify major effect QTL for resistance to white mold disease and candidate genes underlying them in an agronomically adapted pinto RIL population derived from the cross PT12-37xVCP-13 (P3/VCP).

MATERIAL AND METHODS

RIL Population

A pinto RIL population with 164 $F_{5:6}$ lines was developed by single seed descent (SSD) from the cross PT12-37xVCP-13 (P3/VCP). PT12-37 is a high yielding white mold susceptible pinto bean line developed by the USDA-ARS dry bean breeding and genetics program at Prosser, WA, USA. The VCP-13 pinto bean line, jointly developed at the University of Idaho and Colorado State University (Singh et al., 2016), was derived from an interspecific recurrent backcross population between white mold susceptible common bean 'UI 320' (Myers et al., 2001) and resistant *P. coccineus* PI 439534 (UI 320*2/PI 439534, Schwartz et al., 2006). VCP-13 has a high level of resistance to white mold likely derived from the *P. coccineus* parent. Although VCP-13 has a high level of resistance, it exhibits low yield potential, late maturity, and undesirable indeterminate prostrate growth habit Type III (Singh, 1982),

Three seeds of 164 RILs, two parents, and six checks (G122, Othello, PT9-5-6, USPT-WM-12, SR9-5, and Ruby), were planted in separate 10 cm diameter square plastic pots, and after emergence, thinned to one plant per plot. The experimental design was a randomized complete block (RCBD) with five replicates and was conducted during the winter months in 2019-2020, in the USDA-ARS greenhouses at Prosser, WA, USA. The greenhouse temperatures were maintained at 21ºC day and 16ºC night, and HID lamps were utilized to maintain a 12 h day length.

The plants were inoculated with *Sclerotinia sclerotiorum* isolate '1980', 21-28 days after planting. Multiplication of white mold inoculum was performed in two steps in Potato Dextrose Agar (PDA) medium at 20±3 °C. First, disinfected sclerotia were added to plates with PDA for 1 week, then mycelium discs were removed from these plates and placed into new plates with PDA medium. Three days after the second multiplication, mycelium discs were used for inoculation. To inoculate, a 200 ul pipette tip with agar disc containing the

mycelium was placed on the cut stem [the apex of the plant stem was cut at about 2.5 cm above the fifth node]. Seven, twelve, and sixteen-days post-inoculation (dpi), the plants were screened for reaction to white mold disease using a scale from 1 (absence of symptoms) to 9 (maximum infection or dead plant) described by Petzoldt and Dickson (1996) and modified by Téran et al. (2006). Spatial analysis was used to generate best linear unbiased estimates (BLUE) for disease reaction.

SNP Markers Genotyping

The genomic DNA was extracted from <100 mg of leaf tissue using a Qiagen DNeasy 96 Plant Kit (Hilden, Germany), and was quantified by NanoDrop spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE). The samples containing 60 ul of volume at a concentration of 100 ng/ul each were added into plates and sent to be genotyped at USDA-ARS Beltsville Agricultural Research Center, MD. The Illumina BARCBean6K BeadChip (Song et al., 2015) was used to genotype the RIL population. The genotyping was conducted on the Illumina platform by following the Infinium HD Assay Ultra protocol (Illumina Inc., San Diego, CA).

The *P. vulgaris* reference genome for G19833 v2.1 (Schmutz et al., 2014; https://phytozome-next.jgi.doe.gov/info/Pvulgaris_v2_1) was used to align the generated SNPs. The chi-square test for 1:1 segregation ratio was performed and SNP markers with significant deviation from the expected Mendelian segregation ratios or > 20% missing data were removed. Redundant SNP markers showing complete linkage (100%) were filtered during linkage map construction.

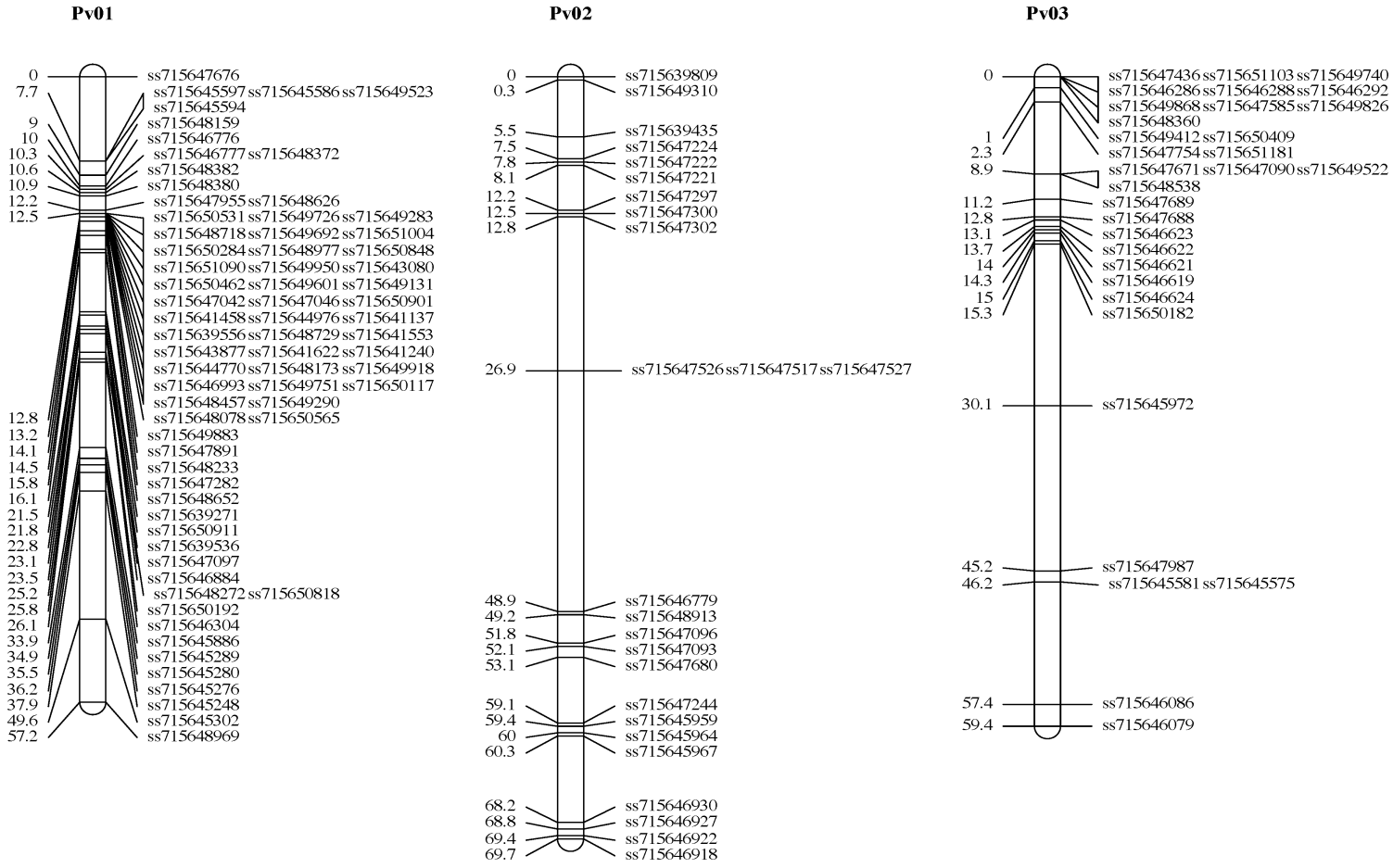
Linkage Map and QTL Analysis

A linkage map for P3/VCP RIL population (Fig. 1) was generated using MapDisto version 1.8.1 (Lorieux, 2012) with parameters r_{max} of 0.24, LOD_{min} of 3, and Kosambi mapping function. The polymorphic markers were filtered by parameter combination of 0 cM

and >100 Kb of genetic and physical distance obtained using a BLASTN from *P. vulgari*s reference genome. Linkage groups were illustrated using MapChart v. 2.30 (Voorrips, 2002).

The QTL analyses were conducted by R/qtl v1.39-5 (Broman et al., 2003) in R v3.3.0 (R Core Team, 2013). Multiple imputations were used to conduct one-dimensional single-QTL genome scans with a scanning interval of 1 cM (imputations = 1,000; error probability = 0.001), and the two- dimensional genome scans were led using Haley-Knott regression (Haley and Knott, 1992) with thresholds based on 1,000 permutations results at 5% significance level to enable assessment of evidence for multi-QTL models involving additive or interacting QTL.

Three GC-Tailed primers were designed under Tm-shift parameters reported by Wang et al., (2005), using the resequencing data from Lobaton et al. (2018) as reference to find SNPs present in *P. coccineus* (G35346). The Tm-shift method uses two forward allele-specific primers with the 3' base of each primer matching one of the SNP allele bases, a reverse common primer, and a fluorescent dye, SYBR® Green I. To each of the two allele-specific primers, GC tails of different lengths were added. The GC-rich tails of unequal length generate PCR products with a distinct Tm that depends on the responsible primer for amplification and genotypes can be determined by inspection of a melting curve on a real-time PCR instrument. All the nine primers were designed using Primer3 software (Untergasser et al., 2012).


