Received: 16 August 2021 | Accepted: 16 December 2021

DOI: 10.1002/tpg2.20190

The Plant Genome

ORIGINAL RESEARCH

New genomic regions associated with white mold resistance in dry bean using a MAGIC population

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Assigned to Associate Editor David Hyten.

Funding information
Northarvest Bean Growers Association, Grant/Award Number: Dry Bean Improvement for the Northern Plains; U.S. Department of Agriculture, Grant/Award Number: National Sclerotinia Initiative, Agreement No. 58

Abstract

Dry bean (*Phaseolus vulgaris* L.) production in many regions is threatened by white mold (WM) [*Sclerotinia sclerotiorum* (Lib.) de Bary]. Seed yield losses can be up to 100% under conditions favorable for the pathogen. The low heritability, polygenic inheritance, and cumbersome screening protocols make it difficult to breed for improved genetic resistance. Some progress in understanding genetic resistance and germplasm improvement has been accomplished, but cultivars with high levels of resistance are yet to be released. A WM multiparent advanced generation inter-cross (MAGIC) population ($n = 1060$) was developed to facilitate mapping and breeding efforts. A seedling straw test screening method provided a quick assay to phenotype the population for response to WM isolate 1980. Nineteen MAGIC lines were identified with improved resistance. For genome-wide association studies (GWAS), the data was transformed into three phenotypic distributions—quantitative, polynomial, and binomial—and coupled with ~52,000 single-nucleotide polymorphisms (SNPs). The three phenotypic distributions identified 30 significant genomic intervals [$-\log_{10}$ (P value) ≥ 3.0]. However, across distributions, four new genomic regions as well as two regions previously reported were found to be associated with resistance. Cumulative R^2 values were 57% for binomial distribution using 13 genomic intervals, 41% for polynomial using eight intervals, and 40% for quantitative using 11 intervals. New resistant germplasm as well as new genomic regions associated with resistance are now available for further investigation.

1 | INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the most essential and ancient worldwide crops (Akibode & Maredia, 2011). One of the most important aspects of dry bean lies in the amount of protein, vitamins, and minerals it provides to humans (Miklas et al., 2006b). The United States is the fourth largest producer worldwide. Like many other crops, common bean faces both abiotic and biotic stresses during crop establishment, vegetative growth, and the reproductive cycle (Fageria & Santos, 2008). In the case of biotic stress, white mold (WM), caused by the fungus *Sclerotinia*

Abbreviations: GEMMA, genome-wide efficient mixed model association; GWAS, genome-wide association studies; MAF, minor allele frequency; MAGIC, multiparent advanced generation inter-cross; QTL, quantitative trait loci; SNP, single-nucleotide polymorphism; WM, white mold.

sclerotiorum (Lib.) de Bary, is considered one of the most important diseases limiting dry bean production in the United States. When susceptible cultivars are planted in previously infected fields, seed yield losses can be up to 100% (Schwartz et al., 2007; Schwartz & Singh, 2013).

Several practices are recommended to manage WM, with chemical control being the most common. However, chemical control has disadvantages such as high costs, environmental pollution, and variable fungicide efficacy (Bales & Chilvers, 2020; Bolton & Nelson et al., 2005; Harveson et al., 2013; Markell et al., 2019). Therefore, genetic resistance has been sought as a better option to control WM (Schwartz & Singh, 2013). When breeding for genetic resistance to WM, two mechanisms need to be considered: physiological and avoidance (Miklas et al., 2001). Greenhouse screening using the straw (Petzoldt & Dickson, 1996) and seedling straw (Arkwazee & Myers, 2017) tests have provided reliable methods for detecting physiological resistance. Conversely, avoidance is related to mechanisms associated with upright plant architecture and reduced lodging, which contributes to a drier canopy less ideal to the pathogen. Field testing is necessary to screen for plant avoidance traits that reduce WM infection (Myers et al., 1998; Carneiro et al., 2011). Therefore, a combination of both resistance mechanisms would be ideal for WM resistance.