Pv01
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7.7 ss715645597 ss715645586 ss715649523
ss715645594
9 ss715648159
10 ss715646776
10.3 ss715646777 ss715648372
10.6 ss715648382
10.9 ss715648380
12.2 ss715647955 ss715648626
12.5 ss715650531 ss715649726 ss715649283
ss715648718 ss715649692 ss715651004
ss715650284 ss715648977 ss715650848
ss715651090 ss715649950 ss715643080
ss715650462 ss715649601 ss715649131
ss715647042 ss715647046 ss715650901
ss715641458 ss715644976 ss715641137
ss715639556 ss715648729 ss715641553
ss715643877 ss715641622 ss715641240
ss715644770 ss715648173 ss715649918
ss715646993 ss715649751 ss715650117
ss715648457 ss715649290
12.8 ss715648078 ss715650565
13.2 ss715649883
14.1 ss715647891
14.5 ss715648233
15.8 ss715647282
16.1 ss715648652
21.5 ss715639271
21.8 ss715650911
22.8 ss715639536
23.1 ss715647097
23.5 ss715646884
25.2 ss715648272 ss715650818
25.8 ss715650192
26.1 ss715646304
33.9 ss715645886
34.9 ss715645289
35.5 ss715645280
36.2 ss715645276
37.9 ss715645248
49.6 ss715645302
57.2 ss715648969
Pv02
0 ss715639809
0.3 ss715649310
5.5 ss715639435
7.5 ss715647224
7.8 ss715647222
8.1 ss715647221
12.2 ss715647297
12.5 ss715647300
12.8 ss715647302
26.9 ss715647526 ss715647517 ss715647527
48.9 ss715646779
49.2 ss715648913
51.8 ss715647096
52.1 ss715647093
53.1 ss715647680
59.1 ss715647244
59.4 ss715645959
60 ss715645964
60.3 ss715645967
68.2 ss715646930
68.8 ss715646927
69.4 ss715646922
69.7 ss715646918
Pv03
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ss715648360
1 ss715649412 ss715650409
2.3 ss715647754 ss715651181
8.9 ss715647671 ss715647090 ss715649522
ss715648538
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12.8 ss715647688
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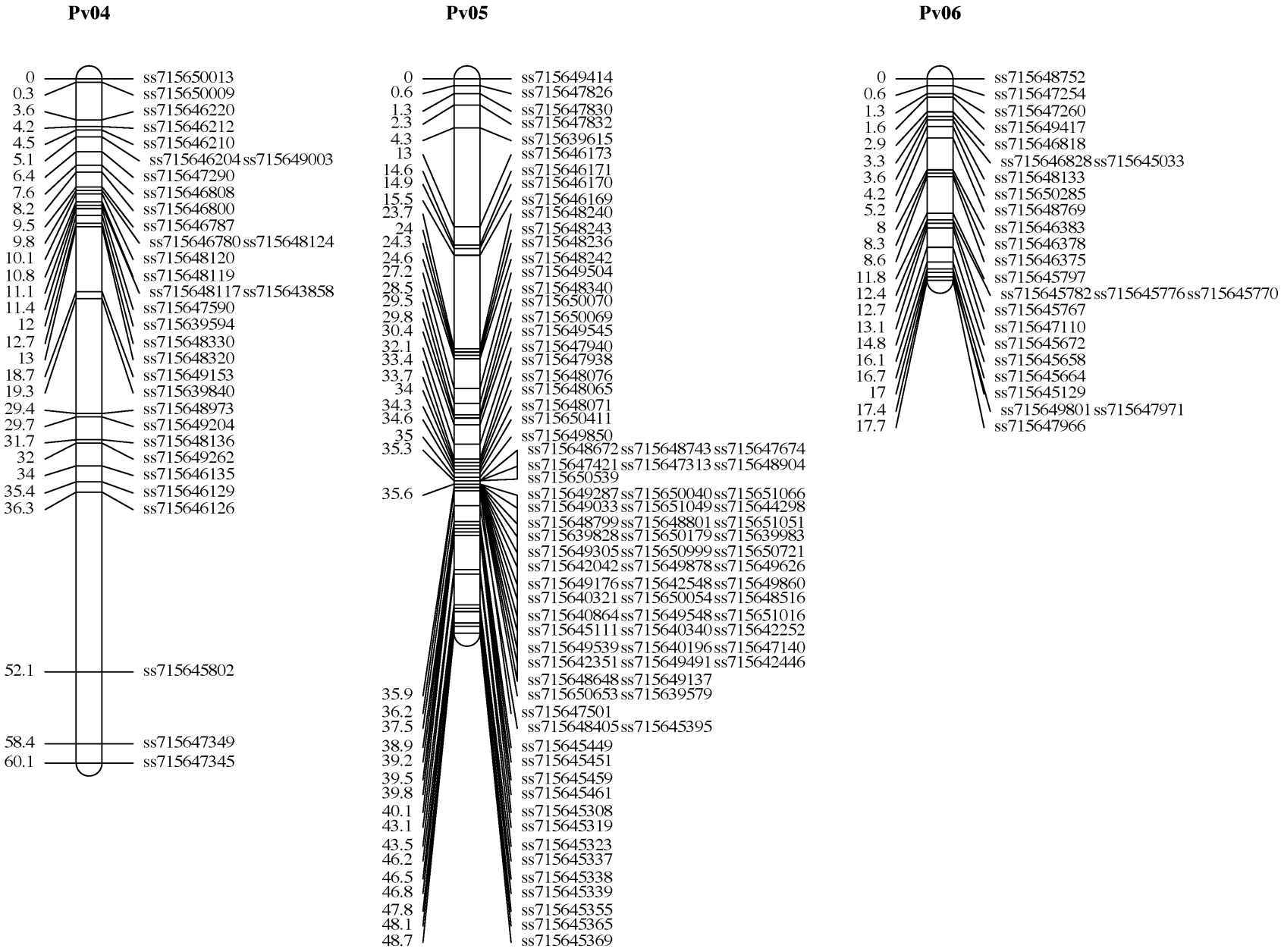
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7.6 ss715646808
8.2 ss715646800
9.5 ss715646787
9.8 ss715646780 ss715648124
10.1 ss715648120
10.8 ss715648119
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11.4 ss715647590
12 ss715639594
12.7 ss715648330
13 ss715648320
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19.3 ss715639840
29.4 ss715648973
29.7 ss715649204
31.7 ss715648136
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34 ss715646135
35.4 ss715646129
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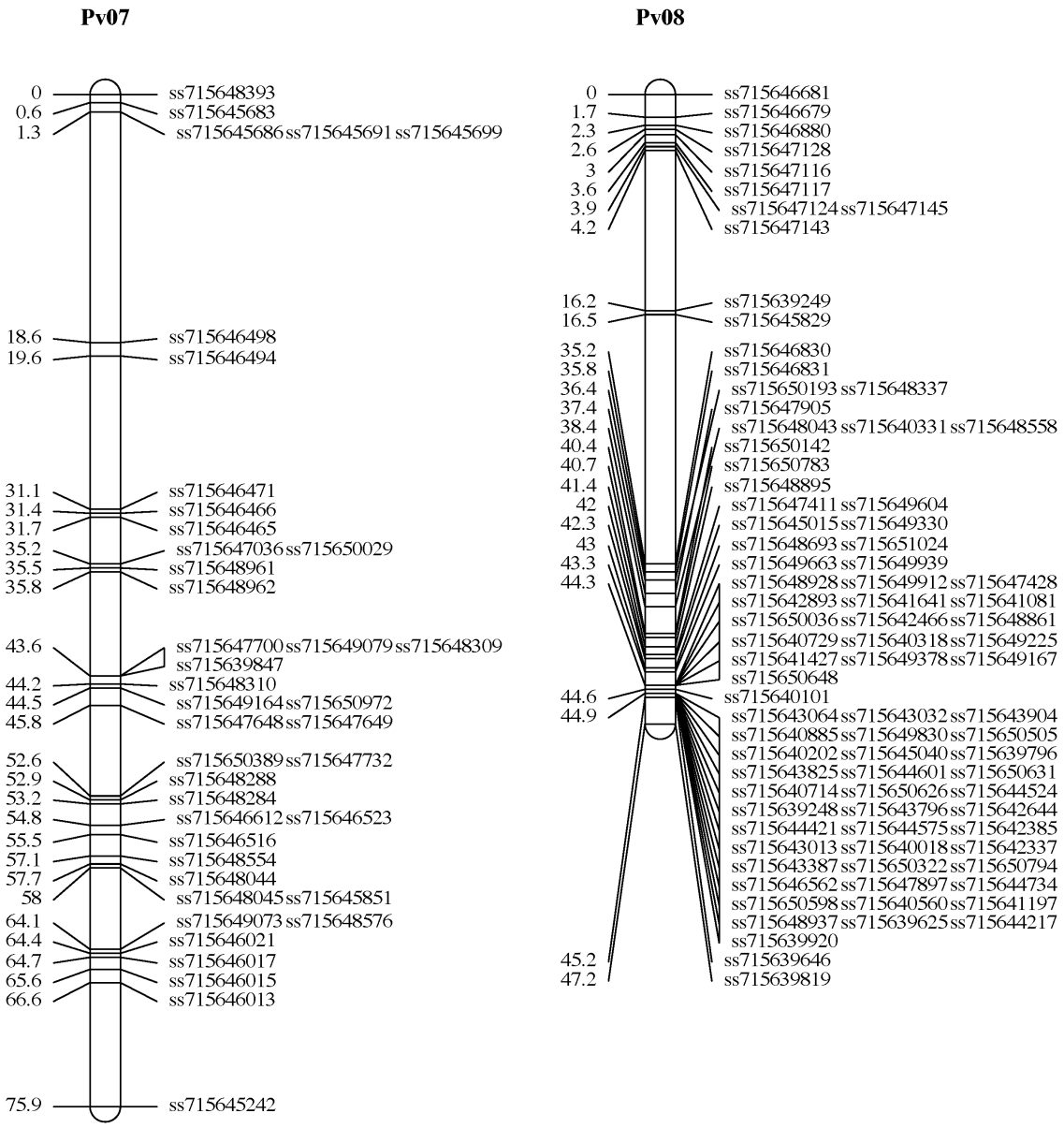
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37.4 ss715647905
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43.3 ss715649663 ss715649939
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44.9 ss715643064 ss715643032 ss715643904 ss715640885 ss715649830 ss715650505 ss715640202 ss715645040 ss715639796 ss715643825 ss715644601 ss715650631 ss715640714 ss715650626 ss715644524 ss715639248 ss715643796 ss715642644 ss715644421 ss715644575 ss715642385 ss715643013 ss715640018 ss715642337 ss715643387 ss715650322 ss715650794 ss715646562 ss715647897 ss715644734 ss715650598 ss715640560 ss715641197 ss715648937 ss715639625 ss715644217 ss715639920
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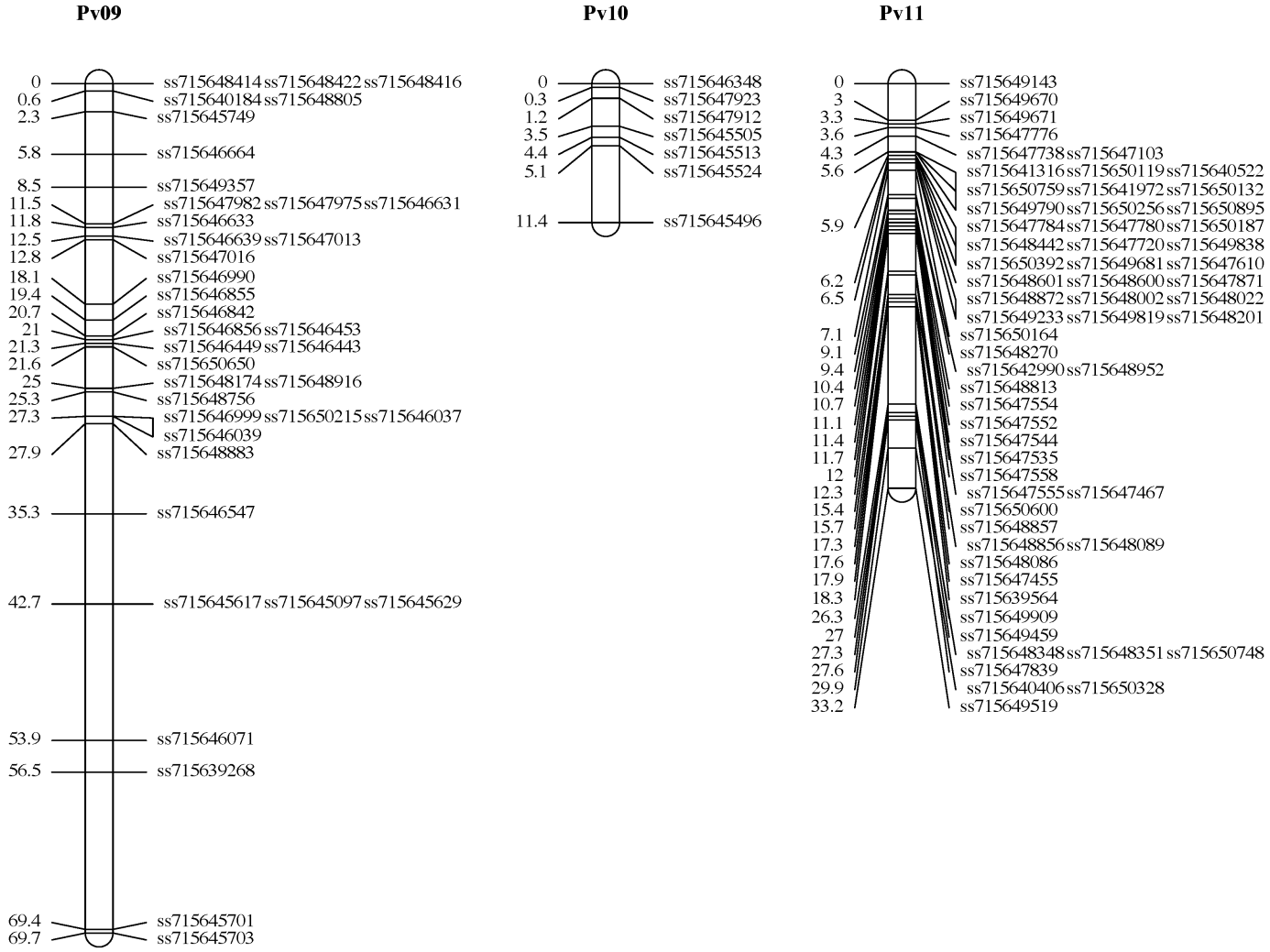

Figure 1. SNP linkage map for PT12-37xVCP-13 RIL population with QTL positions identified for partial resistance to white mold in the straw test.

RESULTS

Figure 2 shows the distribution of the monomorphic SNP markers, the missing data, and the percentage of the SNP markers showing significant deviation from the expected segregation ratios at P < 0.05 to P < 0.00001. A total of 4225 (78%) SNP markers were monomorphic, 778 (15%) presented normal segregation at P < 0.05, and 242 (5%) showed normal segregation at P < 0.01. In all, 5398 SNP markers were used, 1092 of them were polymorphic, and 583 were redundant markers. The genetic linkage map was constructed with 509 non-redundant SNPs. The total map length was 550.2 cM with an average spacing of 1.1 cM, and maximum spacing of 22 cM between markers (Table 1). The highest number of non-redundant markers was identified on chromosome Pv05 with 88 SNP markers, followed by Pv06 with 86 markers, and the lowest number of SNPs was observed on chromosome Pv07 with 10 SNPs.

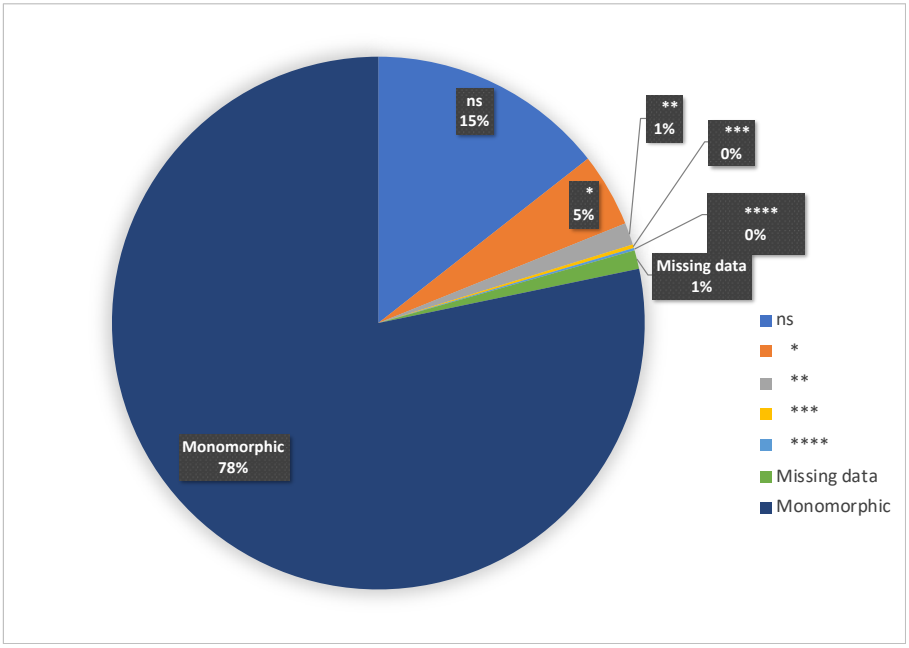

Figure 2. Significant deviation from the expected segregation ratios at P < 0.05, *P < 0.01, **P < 0.001, ***P < 0.0001, ****P < 0.00001 of the SNP markers, missing data, and percentage of monomorphic markers.

The QTL analysis of PT12-37 / VCP-13 RIL population revealed a major QTL on Pv05 (explaining 34% of the phenotypic variation) and two minor QTL on Pv04 (6%) and Pv09 (5%) for white mold disease reaction in the straw test 7 dpi. The three QTL had an additive effect of -0.19, -0.49, and -0.19, respectively (Table 2. Fig. 3). At 12 dpi only the Pv04 (-0.27) and Pv05 (-0.65) QTL were detected, and only Pv05 QTL was observed at 16 dpi with -0.76 additive effect. The logarithm of the odds (LOD) score for Pv04, Pv05, and Pv09 QTL at seven dpi were: 3.70, 16.68, and 3.39.

Table 1. Number of non-redundant, redundant and polymorphic SNP markers mapped to each chromosome and linkage group lengths.

Chromosome	Non-Redundant Markers	Redundant Markers	Polymorphic	Length (cM)	Ave. spacing	Max. spacing
Pv01	71	112	183	57.2	0.8	11.7
Pv02	25	26	51	69.7	2.9	22
Pv03	32	35	67	59.4	1.9	15.1
Pv04	33	59	92	60.1	1.9	15.7
Pv05	88	96	184	48.7	0.6	8.7
Pv06	26	52	78	17.7	0.7	3.2
Pv07	41	36	77	75.9	1.9	17.3
Pv08	86	48	134	47.2	0.6	18.7
Pv09	39	28	67	69.7	1.8	12.9
Pv10	7	3	10	11.4	1.9	6.3
Pv11	61	75	136	33.2	0.6	8.1
Scaffolds		13	13			
TOTAL	**509**	**583**	**1092**	**550.2**	**1.1**	**22**

Table 2. Individual QTL for resistance to white mold identified in common bean RIL population screened in greenhouse straw test (using a scale from 1 to 9) at 7, 12 and 16 dpi.

Trait_WM	Chromosome	LOD	R2 (%)	Additive effect	Start v.2		Peak v.2		End v.2	
7 dpi	4	3.70	6.29	-0.19	ss715650013	2,292,256	ss715646780	4,208,621	ss715648124	4,344,103
	5	16.68	34.33	-0.49	ss715649850	4,935,649	ss715650653	35,507,134	ss715639579	35,911,616
	9	3.39	5.74	-0.19	ss715649357	12,492,103	ss715646855	16,941,658	ss715648756	21,117,127
12 dpi	4	3.58	6.39	-0.27	ss715646220	2,880,996	ss715646780	4,208,621	ss715648124	4,344,103
	5	15.70	33.52	-0.65	ss715650411	4,845,473	ss715650653	35,507,134	ss715639579	35,911,616
16 dpi	5	16.54	37.14	-0.76	ss715650411	4,845,473	ss715650653	35,507,134	ss715639579	35,911,616

Figure 3 shows the LOD and disease scores, the RIL population genotype at seven dpi, and chromosomes Pv04, Pv05, and Pv09. The plants that presented the resistant parent genotype in all three chromosomes have a lower score, which means that they have more resistance to white mold. In addition, what can be observed is that, by presenting a major effect QTL, individuals that had the resistant parental genotype on chromosome Pv05, obtained lower scores on the straw test, even with the presence of the susceptible genotype on another chromosome.

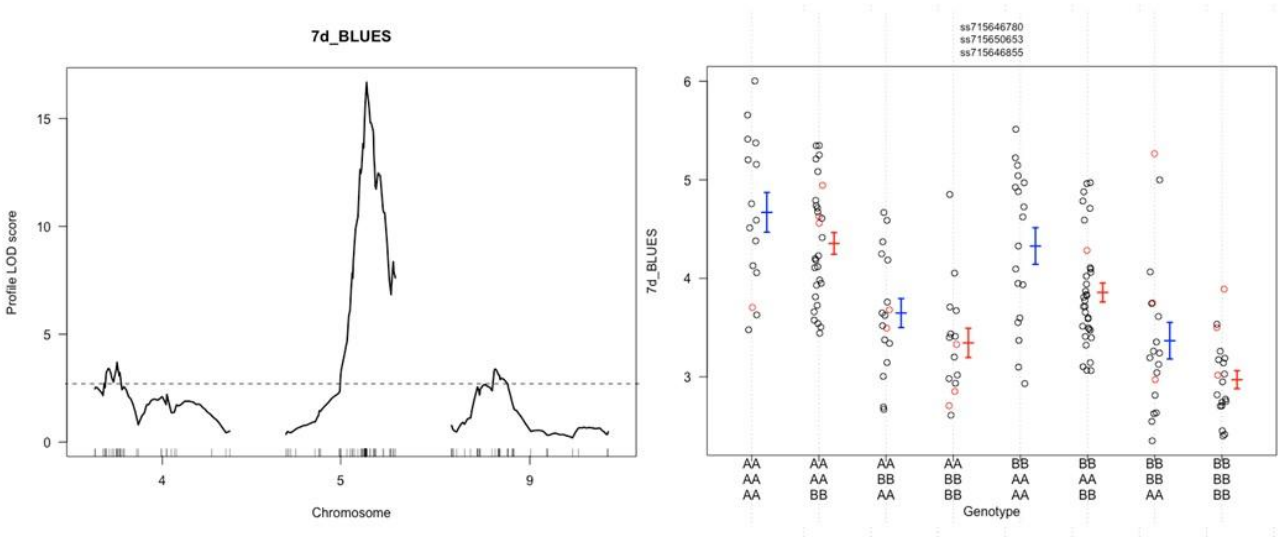

Figure 3. QTL for resistance to white mold (1 to 9 score) identified at seven dpi in PT12-37xVCP-13 RIL population and QTL effect(s) on mean disease score.

Three SNP markers were developed under Tm-shift parameters reported by Wang et al., (2005), using as reference the *P. coccineus* landrace G35346 (Table 3). The primers *C05_34901413*, *C05_34927709*, and *C05_35044557* were tested in PT12-37/VCP-13 on Pv05 QTL and were polymorphic.

The major QTL on Pv05 corresponds to WM5.4 from Vasconcellos et al., (2017) paper when converted to version 2.1 of the reference genome G19833. The QTL was found in three RIL populations described by Vasconcellos et al. (2017): Montrose x I9365-25 (M25), Raven x I9365-31 (R31), and UI-537 x I9365-25 (U25).

Table 3. Three GC-Tailed Primers designed under Tm-shift parameters using as reference the *P. coccineus* landrace G35346.

ID name	Sequence
C05_34901413_Fa	**gcgggc**TAGTACATAATACGGGGCATGa
C05_34901413_R	TGGAATGCGTTAGTTCCTGAAT
C05_34901413_Fb	**gcgggcagggcggc**GTACATAATACGGGGCATGg
C05_34927709_Fa	**gcgggcagggcggc**CTCTACTCGTTCCACCTCTCGg
C05_34927709_R	CTGACTTGAGCGTTGGAGTG
C05_34927709_Fb	**gcgggc**TCGTTCCACCTCTCGaTCTT
C05_35044557_Fa	**gcgggcagggcggc**CGTGACGATATGGGGg
C05_35044557_R	AGCTAGGGCAAGCCTATTGA
C05_35044557_Fb	**gcgggc**ACGTGACGATATGGGGa

DISCUSSION

The construction of a linkage map is an important step in QTL analysis, as it provides a framework that improve QTL location (Doerge, 2002). For accurate linkage map construction, genotyping errors, missing data, or segregation distortion must be avoided. The 5,398 SNPs markers used in this study, were filtered by eliminating all the monomorphic and segregation distorted SNPs which resulted in 509 SNPs being effectively used to construct a linkage map with 550.2 cM length.

The PT12-37 / VCP-13 RIL population was evaluated for reaction to white mold disease at 7, 12, and 16 dpi. At 7 dpi, three major QTL for white mold disease were found on Pv04 (WM4.3), Pv05 (WM5.4), and Pv09 (WM9.4). In previous studies, QTL have been found in all three chromosomes. Mkwaila et al. (2011) identified QTL associated with white mold resistance in two inbred backcross line populations on Pv02, Pv03, Pv04, Pv07, Pv09, and Pv11 that explained for up to 32% observed variation in straw test and field disease incidence. Soule et al. (2011) reported two independent QTL (WM4.1 and WM4.2) that were initially detected in two different bi-parental populations: PC-50/XAN-159 (Park et al., 2001) and Raven/I9365-31 (Soule et al., 2011). The QTL on B4 in the TL population described by Mkwaila et al. (2011) is probably the same as WM4.2 identified in R31 population by Soule et al. (2011) as it is in the vicinity of SSR marker BMd-15 in the DOR364/G19833 mapping

population. However, no QTL in this linkage group was detected in Raven/I9365-31 when it was re-genotyped for meta-QTL analysis (Vasconcellos et al., 2017), possibly the result of a QTL × environment interaction. The WM4.3 QTL observed in this study cannot be compared to designated WM4.1 and WM4.2 from Soule et al. (2011) since their physical position was not the same.

Using the genotypic data of R31, M25 (Vasconcellos et al., 2017), and PT12-37xVCP-13 populations was possible to refine the WM5.4 QTL from 34,338,257 to 36,112,535 Mb on Pv05 where there are 111 gene models in the reference genome G19833 v2.1. This indicates that the major QTL on Pv05 of the current study, corresponds to Meta-QTL WM5.4 from Vasconcellos et al. (2017). Furthermore, the Meta-QTL WM5.4 was detected for QTL conditioning partial resistance in the greenhouse and field identified across the Montrose/I9365-25, UI-537/I9365-25 (Vasconcellos et al., 2017), and Raven/I9365-31 populations. Both I9365-25 and I9365-31, are resistant to white mold and derived from an interspecific population between *P. vulgaris* and *P. coccineus* suggesting that WM5.4 may derive from the *P. coccineus* gene pool. Vasconcellos et al. (2017) also detected the WM5.5 QTL in A195/OSU6137 (Singh et al., 2007), and four other QTL detected in bi-parental populations (WM5.1, WM5.2, WM5.3, and WM5.4) were previously identified on Pv05 (Park et al., 2001; Ender and Kelly, 2005; Soule et al., 2011).

The same comparative study of bi-parental populations conducted by Soule et al. (2011), detected a QTL on Pv09. The WM9.1 was originally identified in the G122/CO72548 population by Maxwell et al. (2007). A second QTL on Pv09 was reported by Mkwaila et al. (2011) and designated as WM9.2 by Schwartz and Singh (2013). The WM9.3 was described by Vasconcellos et al. (2017), as a meta-QTL identified in A195/OSU6137 and Orion/USPT-WM-12 populations. The WM9.4 found in the current study does not overlap with any of the previously identified regions.

Analyzing the RIL population genotyping, it can be observed that some SNP markers from the BARCBean6K BeadChip were dominant for the susceptible parent PT12-37. A blast search of potential bean white mold resistance candidate genes identified 8 different genes on Pv05 (Table 4). The putative candidate genes are related to defense against pathogen attack, disease resistance, participation in pathogen recognition, response to biotic and abiotic stresses, and activation of innate immune responses. The Phvul.005G116200.1 and Phvul.005G116300.1 genes were linked to markers *C05_34901413* and *C05_34927709*, respectively, and both are cellulose synthase-like (CSL) genes. The CSL genes encode glycosyltransferases thought to be involved in the biosynthesis of cell wall non-cellulosic polysaccharides (Galinousky et al., 2020), which could possibly improve the first defense barrier against pathogen attack. The marker *C05_35044557* is linked to a protein of unknown function at Phvul.005G116200.1 (35.043.973 - 35.046.282 Mb).

The potential genes Phvul.005G115000.1 (MATE efflux family protein) and Phvul.005G117200.1 (P-loop containing nucleoside triphosphate hydrolases superfamily protein) are related to disease resistance reaction (Tiwari et al., 2014; Zheng et al., 2004). The candidate gene Phvul.005G113800.1 is a Peroxidase superfamily protein, and according to Hiraga et al. (2001), plant peroxidases have been implicated in a broad range of physiological processes such as cross-linking of cell wall proteins, salt tolerance, lignification, suberization, auxin metabolism, oxidative stress, and defense against pathogen attack.