Historically, WM resistance has been difficult to incorporate into breeding materials because of low heritability, cumbersome screening methods, and few sources of resistance. Studies only identified quantitative trait loci (QTL) with minor effects that are highly affected by the environment (Ender & Kelly, 2005). Combining multiple QTL with minor effects to enhance resistance levels while maintaining agronomic performance has been a challenge using conventional breeding methods (Miklas, 2007). New genetic strategies that use multiparent advanced generation inter-cross (MAGIC) populations would assist the search for quantitatively inherited genes that affect WM resistance (Pascual et al., 2015) and facilitate combining multiple QTL contributing to WM resistance in lines with acceptable agronomic performance.

The inclusion of multiple parents and the additional intermating among their offspring increases recombination in the MAGIC population (Osorno et al., 2017), leading to improved mapping resolution (Islam et al., 2016). The first MAGIC population developed for plant genetics analysis was used to discover QTL conditioning germination and bolting time in *Arabidopsis thaliana* (L.) Heynh. (Kover et al., 2009). Since then, MAGIC populations have been developed to investigate genetics of plant height and hectoliter weight in wheat (*Triticum* spp.) (Huang et al., 2012), biotic and abiotic stresses in rice (*Oryza sativa* L.) (Bandillo et al., 2013), and fiber quality and yield in cotton (*Gossypium hirsutum* L.) (Islam et al., 2016). Others, like Huynh et al. (2019) for cowpea [*Vigna unguiculata* (L.) Walp.] and Ongom and Ejeta (2018) for sorghum [*Sorghum bicolor* (L.) Moench] developed MAGIC populations for the sole purpose of germplasm development. Recently, the first MAGIC population developed in common bean was used to both map and introgress QTL associated with drought tolerance (Diaz et al., 2020). Additional examples in other crop species exist in the literature (see reviews from Arrones et al. [2020] and Scott et al. [2020]).

Core Ideas

- A MAGIC population was developed to facilitate mapping and pyramiding of QTL related to white mold resistance.
- New genomic regions associated with white mold resistance are reported while some known regions are confirmed.
- Pinto and great northern beans with improved resistance to white mold were identified.
- Disease-related candidate genes for white mold resistance were identified.

Our goal was to develop a WM-MAGIC population for germplasm development and genetic discovery of WM resistance with a focus on pinto and great northern market classes (Durango race), since pinto is the most grown and WM-susceptible market class in the United States (Miklas et al., 2004; Schwartz & Singh, 2013). The specific objectives of this research were to (a) identify resistant lines from the WM-MAGIC population with good agronomic performance and (b) identify both new and previously reported genomic regions associated with WM resistance.

2 | MATERIALS AND METHODS

2.1 | MAGIC population development

The WM-MAGIC population development had two primary purposes: gene mapping and the production of inbred lines with improved WM resistance combined with good agronomic performance for primarily the pinto bean market class. The pinto market class was targeted because it is the most produced and consumed market class in the United States and one of the most susceptible market classes to WM. Therefore, the founders of this WM-MAGIC population were all from race Durango of the Middle American gene pool and the majority were pinto bean seed types. The founders represent improved germplasm or cultivars released by different breeding programs across the United States that were selected for high seed yield potential, acceptable seed quality, partial physiological resistance to WM, or upright plant architecture, which contributed to WM disease avoidance (see Table 1). Four of the

TABLE 1 Multiparent advanced generation inter-cross population founders background information

Genotype	Origin	Market class	Straw test	Field test
USPT-WM-12	USDA–ARS	Pinto	Good	Good
PT 7-2	USDA–ARS	Pinto	Susceptible	Susceptible
'El Dorado'	MSU	Pinto	Intermediate	Very good
CO16079	CSU	Pinto	Good	No data
ID14-4	USDA–ARS	Pinto	Good	No data
'La Paz'	Provita	Pinto	Susceptible	Good avoidance
'Lariat'	NDSU	Pinto	Susceptible	Some avoidance
'Powderhorn'	MSU	Great Northern	No data	Good avoidance

Note. MSU, Michigan State University; CSU, Colorado State University. Reactions from each genotype to white mold (straw and field test) are based on previously recorded data by the authors and also based on the studies from Osorno et al. (2010), Kelly et al. (2012, 2014), and Miklas et al. (2014).