During their evolution, plants developed a series of tolerance mechanisms to biotic and abiotic stresses. At the molecular level, plant exposure to these stresses activates signals responsible for inducing or repressing target gene expression (Finatto et al., 2018). In the present study, two candidate genes are related to these responses. Phvul.005G116100.1 (alpha/beta-Hydrolases superfamily protein) is related to survival responses to widely varying biotic and abiotic ecologies (Mindrebo et al., 2016), and Phvul.005G116000.1 gene is

responsible for the production of WRKY family transcription factors, which operate in the regulation of genes responsive to many plant growth and developmental cues, as well as to biotic and abiotic stresses (Finatto et al., 2018).

To deal with pathogens, plants have an advanced immune system that induce specific defense responses. A range of pathogens are recognized by plants through pathogen-associated molecular patterns (PAMPs), in addition to resistance (R) proteins. The genes Phvul.005G117900.1 and Phvul.005G115900.1 are involved in pathogen signaling and recognition through the production of NB-ARC domain-containing disease resistance protein (van Ooijen et al., 2008) and mitogen-activated protein kinase kinase kinase (Dóczi et al., 2007), respectively.

DNA markers are an important tool to track resistance alleles in segregating populations. Molecular markers are not affected by environmental variation and allow selection in the absence of the pathogen. The use of MAS in breeding is recommended in cases where the trait of interest is difficult to be evaluated using traditional phenotypic selection methods. All three designed SNP markers worked well, however, the SNP marker *C05_35044557* was found in some lines with Mesoamerican background that were absent interspecific introgression for *P. coccineus*. Therefore, it is suggested that only SNP markers *C05_34901413* or *C05_34927709* be used for marker-assisted selection (MAS) of the WM5.4 QTL. Resistance to white mold in beans, as a quantitative characteristic, should offer great potential for the use of MAS, as the interaction between environmental effects and plant architecture can hinder direct selection in the field (Miklas, 2007).

Defense against white mold in the common bean is conditioned by avoidance and physiological resistance mechanisms. Several QTL have already been mapped for *S. sclerotiorum* resistance and disease avoidance. The results from this study verifies the complexity of resistance to white mold, validates the WM5.4 QTL and its likely derivation

from *P. coccineus*, and provides new QTL targets that could be of major interest to bean breeders.

Table 4. WM5.4 QTL potential genes.

Pv gene model_v.2	Start	End	arabi-defline
Phvul.005G113800.1	34.355.270	34.357.322	Peroxidase superfamily protein
Phvul.005G115000.1	34.572.752	34.577.499	MATE efflux family protein
Phvul.005G115900.1	34.830.436	34.831.635	mitogen-activated protein kinase kinase kinase 15
Phvul.005G116000.1	34.849.823	34.852.957	WRKY family transcription factor
Phvul.005G116100.1	34.877.924	34.881.291	alpha/beta-Hydrolases superfamily protein
Phvul.005G116200.1	34.899.296	34.903.096	cellulose synthase-like B4
Phvul.005G116300.1	34.928.989	34.932.748	cellulose synthase-like B4
Phvul.005G116400.1	34.957.178	34.960.719	cellulose synthase-like B3
Phvul.005G116450.1	34.978.614	34.982.473	cellulose synthase-like B4
Phvul.005G116501.1	35.008.350	35.016.525	cellulose synthase-like B3
Phvul.005G116551.1	35.029.075	35.034.296	cellulose synthase-like B4
Phvul.005G116700.1	35.052.364	35.056.486	cellulose synthase-like B4
Phvul.005G117200.1	35.105.075	35.115.678	P-loop containing nucleoside triphosphate hydrolases superfamily protein
Phvul.005G117833.1	35.238.315	35.243.275	cellulose synthase-like B4
Phvul.005G117900.1	35.309.250	35.314.337	NB-ARC domain-containing disease resistance protein

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ACKNOWLEDGMENTS

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ARTICLE 2 - QTL ANALYSIS FOR AGRONOMIC TRAITS AND RESISTANCE TO WHITE MOLD IN PINTO BEAN RIL POPULATION

The preliminary version of the article was written according to the standard of the scientific journal Crop Science. The editorial board of the journal can recommend changes to adjust it to its style.

QTL ANALYSIS FOR AGRONOMIC TRAITS AND RESISTANCE TO WHITE MOLD IN PINTO BEAN RIL POPULATION

Core ideas

- The identification of new (and validated) QTL can assist identify pinto bean cultivars agronomically adapted for maturity and lodging.
- The identification of major effect QTL for resistance to white mold in Pinto RIL population can assist to obtain new cultivars resistant to the fungus.

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ABSTRACT

White mold disease caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary limits common bean (*Phaseolus vulgaris* L.) production worldwide. Integrated strategies which optimize the use of irrigation, crop rotation, fungicides, and cultivars with disease avoidance characteristics and physiological resistance contribute to disease control. However, developing less susceptible cultivars with good agronomic characteristics, is a difficult task because resistance is often from exotic sources and is polygenic with low to moderate heritability. Our objective was to characterize physiological resistance and disease avoidance in pinto bean with good agronomic performance. A pinto RIL population with 196 $F_{5:6}$ lines

from the cross PT9-5-6/USPT-WM-12 was screened in the greenhouse for physiological reaction to white mold using the straw test and for disease reaction in the field. The RIL population was genotyped with ~12,000 SNPS from the Illumina BARCBean12K BeadChip. A genetic linkage map of 533 cM was constructed with 661 SNPs. Three QTL WM1.2, WM7.4 and WM11.1 on chromosomes Pv01, Pv07, and Pv11, respectively, influenced physiological resistance in the straw test. WM1.2 with minor (11% phenotypic variance explained) and WM7.4 with moderate effect (19%), derived from the resistant parent USPT-WM-12, validates their effect from a previous study. The WM11.1 QTL derived from the susceptible parent PT9-5-6 had a minor effect (11%) and epistatic interaction with WM1.2. In the field, each parent contributed a QTL LDG7.1 and LDG11.1 for resistance to lodging. The LDG7.1 QTL with major effect (41%) is well known from previous studies. The difference in maturity between the early susceptible and late resistant parent was influenced by two QTL, MAT2.1 with major (47%) and MAT3.1 with minor (10%) effect. Field reaction to white mold was conditioned by WM2.2 (12%) and WM11.1 (10%) with minor effect. The WM2.2 QTL was previously identified in other populations. The new and validated QTL identified for reduced lodging, early maturity, and resistance to white mold in greenhouse and field environments provides breeders with new information for generating white mold resistant pinto bean cultivars with better agronomic traits.

INTRODUCTION

The bean crop is highly influenced by the action of phytopathogenic organisms that cause significant damage. According to Miklas et al. (2006a), there is a decrease in bean yield due to biotic and abiotic stresses suffered by the common bean. Biotic stresses are related to the occurrence of pests and diseases caused by fungi, bacteria, viruses, and nematodes. Among the diseases commonly found in beans, white mold, caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, stands out. This fungus has resistance structures, called sclerotia,

that ensure its survival in unfavorable environments. Sclerotia survives in the soil for several years and vary in shape and size.

Under favorable climate to the fungus, bean crop may suffer losses of 30% or even 100% if no action is taken. The dead flower is the primary energy source to the fungus at the beginning of the infection (Oliveira, 2005). After infecting the host, *S. sclerotiorum* can spread to other plants through plant-to-plant contact (Bolton et al., 2006).

There is little genetic diversity for white mold resistance in the *Phaseolus vulgaris* gene pool. The highest resistance levels are found in secondary gene pools such as *P. coccineus* L. (Singh et al., 2014). Resistance to white mold is linked to the intrinsic characteristics of the genotype, and also to plant characteristics such as shrub growth habit, which reduce the development of the disease and correspond to escape mechanisms (Miklas et al., 2013). Integrated strategies which optimize the use of irrigation, crop rotation, fungicides, and use of upright cultivars with a more open canopy and cultivars with more physiological resistance contribute to disease control. However, developing less susceptible cultivars with good agronomic characteristics is a difficult task due to resistance is often from exotic sources. In addition, resistance to white mold is polygenic with low to moderate heritability and suffers significant traits influence in morphological characters that confuse the expression and detection of this mechanism. (Miklas et al., 2004).

It is of fundamental importance to understand the interaction between host resistance alleles and pathogen virulence alleles for the definition of plant breeding strategies for resistance to phytopathogens (Melo and Santos, 1999). According to Xu and Crouch (2008), the use of markers helps the identification, quantification, and characterization of a genetic variation of available germplasms, in addition to speeding up the identification, cloning, and introgression of alleles and/or useful QTL (Quantitative trait loci) for the improvement of a given characteristic.

Genetic linkage maps can provide improved insight into genome evolution and organization through comparative mapping and serve as the basis for studying complex and polygenic forms of resistance to disease through the localization of major genes and quantitative trait loci. To construct a high-density genetic map, additional molecular markers need to be developed. Single-nucleotide polymorphisms (SNPs) are one of the most efficient markers for identifying candidate genes associated with QTL.

The main objective of this research was to identify major effect QTL for maturity, lodging, and resistance to white mold in an agronomically adapted pinto RIL population derived from the cross PT9-5-6/USPT-WM-12 (PT/US).

MATERIAL AND METHODS

RIL Population

A pinto RIL population with 196 $F_{5:6}$ lines was developed by single seed descent (SSD) from the cross PT9-5-6/USPT-WM-12 (PT/US). PT9-5-6 is an advanced pinto bean line with upright architecture and high yield potential but susceptible to white mold, developed by the dry bean breeding and genetics program of USDA-ARS at Prosser, WA, USA. USPT-WM-12 is a pinto bean germplasm developed by modified pedigree breeding method from the cross G99750/USPT-WM-1 and tested as PS02-037- 2 or 37-2 in early generations. USPT-WM-12 exhibited high levels of resistance to white mold disease in field and straw tests conducted by the national Bean White Mold Nursery (BWMN) from 2008 to 2012 (McCoy et al., 2009, 2011, 2012; Jhala et al., 2013). Compared to the other BWMN entries of Middle American gene pool, USPT-WM-12 was the most resistant to white mold. USPT-WM-12 also exhibited high yield potential under severe disease pressure in Michigan, ranking the highest for yield of 64 lines tested for two consecutive years in 2010 and 2011. Despite having commercially acceptable agronomic characteristics, including upright

architecture and moderate maturity, its seed is too dark for commercial acceptance (Miklas et al., 2014).

The 196 RILs, parents, and four checks (G122, Othello, PT12-37, and VCP-13), were screened for physiological reaction to white mold using the straw test in the USDA-ARS greenhouses at Prosser, WA, USA. The plants were inoculated with *Sclerotinia sclerotiorum* isolate 1980 approximately 21-28 days after planting. Inoculum multiplication was performed in two steps to obtain greater uniformity in Potato Dextrose Agar (PDA) medium at 20±3 °C. In the first step, sterilized sclerotia were added to plates with PDA for 1 week, then discs containing the mycelium were removed from these plates and placed in new plates with the same medium. Three days after the second multiplication, the mycelium was inoculated into the plants. For inoculation, the apex of the stem was cut at about 2.5 cm above the fifth node, a pipette tip with an agar disc containing the mycelium was placed on the cut stem. Seven and ten days post-inoculation (dpi), each plant was evaluated for reaction to white mold, using a scale from 1 (absence of symptoms) to 9 (maximum infection or dead plant) described by Petzoldt and Dickson (1996) and modified by Téran et al. (2006).

The experimental design was a randomized complete block (RCBD) with five replications and was conducted during the winter months in 2019-2020. The greenhouse temperatures were maintained at 21ºC day and 16ºC night, and HID lamps were utilized to maintain a 12 h day length. Three seeds were planted in a 10 cm diameter square plastic pot, and after their emergence, pots were thinned to one plant.

The RILs, parents, and checks were evaluated for white mold disease reaction in the field in June-September 2020 at the USDA-ARS Research Farm near Paterson, WA, USA. The experimental design was an RCBD with two replications. Plots were three rows wide with 3 m length and row spacing of 0.56 m. A single row of a susceptible line PK7-4 was planted between plots. Lodging on a plot basis (1 = none to 9 = completely lodged) was

recorded at R6 growth stage and maturity was recorded on a plant basis as days after planting (DAP) till harvest maturity. Disease reaction (from 1 = no symptoms to 9 = completely diseased) described by Petzoldt and Dickson (1996) and modified by Téran et al. (2006) normally recorded on a plot basis was instead recorded on an individual plant basis for the most susceptible plant within the plot, due to poor stand establishment for ~30% of the plots.

Spatial analysis was used to generate best linear unbiased prediction (BLUP) for disease reaction.

Genotyping with SNP Markers

The genomic DNA was isolated from ~100 mg of leaf tissue using a Qiagen DNeasy 96 Plant Kit (Hilden, Germany), and the DNA was quantified by NanoDrop spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE). The samples were added into 3 plates containing 60 ul of volume at a concentration of 100 ng/ul each.

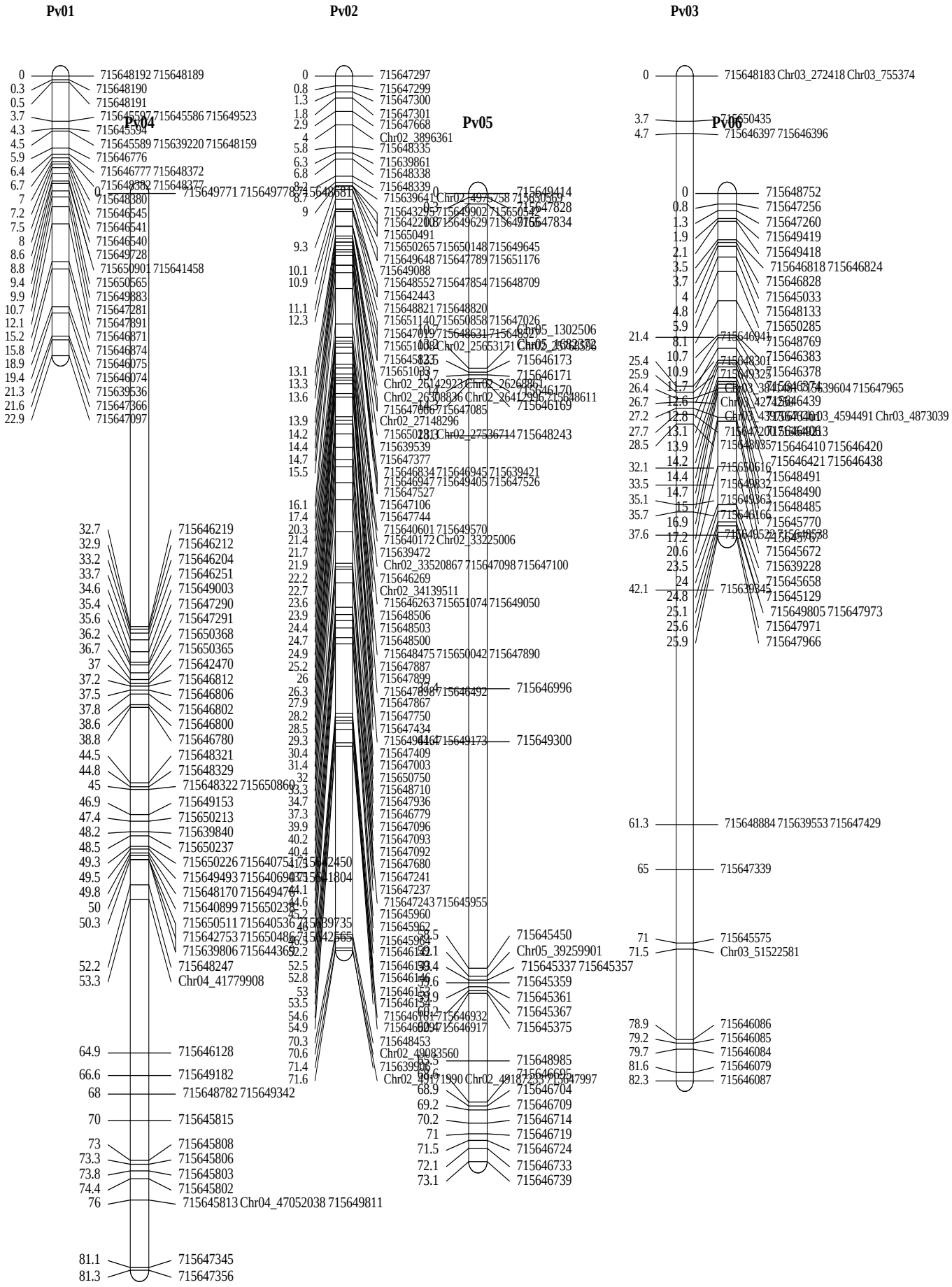
The RIL population was genotyped using the Illumina BARCBean12K BeadChip at USDA-ARS Beltsville Agricultural Research Center, MD (Song et al., 2015; Miklas et al., 2020). The genotyping was conducted on the Illumina platform by following the Infinium HD Assay Ultra protocol (Illumina Inc., San Diego, CA). The SNPs probe sequences were aligned with *P. vulgaris* reference genome for G19833 v2.1 (Schmutz et al., 2014; https://phytozome-next.jgi.doe.gov/info/Pvulgaris_v2_1). SNP markers with > 20% missing data or significant deviation from the expected Mendelian segregation ratios as determined by chi-square analysis were removed. Additionally, redundant SNP markers showing complete linkage (100%) were filtered during linkage map construction.

Genetic Linkage Map and QTL Analysis

A genetic linkage map for PT/US RIL population (Figure 1) was generated using MapDisto version 1.8.1 (Lorieux, 2012) with parameters r_{max} of 0.24, LOD_{min} of 3, and Kosambi mapping function. Linkage groups were assigned according to physical positions

obtained using a BLASTN from *P. vulgaris* reference genome. Linkage groups were illustrated using MapChart v. 2.30 (Voorrips, 2002).

QTL analyses were conducted using R/qtl v1.39-5 (Broman et al., 2003) in R v3.3.0 (R Core Team, 2013). One-dimensional, single-QTL genome scans were conducted using multiple imputations with a scanning interval of 1 cM (imputations = 1,000; error probability = 0.001). Two- dimensional genome scans were conducted using Haley-Knott regression (Haley and Knott, 1992) with the thresholds based on the results of 1,000 permutations at a 5% significance level to enable assessment of evidence for multi-QTL models involving additive or interacting.


Pv01
Pv02
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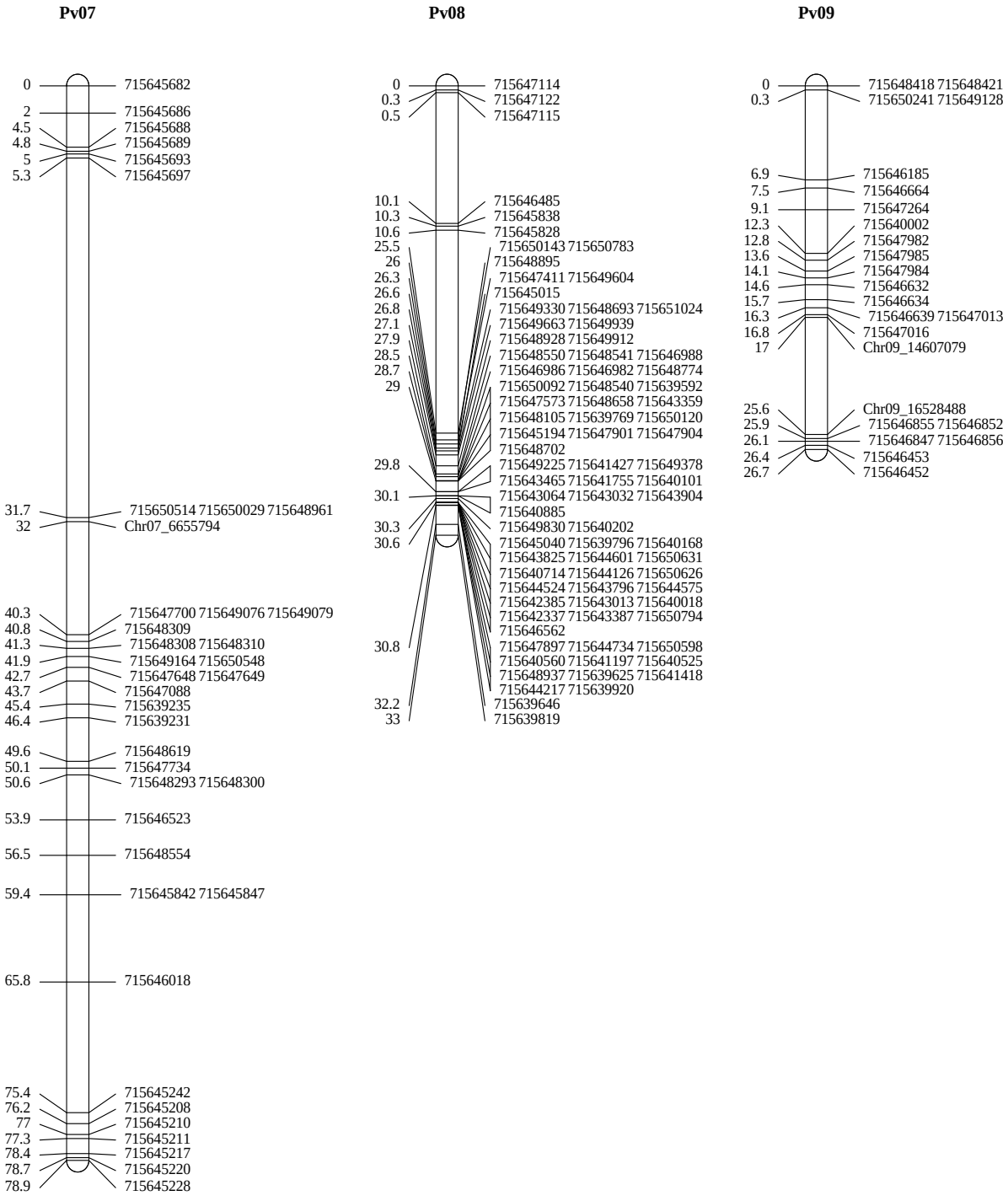
Pv05
Pv06


Pv07
0 715645682
2 715645686
4.5 715645688
4.8 715645689
5 715645693
5.3 715645697
31.7 715650514 715650029 715648961
32 Chr07_6655794
40.3 715647700 715649076 715649079
40.8 715648309
41.3 715648308 715648310
41.9 715649164 715650548
42.7 715647648 715647649
43.7 715647088
45.4 715639235
46.4 715639231
49.6 715648619
50.1 715647734
50.6 715648293 715648300
53.9 715646523
56.5 715648554
59.4 715645842 715645847
65.8 715646018
75.4 715645242
76.2 715645208
77 715645210
77.3 715645211
78.4 715645217
78.7 715645220
78.9 715645228
Pv08
0 715647114
0.3 715647122
0.5 715647115
10.1 715646485
10.3 715645838
10.6 715645828
25.5 715650143 715650783
26 715648895
26.3 715647411 715649604
26.6 715645015
26.8 715649330 715648693 715651024
27.1 715649663 715649939
27.9 715648928 715649912
28.5 715648550 715648541 715646988
28.7 715646986 715646982 715648774
29 715650092 715648540 715639592
715647573 715648658 715643359
715648105 715639769 715650120
715645194 715647901 715647904
715648702
29.8 715649225 715641427 715649378
715643465 715641755 715640101
30.1 715643064 715643032 715643904
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30.6 715645040 715639796 715640168
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30.8 715647897 715644734 715650598
715640560 715641197 715640525
715648937 715639625 715641418
715644217 715639920
32.2 715639646
33 715639819
Pv09
0 715648418 715648421
0.3 715650241 715649128
6.9 715646185
7.5 715646664
9.1 715647264
12.3 715640002
12.8 715647982
13.6 715647985
14.1 715647984
14.6 715646632
15.7 715646634
16.3 715646639 715647013
16.8 715647016
17 Chr09_14607079
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26.7 715646452