founders are reported to have partial resistance to WM. USPT-WM-12 is an upright pinto bean germplasm release (Miklas et al., 2014) with partial resistance to WM expressed in the greenhouse straw test and under field conditions. Its resistance is derived in part from 'ICA Bunsi' navy bean. CO16079 is an advanced pinto breeding line from Colorado State University with partial resistance to WM derived from three different sources: USPT-WM-1 pinto bean (Miklas et al., 2006a); PI 255956 scarlet runner bean (*P. coccineus* L.) (Brick et al., 2008); and G122 ('Jatu Rong'), a cranberry bean landrace from India (Miklas et al., 2001). ID14-4 is an advanced pinto breeding line from the USDA–ARS in Prosser, WA, with partial resistance to WM in the straw test. The source of this resistance is unknown. 'Eldorado' pinto bean, released by Michigan State University (Kelly et al., 2012), has field resistance to WM derived in part from USPT-WM-1 (Miklas et al., 2006a). The two commercial pinto cultivars, 'Lariat' (Osorno et al., 2010) and 'LaPaz', and the great northern 'Powderhorn' (Kelly et al., 2014) have upright architecture that contributes to disease avoidance under low to moderate disease pressure. Lariat, when grown under high nitrogen conditions, generates excess biomass and lodges, which negates its disease avoidance attributes. PT7-2 is a pinto breeding line from USDA–ARS, Prosser, WA, with high yield potential under abiotic stress conditions. PT7-2 is susceptible to WM in the field, so it was used as a contrasting parent.

All crosses were made at the North Dakota State University greenhouse complex and initial single crosses were made in the fall of 2014. Figure 1 shows the crossing scheme among the eight founder lines. The F_1 plants of each initial cross were crossed using a one-way funnel [(A×B) (C×D)]. The F_1 plants were tested with a polymorphic subset of insertion–deletion markers evenly distributed across all chromosomes (Moghaddam et al., 2014) to confirm they were true hybrids. For each cycle, reciprocal crosses were conducted to offset potential maternal effects and maternal inheritance. Only the true F_1 hybrids were used in subsequent crosses. After the final crosses, the F_1 seeds were planted to produce the F_2 generation, which then went through three rounds of single-seed descent from F_2 to F_5 populations. A total of 1,050 $F_{5:7}$ inbred lines were developed for this WM-MAGIC population.

2.2 | Phenotypic evaluation in greenhouse

A subset of 500 lines was selected out of the original 1,050 lines from the WM-MAGIC population making sure that all the original gametic combinations were represented in this set. We included reciprocal F_1 crosses, as well, to balance any maternal effects or trait with maternal inheritance. Selected lines were screened with *S. sclerotiorum* (ATCC 18683D-2), better known as 'isolate 1980'. This isolate was collected by J. Steadman from an infected great northern cultivar in a field near Mitchell, NE, in 1980. It has been widely used as control or type in many WM studies in common bean (Mamidi et al., 2016; Otto-Hanson, et al., 2011), providing consistent results, especially in the checks. In addition, this isolate of *S. sclerotiorum* was selected for whole-genome sequencing (Amselem et al., 2011; Derbyshire et al., 2017).

The experimental design was an augmented randomized block with four replications and two samples (plants within each replication). Two susceptible checks ('Beryl' and 'Othello') (Burke et al., 1995), two resistant checks (USPT-WM-12 and 'PC-50') (Park et al., 2001; Miklas et al., 2014), and 50 genotypes of the WM-MAGIC population were included within each incomplete block. Plants were grown in plastic trays (10 by 15 cm) with Promix substrate. The greenhouse temperature was set at 24 ± 2 ˚C during the day and 16 ± 2 ˚C during the night. To prepare the inoculum, WM was first grown directly from sclerotia in PDA media in a 100- by 20-mm petri dish at 23 ˚C for 3 d. Then, mycelia from the most outer part of the growth were transferred to a new petri dish with agar. The new plates with the mycelia were placed in a dark environment at room temperature for 2 d, after which they were grown until mycelia covered 75% of the petri dish (Petzoldt & Dickson, 1996; Arkwazee & Myers, 2017).