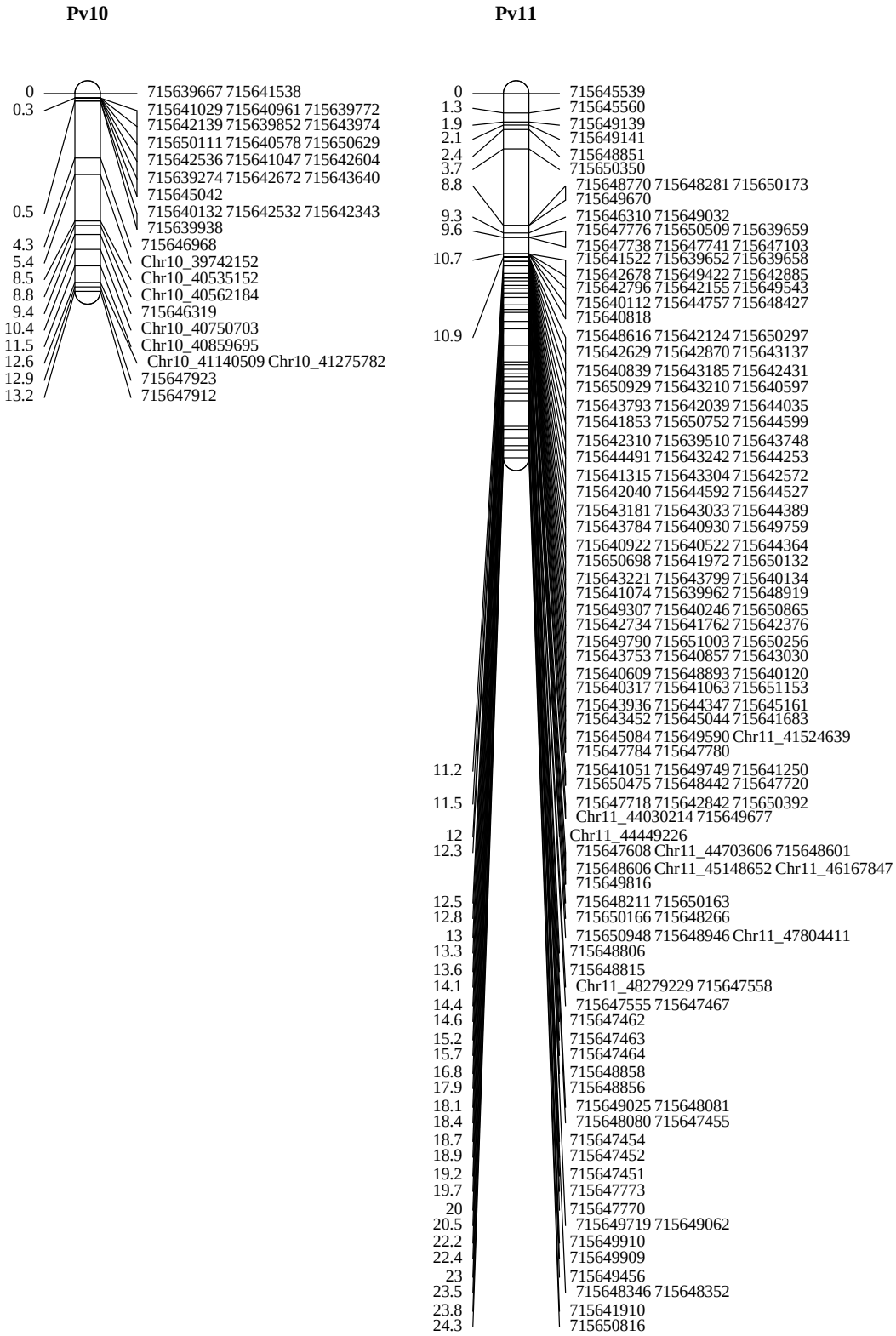

Figure 1. SNP linkage map for PT9-5-6/USPT-WM-12 (PT/US) RIL population with QTL positions identified for partial resistance to white mold, lodging and maturity.

RESULTS

The SNP-based linkage map was constructed with 661 SNPs, and it covers 533.3 cM. The highest number of markers was identified on chromosome Pv11 with 163 SNP markers, followed by Pv02 with 125 markers and the lowest number of SNPs was observed on chromosome Pv09 with 24 SNPs (Table 1).

Table 1. Number of SNPs mapped to each chromosome and linkage group lengths.

Chromosome	N. markers	Length (cM)	Average spacing (cM)	Maximum spacing (cM)
Pv01	34	22.9	0.7	3.2
Pv02	125	71.6	0.6	15.4
Pv03	37	82.3	2.3	19.3
Pv04	60	81.3	1.4	32.7
Pv05	29	73.1	2.6	19
Pv06	35	25.9	0.8	3.4
Pv07	39	78.9	2.1	26.4
Pv08	82	33	0.4	14.9
Pv09	24	26.7	1.2	8.6
Pv10	33	13.2	0.4	3.8
Pv11	163	24.3	0.1	5.1
Overall	**661**	**533.3**	**0.8**	**32.7**

The Rqtl program identified two major QTL on Pv01 (45.95 Mb) and chromosome Pv07 (32.05 Mb) for disease reaction in the straw test 7 dpi, and these QTL had an additive effect (-0.17) on resistance. These same two QTL and a third on Pv11 (12.22 Mb) was observed at 10 dpi (Table 2). The LOD score is the significance level for the presence of a QTL at a particular location. The LOD values for Pv01, Pv07, and Pv11 QTL were: 6.68, 11.04, and 6.91.

Figure 2 shows the LOD and disease scores, the RIL population genotype, and chromosomes Pv01 and Pv07 seven days post inoculation. The plants that presented the susceptible parent genotype in both chromosomes (Pv01 and Pv07) have a higher disease score, which means that they are more susceptible to white mold. In contrast, plants with the

same parental genotype resistant to *S. sclerotiorum* on chromosomes Pv01 and Pv07, obtained lower scores in the straw test.

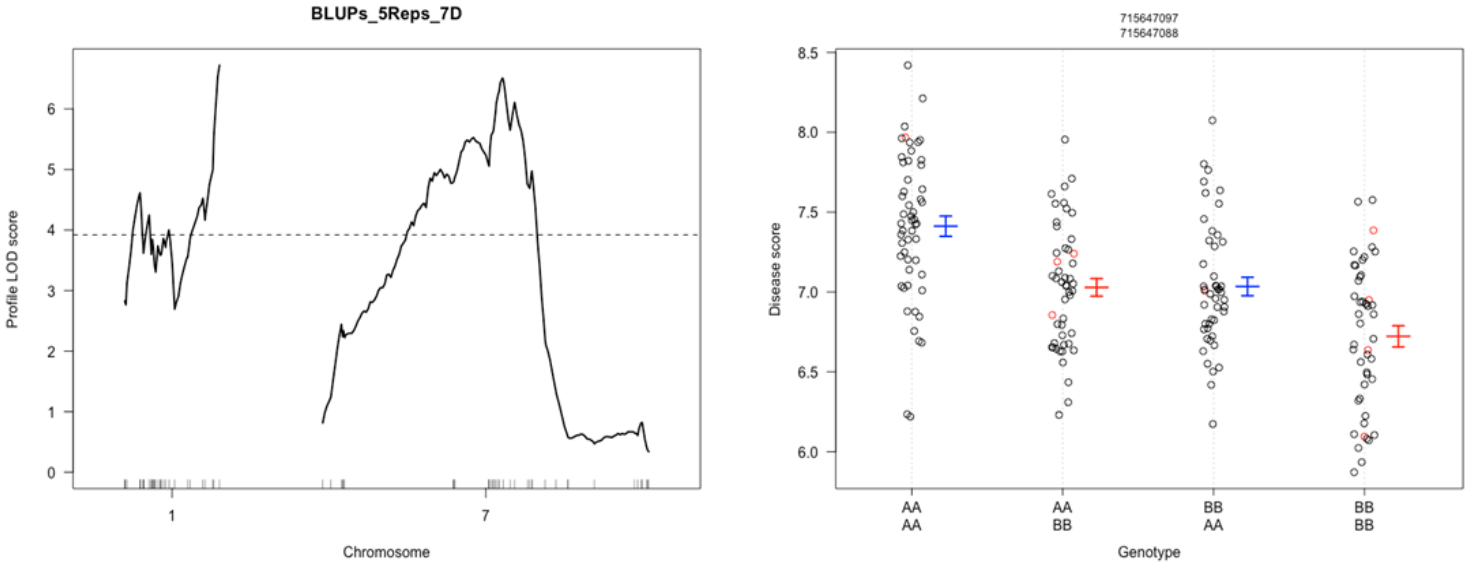

Figure 2. QTL for resistance to white mold (1 to 9 score) identified at seven dpi in PT9-5-6/USPT-WM-12 RIL population and QTL effect(s) on mean disease score.

The Figure 3 presents the LOD and disease scores, the RIL population genotype, and chromosomes Pv01, Pv07 and Pv11 ten days post inoculation. Plants with susceptible parent genotypes in the three chromosomes obtained a higher score in the straw test. On the other hand, plants with resistant parental genotypes in Pv01 and Pv07 and susceptible parental genotype in Pv11 showed greater resistance to white mold than plants with the identical resistant parental genotype for all three QTL. This means that Pv11 QTL had an epistatic interaction with Pv01 and Pv07 QTL and the pathogen resistant allele on chromosome Pv11 was from the susceptible parent.

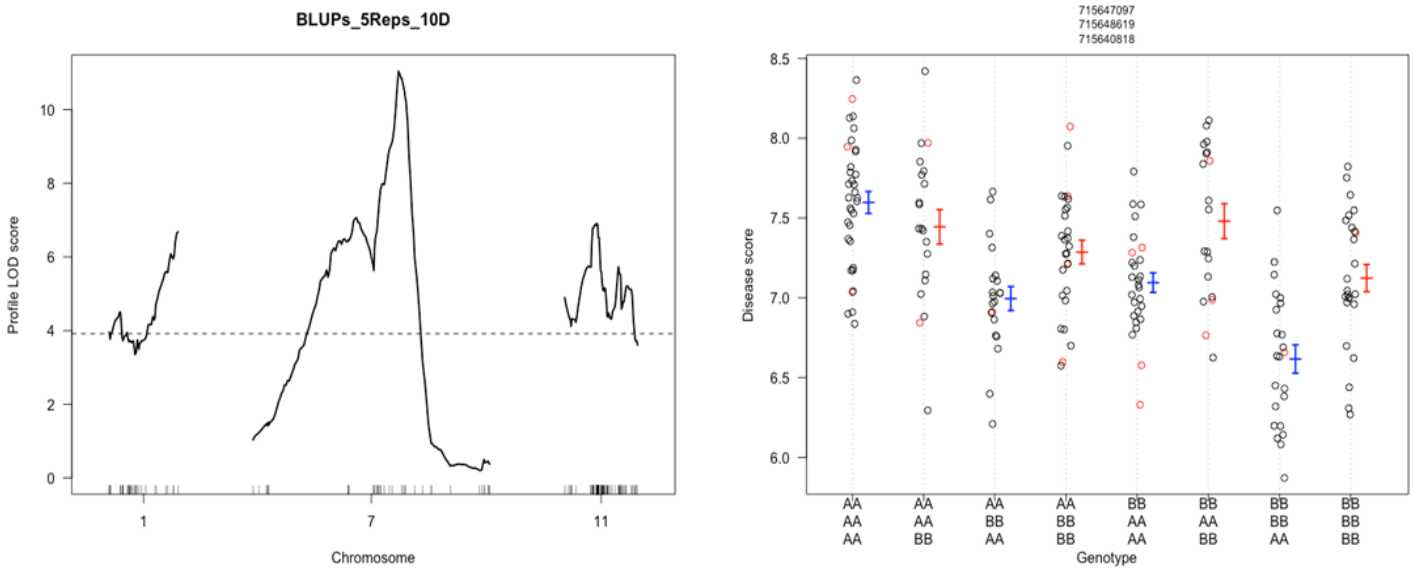

Figure 3. QTL for resistance to white mold (1 to 9 score) identified at ten dpi in PT9-5-6/USPT-WM-12 RIL population and QTL effect(s) on mean disease score.

Plants with upright architecture and less lodging contribute to an open canopy less favorable for the pathogen which lessens white mold disease pressure and development. Two main QTL on Pv07 (34.45 Mb) and Pv11 (5.21 Mb) were found and both had an additive effect for lodging in the RIL population in the field assessments. The LOD scores for these chromosomes were 22.25 and 8.15, consequently (Table 3). In figure 4, it can be seen that the individuals which had the lowest lodging score had susceptible and resistant genotypes, with the genotype coming from the parental susceptible to *S. sclerotiorum* on chromosome Pv07 and the resistant genotype on Pv11.

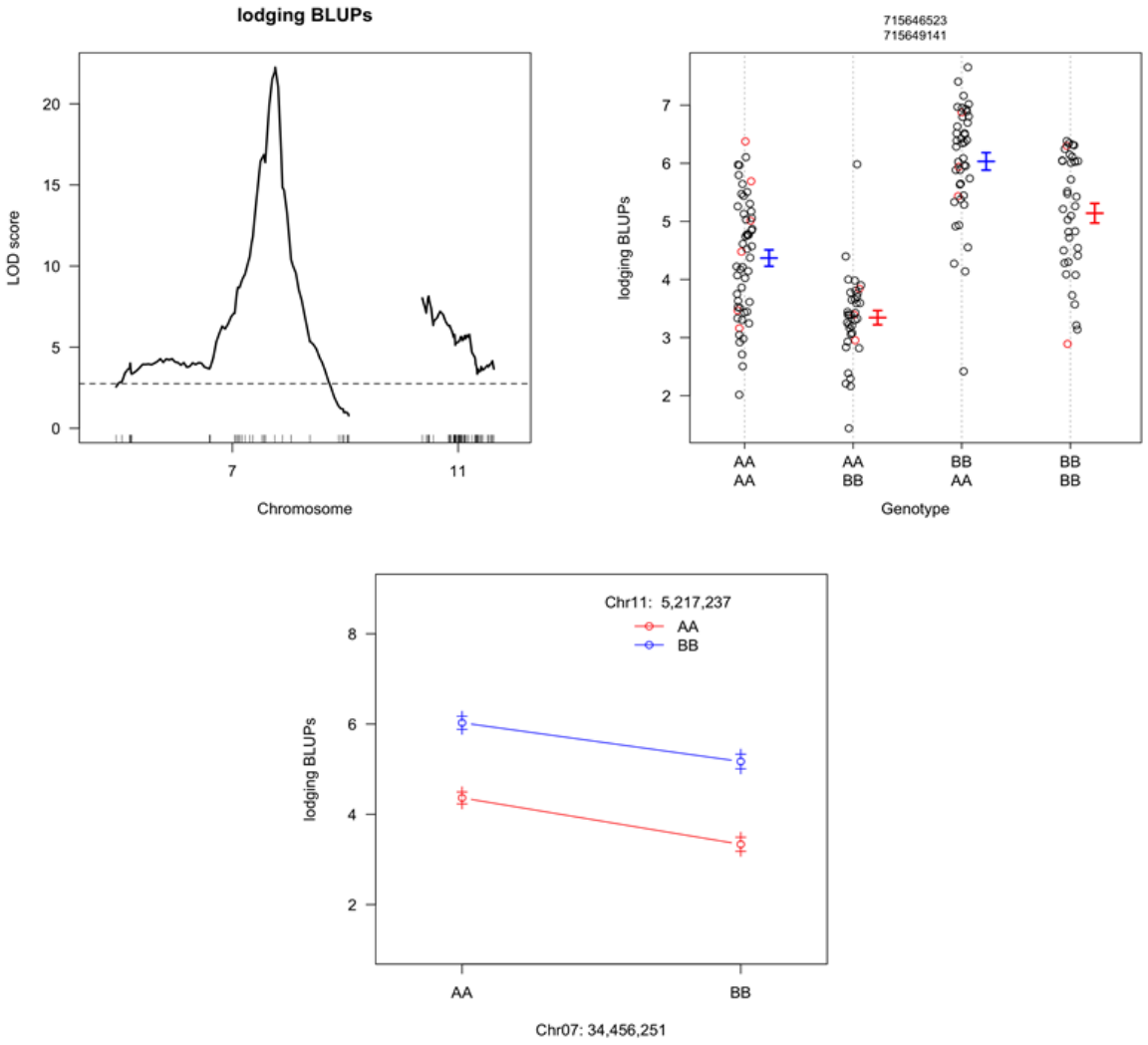

Figure 4. QTL for resistance to lodging (1 to 9 score) recorded at R6 growth stage in PT9-5-6/USPT-WM-12 RIL population and QTL effect(s) on mean score.

The RIL population was also evaluated for harvest maturity resistance to white mold and *S. sclerotiorum* disease score (Table 3). Regarding disease score, two QTL were observed: one present on Pv02 (4.62 Mb) with additive effect -0.100 and other on chromosome Pv11 (49.43 Mb) with an additive effect -0.085. The LOD scores for these chromosomes are 5.35 and 4.25, consequently. In the field evaluation for white mold, the presence of a QTL was observed again on chromosome Pv11 and another on Pv02, however, in this case, there is no epistatic interaction between the two chromosomes (Fig. 5). The effect

of either QTL is minimal and with minimal additive effect detected when combined. Individuals with susceptible genotypes on both chromosomes have higher scores for the incidence of white mold, and plants with resistant genotype had the lowest scores for the disease caused by the fungus.

The two main QTL for harvest maturity resistance to white mold were found on chromosome Pv02 (14.23 Mb) with additive effect 3.478 and 26.82 for LOD score. The other observed QTL was on chromosome Pv03 (5.04 Mb) with an additive effect of 1.632 and 7.79 LOD score.

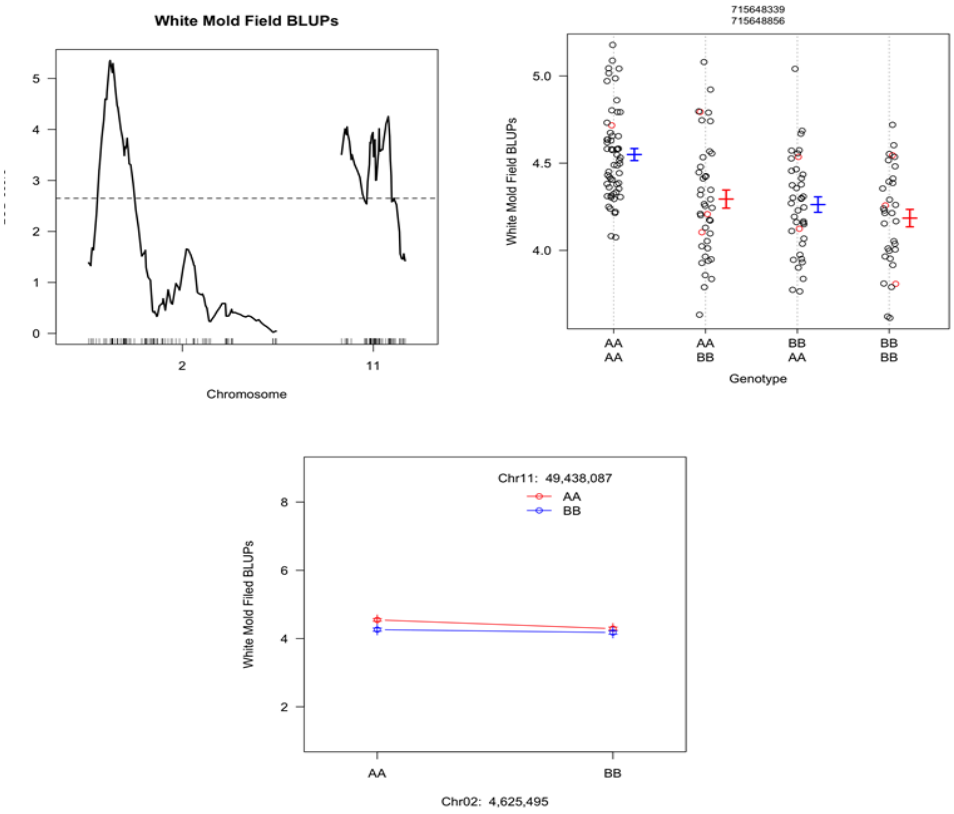

Figure 5. QTL for resistance to white mold (1 to 9 score) in the field recorded on an individual plant basis for the most susceptible plant within the plot in PT9-5-6/USPT-WM-12 RIL population and QTL effect(s) on mean score.

Table 2. Individual QTL for resistance to white mold in common bean identified across RIL population in greenhouse straw test 10 dpi.

Trait	QTL	Chromosome	LOD	R^2(%)	Additive effect	Start		Peak		End	
						(G19833 v2.1)		(G19833 v2.1)		(G19833 v2.1)	
White mold score (1–9)	WM1.2	Pv01	6.68	10.90	-0.15	715646874	42,906,200	715647097	45,952,069	715647097	45,952,069
	WM7.4	Pv07	11.04	19.01	-0.23	715639231	30,853,430	715648619	32,054,321	715646523	34,456,251
	WM11.1	Pv11	6.91	11.32	0.14	715650350	5,519,487	715641522	12,221,282	715649910	51,904,324
	WM1.2 x WM11.1	Pv01 x Pv11	2.10	3.24	0.10	NA	NA	NA	NA	NA	NA

Table 3. Individual QTL for lodging, maturity, and white mold disease in common bean identified across RIL population in the field.

Trait	QTL	Chromosome	LOD	R2 (%)	Additive effect	Start		Peak		End	
						(G19833 v2.1)		(G19833 v2.1)		(G19833 v2.1)	
Lodging	LDG7.1	Pv07	22.25	40.69	0.871	715648300	32,697,386	715646523	34,456,251	715646523	34,456,251
	LDG11.1	Pv11	8.15	12.15	-0.471	715645539	4,428,132	715649141	5,217,237	715649670	8,131,154
Maturity	MAT2.1	Pv02	26.82	46.94	3.478	715649088	11,039,483	715642443	14,233,283	715648820	23,106,531
	MAT3.1	Pv03	7.79	10.32	1.632	715646941	2,777,244	715647200	5,047,389	715649213	5,174,889
White mold score	WM2.2	Pv02	5.35	12.40	-0.100	Chr02_3896361	3,896,361	715648339	4,625,495	715651033	26,118,174
	WM11.2	Pv11	4.25	9.71	-0.085	715645539	4,428,132	715648856	49,438,087	715647451	50,349,429

DISCUSSION

According to Gaitan-Solis et al. (2008), a set of 239 common bean SNPs was first reported by comparing sequences from genomic DNA and non-coding and coding regions of a set of 10 diverse genotypes. It is known that Single Nucleotide Polymorphism is the most abundant form of DNA polymorphism in eukaryotic genomes (Brookes 1999), and according to Ding and Jin (2009), the SNPs are easy-to-automate genotyping methods and suitable for the development of high-throughput.

In this study, 661 SNPs constructed the SNP-based linkage map, and it covers 533.3 cM. The low coverage is due to this narrow cross between two pinto beans which likely have a lot of their genome in common. A better coverage can be attained from wide crosses, eg. Andean x Mesoamerican beans. The genetic linkage maps are a basic tool to localize genes or QTL in the genome, and mapping QTL is important to understand the genetic architecture underlying complex traits.

Genetic resistance is the most effective way of controlling white mold in common beans. The three major QTL identified by the Rqtl program for *Sclerotinia sclerotiorum* evaluated in the greenhouse, are present in chromosomes Pv01, Pv07 and Pv11 (Table 4). The WM1.2 and WM7.4 were found seven days post inoculation and WM11.1, 10 dpi. WM1.2 and WM7.4 were investigated to identify potential candidate genes involved in the white mold response.

Table 4. Individual QTL for resistance to white mold, lodging, and maturity in common bean identified in the RIL population.

Trait	Straw Test/Field	Chromosome	QTL	R2 (%)	Peak (Mb)
White mold	Straw Test	Pv01	WM1.2	10.90	45,952,069
	Straw Test	Pv07	WM7.4	19.01	32,054,321
	Straw Test	Pv11	WM11.1	11.32	12,221,282
White mold	Field	Pv02	WM2.2	12.40	4,625,495
	Field	Pv11	WM11.2	9.71	49,438,087
Lodging	Field	Pv07	LDG7.1	40.69	34,456,251
	Field	Pv11	LDG11.1	12.15	5,217,237
Maturity	Field	Pv02	MAT2.1	46.94	14,233,283

	Field	Pv03	MAT3.1	10.32	5,047,389

Potential genes coding for disease resistance-responsive proteins, antifungal-stress responses, and pathogenesis-related proteins were found on both chromosomes. In WM1.2, nine genes that code for four different proteins can be observed (Table 5): dirigent-like protein, Glutathione S-transferase family protein, Leucine-rich repeat protein kinase family protein, and RING/U-box superfamily protein.

Dirigent (DIR) proteins are responsible to mediate bimolecular phenoxy radical coupling during lignan biosynthesis, and according to Davin and Lewis (2005), lignans have an important role in plant defense against pathogens, by inhibiting degradative enzymes such as laccases, glucosidases, cellulase, and polygalacturonases (MacRae and Towers, 1984). The glutathione S-transferase (GST) gene family encodes genes that are critical for certain life processes, as well as for detoxication (Nebert and Vasiliou, 2004). The GST genes are upregulated in response to oxidative stress, and as is known, a large number of phytopathogenic fungi secrete oxalic acid including *S. sclerotiorum*. This secreted acid, in addition to exerting a toxic effect on the plant, acts as a suppressor of the oxidative stress generated by the plant. The Leucine-Rich Repeats Receptor-Like Kinase genes represent a complex gene family in plants involved in development and stress responses (Dufayard et al., 2017). And the RING / U-box superfamily protein is related to a role in the response of plants to various environmental stresses, with its expression increased due to host-pathogen interaction (Zeng et al., 2008).

In WM7.4, eight genes that code for four different proteins can be observed (Table 6): Alba DNA/RNA-binding protein, Glutathione S-transferase family protein, RING/U-box superfamily protein, and Chitinase family protein. The Alba DNA/RNA-binding protein is recognized as chromosomal protein, speculated to help in maintaining chromatin architecture, transcriptional repression and it might play a crucial role for stress adaptation (Verna et al.,

2018). The Chitinase family protein catalyzes the hydrolysis of chitin β-1,4 linkages, however, plants do not produce chitin, therefore, plant chitinases have been indicated to hydrolyze the pathogens cell walls and to release elicitors for defense reactions (Hadwiger and Beckman, 1980). Through blocking mycelium cells and inducting downstream defense pathways, chitinase activity can be induced due to defense response against pathogen and fungal infection (Gerhardt et al., 1997).

An epistatic interaction between WM11_ST and WM1.2 and WM7.4 was observed (Fig. 3), in which individuals with resistant genotype in chromosomes Pv01 and Pv07 and susceptible genotype in Pv11 showed greater resistance to white mold than individuals with the resistant parental genotype in all three chromosomes. Epistatic effects are often involved in complex traits, but they are difficult to confirm because of their environmental interaction and small effects. Epistasis has shown an important role in the genetic control of several quantitative traits of common bean, therefore, to retain the smaller effect of QTL and select the epistatic interactions that contribute to increasing resistance, it is necessary to use phenotypic selection (Miklas et al., 2006b).