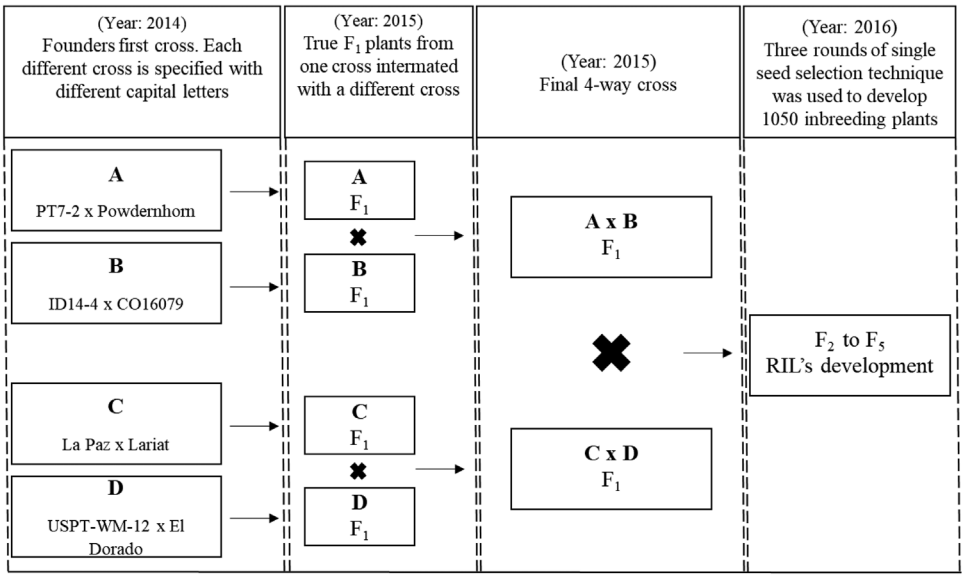

FIGURE 1 Crossing plan for white mold multiparent advanced generation inter-cross population development

The seedling straw test method described by Arkwazee and Myers (2017) was used to screen the selected lines under WM pressure. For this method, 10-d-old seedlings were inoculated. By that time, the apical meristem had grown at least 2 cm above primary leaves. Stems were cut 2–3 cm above primary leaves. A straw of ~5 mm diam. and 1–2 cm length was sealed on one end and plugged with one plug of agar in the other end. The mycelia plug was collected from the outer side of the plate where the fungus was actively growing in the petri dish. The straw with the plug was placed on the decapitated part of the stem. The plants were scored 4 d after inoculation using the disease severity scale described in the protocol. Following the standard disease visual scale, lines were considered resistant with values from 1 to 3, intermediate with a value of 4, and susceptible with values from 5 to 9. Adjusted means (least square means) were calculated using a linear mixed model in which genotypes were considered fixed effects and reps, blocks, and samples were considered random effects. Statistical analyses were made using JMP 14 Pro software (https://www.jmp.com/en_us/software/data-analysis-software.html). Phenotypic means were considered significantly different at $\alpha \leq 0.05$. Broad-sense heritability (H^2) (plot-mean based) was calculated from the seedling straw test results from the greenhouse by estimating the variance components from the resulting ANOVA table following the method by Annicchiarico (2002) and Falconer and Mackay (1996). All phenotypic data from this greenhouse screening is available online (https://hdl.handle.net/10365/32231).

2.3 | Phenotypic evaluation of selected lines in the field

Based on the greenhouse screening, out of the 500 lines evaluated, a total of 19 WM-MAGIC lines were identified as resistant (score 3 or less). Therefore, during the 2019 growing season, 12 of these WM-MAGIC resistant lines that had enough seed availability were tested in the field at the Carrington Research and Extension Center in North Dakota using a randomized complete block design with three replications. The study was planted on land with a prior history of *Sclerotinia* epidemics. To facilitate disease development, supplemental overhead irrigation was applied with low output rotating sprinklers with a 6-m (20-foot) spray radius established on a 6.096-m offset grid. Irrigation was applied as needed to keep the top 1-cm (one-half inch) of the soil moist from late vegetative growth through late pod-fill and to facilitate extended periods of canopy wetness (>24 h) during bloom. White mold reaction was evaluated during the R6 stage using the standard field visual disease scale (1–9) as described by Miklas et al. (2001). Disease reaction within each plot was scored from 1 to 9 on the basis of combined incidence and severity of infection at physiological maturity where 1 indicates no diseased plants, 2 indicates 1–20% diseased plants or 1–5% infected tissue, 3 indicates 20–30% diseased plants or 5–10% infected tissue, 4 indi cates 30–40% diseased plants or 10–20% infected tissue, 5 indicates 40–50% diseased plants or 20–30% infe cted tissue, 6 indicates 50–60% diseased plants or 30–40%

infected tissue, 7 indicates 60–70% diseased plants or 40–50% infected tissue, 8 indicates 70–80% infected plants or 50–60% infected tissue, and 9 indicates 80–100% diseased plants or 60–100% infected tissue.