Three characteristics of the RIL population were evaluated in the field: lodging, time to maturity, and resistance to white mold (Table 4). In this study, it can be seen that individuals with susceptible genotype on LDG7.1 and resistant genotype on LDG11.1 have lower lodging scores, followed by susceptible parental genotypes in both chromosomes. Thus, each parent apparently has a different QTL that influences upright architecture which exhibits an additive effect when combined. The importance of the plant lodging characteristic is evidenced in various studies of QTL identification for white mold resistance that mention the presence of escape mechanisms, such as plant architecture, located together with the QTL that confer resistance to white mold in beans (Soule et al., 2011).

The QTL for lodging on Pv07 is well known and was described previously. Moghaddam et al. (2016) found a strong and consistent Pv07 (46 Mb) peak for lodging at Colorado, Michigan, Nebraska, and North Dakota as well as across all locations. The peak consists of multiple SNPs, in which there are two smaller signals at Pv07 with 45 Mb and 48 Mb, that are not in LD with the Pv07 (46 Mb) region. The marker at 46.11 Mb, which resides in *Phvul.007G221700* if converted to version 2.1 of the reference genome G19833, is in the same position as LDG7.1 found in the study. Parker et al. (2020) observed 3 QTL related to plant height. One QTL located on Pv09, which was related to drone-measured maximum canopy height at 42 DAP, another QTL on Pv10, and a major QTL for hand-measured height 42 DAP on Pv07. The authors identified the same QTL for lodging scores, indicating that the variation in canopy height was the result of canopy architecture changes.

Lodged beans create a denser environment which results in cooler and humid places favorable to the pathogen, and according to Miklas et al. (2013), the plant's organs with a high level of lodging come into contact with the soil, making them vulnerable to infections by white mold mycelia from colonized senescent blossoms and leaf litter on the soil surface. Plant architectural traits such as upright determinate growth habit Type I and upright indeterminate Type II with open porous canopy, lodging resistance, stay-green stem characteristic, and late maturity help avoid/minimize *S. sclerotiorum* incidence and severity.

Partial physiological resistance to white mold is controlled by major dominant and recessive genes (Schwartz and Singh, 2013). There is some variability in the bean cycle, however, most bean types require a frost-free growing season of 85 to 120 days. Figure 6 shows the days to maturity, the RIL population genotypes, and their respective chromosomes. Plants with the susceptible parental genotype had the shortest physiological maturation time. In contrast, individuals with the longest maturation stage were those with the resistant parental genotype on both chromosomes. The presence of the susceptible genotype on

MAT2.1 confers a decrease in the days of maturation, since, when individuals have a resistant genotype in MAT2.1, the average number of days to maturation increases, and this is probably due to the fact that the MAT2.1 QTL has a larger effect than MAT3.1 QTL.

Different numbers and relative positions of QTL involved in genetic control of maturity have already been reported. For example, concerning days to maturity, Beattie et al. (2003) mapped three QTL on B4, B6 and B8, and Blair et al. (2006) mapped two QTL for the trait on B5 and B7. Silva et al. (2018) detected three QTL for days to maturity on Pv01, Pv03, and Pv09, which explained between 4.38 and 13.79% of the phenotypic variation. Moghaddam et al. (2016) also described QTL for days to maturity. The most noticeable GWAS peaks were on Pv04 (originated from Michigan) and Pv11 (from North Dakota, Nebraska, and Michigan). The authors also found a Nebraska-specific peak on Pv01 and a Colorado-specific peak on Pv07. Perez-Vega et al. (2010), identified five QTL for days to maturity (DM). One QTL (DM1) was located in linkage group B1, two QTL (DM2.1 and DM2.2) in B2 at relatively distant positions from one another, and two QTL in B6 (DM6.1 and DM6.2). The DM2.1 is located near the upper end of linkage group B2, close to the *I gene*. Nkhata et al. (2021), mapped significant markers for days to maturity on Pv02, Pv05, Pv06, and Pv07. On Pv02, two markers were found, one located at the beginning of the chromosome (623,837 Mb) and other near the lower end (48,039,523 Mb). Miklas et al. (2013), carried out a study with phenotypic association of agronomic characteristics and white mold disease severity in common beans in Washington, Oregon, and Michigan during the years 2000 to 2011. In this work, they could observe that the developing resistant lines with mid-season maturity have been a battle for breeders, as most lines with partial resistance to white mold are later maturing. The association of later maturity with less disease in the Michigan trials may result from the inclusion of advanced lines that were late due to they possess the stay-green stem trait which contributes to physiological resistance.

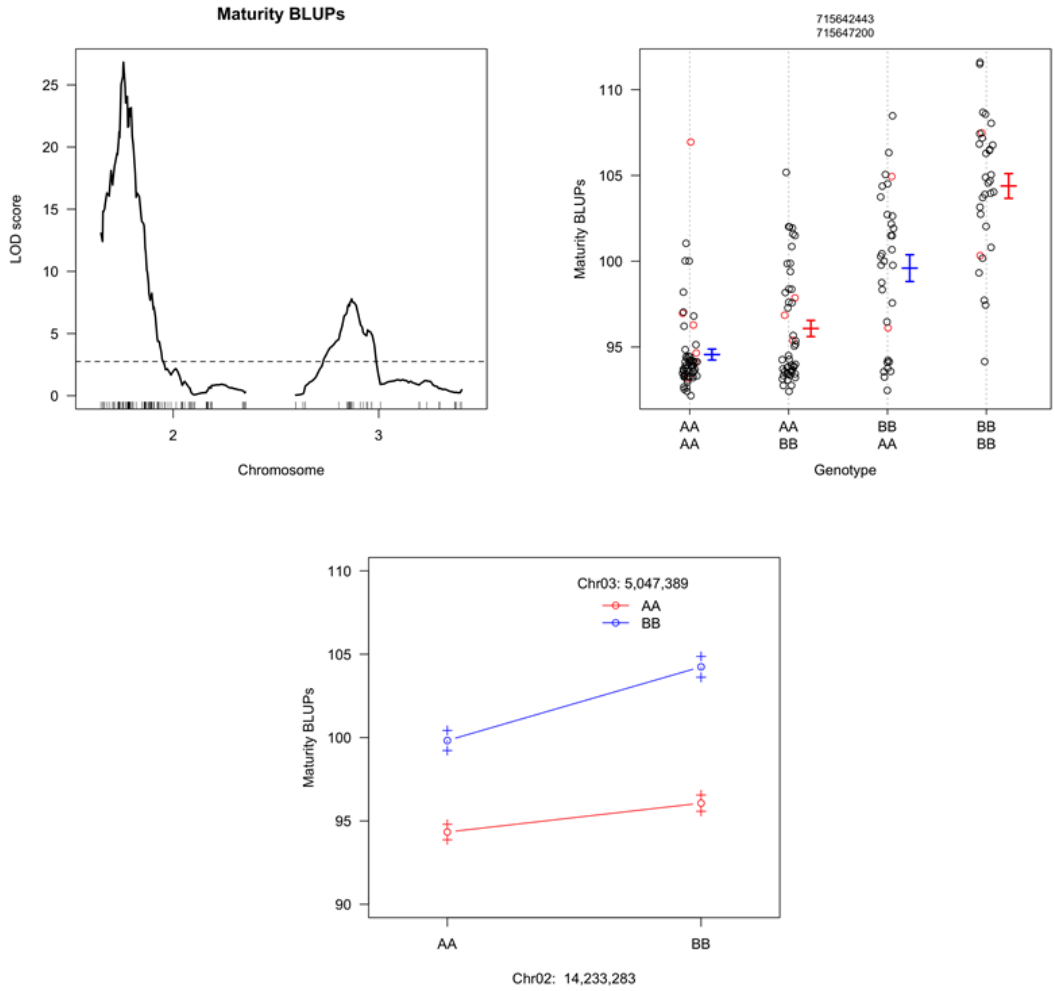

Figure 6. QTL for maturity recorded on a plant basis as days after planting (DAP) till harvest maturity in PT9-5-6/USPT-WM-12 RIL population and QTL effect(s) on mean score.

Two main QTL were found for evaluation of white mold in the field. Contrary to what occurred in the greenhouse, no epistasis was observed between WM2.2 QTL and WM11.2 QTL, and individuals with the resistant parental genotypes on both chromosomes had a lower score disease. The straw test QTL on Pv07 was named previously and this study validates it. Pérez-Vega et al. (2012) detected two QTL on Pv07 in the XC population, one QTL named WM7.1 and the other WM7.4. The second QTL may be associated with taller plants, and is located between the previously detected WM7.2 (Kolkman and Kelly 2003; Ender and Kelly 2005) expressed only in the field and WM7.3 expressed in the straw test. Miklas et al. (2013), placed five QTL on Pv07, and three of those five QTL (WM7.1, WM7.4, and WM7.5) were defined as meta-QTL by Vasconcellos et al. (2017). WM7.4 QTL is detected in Orion/USPT-

WM-12 (WM7.4 meta-QTL) and PT9-5-6/USPT-WM-12 at same position in version 2.1 of the reference genome G19833.

Vasconcellos et al. (2017) identified 37 QTL which condition partial resistance to white mold from 14 mapping populations. Two Pv01 QTL and 2 Pv02 QTL have a similar location to the QTL found in this study. The Pv01 QTL is between WM1.1, in which was observed in the Xana/Cornell 49242 RIL population (Pérez-Vega et al., 2012) and WM1.2, observed in the Orion/USPT-WM-12 population. WM1.1 and WM1.2 were conditioned partial physiological resistance in the greenhouse straw test. Due to its proximity the observed Pv01 QTL was named as WM1.2. Both WM2.2, one reported previously for the Aztec/ND88-106-04 RIL population (Miklas et al., 2007) and the other from Raven/I9365-31 (Soule et al., 2011) were associated with disease response in field trials.

The genetics of resistance to white mold is divided into two main categories: disease avoidance and physiological resistance. The different results between the greenhouse and field evaluation could be related to the fact that the evaluations of the bean resistance to infection by *S. sclerotiorum* made by straw test (in a greenhouse), takes into account only the physiological resistance, which is related to the defense mechanisms of the plant, which inhibit the spread of the pathogen in plant tissues. Furthermore, in the greenhouse, the environment is controlled and totally favorable to the development of the disease. In the field, the bean resistance to white mold is associated with morphological factors, and there may be genotype x environment interaction.

Lopes et al. (2019) in their work with recurrent selection in common bean aiming at resistance to white mold in a greenhouse, observed a significant gain and greater efficiency in mass selection for *S. sclerotiorum* in the greenhouse than in the field. This is due to in the field, it is difficult to discriminate the effects of physiological resistance and escape mechanisms, as both are interlinked. Furthermore, it is often difficult to obtain areas that are

uniformly infected with the pathogen to allow an adequate assessment of resistance (Schwartz and Singh, 2013).

In summary, our research has identified QTL controlling white mold resistance and important morpho-agronomic traits, such as lodging and maturity in a pinto RIL population. The genotyping tools can provide a starting point for the functional investigation, and validation for generating cultivars resistant to *S. sclerotiorum* with earlier maturity and more resistant to lodging.

Table 5. WM1.2 QTL potential genes.

Pv gene model_v.2	Chromosome	Start	End	
Phvul.001G173000	Chr01	42.988.471	42.990.051	Disease resistance-responsive (dirigent-like protein) family protein
Phvul.001G173300	Chr01	43.013.676	43.015.747	Glutathione S-transferase family protein
Phvul.001G173500	Chr01	43.023.123	43.025.799	Glutathione S-transferase family protein
Phvul.001G174500	Chr01	43.115.764	43.123.172	Leucine-rich repeat protein kinase family protein
Phvul.001G176000	Chr01	43.261.947	43.270.860	RING/U-box superfamily protein
Phvul.001G176700	Chr01	43.359.597	43.364.531	RING/U-box superfamily protein
Phvul.001G185400	Chr01	44.327.823	44.330.316	Leucine-rich repeat protein kinase family protein
Phvul.001G195500	Chr01	45.450.070	45.453.686	Leucine-rich repeat protein kinase family protein
Phvul.001G197000	Chr01	45.646.781	45.647.560	RING/U-box superfamily protein

Table 6. WM7.4 QTL potential genes.

Pv gene model_v.2	Chromosome	Start	End	
Phvul.007G188600	Chr07	30.850.522	30.853.090	Alba DNA/RNA-binding protein
Phvul.007G189800	Chr07	30.974.109	30.975.777	Glutathione S-transferase family protein
Phvul.007G194100	Chr07	31.609.462	31.611.119	RING/U-box superfamily protein
Phvul.007G197300	Chr07	32.054.881	32.065.454	RING/U-box superfamily protein
Phvul.007G199500	Chr07	32.278.047	32.282.125	RING/U-box protein
Phvul.007G208900	Chr07	33.118.867	33.120.096	Chitinase family protein
Phvul.007G220900	Chr07	34.403.549	34.408.140	RING/U-box superfamily protein
Phvul.007G221300	Chr07	34.434.941	34.440.831	RING/U-box superfamily protein

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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