In 2020, additional field and greenhouse validation tests were conducted for 19 resistant lines: 12 lines evaluated in 2019 plus seven new lines that could not be evaluated in the field in 2019 because of low seed availability. Regular straw tests (instead of the seedling test) were conducted twice in greenhouses at Prosser, WA, using isolate 1980 again. In addition, a field trial was conducted at the North Dakota State Univ. Robert Titus Irrigation Research Site, 6.4 km (4 miles) south of Oakes, ND. The field study was established on land with a prior history of WM epidemics. White mold pressure was facilitated by applying supplemental irrigation with the overhead linear irrigation system; during bloom, all normally scheduled irrigation was supplemented with the delivery of an additional 0.64 cm of water applied within 24 h. A total of 30 genotypes (19 resistant WM-MAGIC lines plus both resistant and susceptible checks) were tested in this trial using a randomized complete block design with three replications. White mold reaction was evaluated during the R8 stage instead of R6 because of challenges related to SARS-COVID-19 logistics during the 2020 growing season. Once again, the standard 1–9 field visual disease scale described above (Miklas et al., 2001) was used to evaluate resistance and tolerance.

2.4 | Genotyping-by-sequencing, sequence alignment, SNP calling, and SNP imputation

Genomic libraries were developed using the optimized protocol developed by Schröder et al. (2016). These libraries were sequenced at HudsonAlpha Institute for Biotechnology, Huntsville, AL. The sequence of each genotype was separated based on the barcode information using the FastX toolkit barcode splitter (http://hannonlab.cshl.edu/fastx_toolkit/). After obtaining a unique fastq file for each genotype, barcodes were trimmed from the sequences using FastA/Q Trimmer from the FastX toolkit. Low-quality bases were trimmed using SICKLE software developed by Joshi and Fass (2011) at the University of California–Davis. With this software, reads with <80 bp length were discarded, and a default quality threshold of 20 was used.

The Burrows–Wheeler Alignment Tool (BWA-mem) (Li & Durbin, 2009) and SAMtools (Li et al., 2009) were used to map and sort the reads against the reference genome available for common bean (Schmutz et al., 2014; https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Pvulgaris). Read group information for each genotype, including the library, platform, and platform unit, provided by HudsonAlpha Institute, were added before SNP calling using Picard tools. (http://broadinstitute.github.io/picard).

UnifiedGenotyper from Genome Analysis Toolkit v3.3 (GATK) (Mckenna et al., 2010) was used for calling SNPs. The VCF file was filtered for the minimum read depth of two using the GATK variant filtration algorithm. Then using an in-house script, markers with 25% missing data were removed. Finally, genotypes missing more than 90% of genetic information were discarded. FastPHASE (Scheet & Stephens, 2006) was used for imputing SNPs with missing data. The final HapMap was generated from SNPs with a minor allele frequency (MAF) > 0.01. This MAF was used instead of standard MAF > 0.05 since only 4% of the population was resistant. A hapmap file with all the SNP data is available online (https://hdl.handle.net/10365/32231).

2.5 | Genome-wide association study

Phenotypic screening results obtained using the seedling straw test data and sequencing data obtained from genotyping-by-sequencing and SNP calling were used to perform the GWAS. The adjusted means (lsmeans) obtained from the quantitative (1–9) visual scale data from resistance–susceptibility screening was used as the phenotypic data for the GWAS. In addition, the data was also transformed into polynomial and binomial distributions as suggested in the GWAS pipeline described by Oladzad et al. (2019). For polynomial, genotypes with a WM response of 1, 2, or 3 were grouped in the resistant category; genotypes with a mean score of 4 were grouped in the intermediate resistance category; and genotypes with a WM response from 5 to 9 were grouped in the susceptible category. For binomial, genotypes with a mean score of 1, 2, or 3 were grouped as resistant, and the rest of genotypes were grouped as susceptible. This transformation was done to find alleles with minor and major effects on the disease reaction. When GWAS is performed using two categories (binomial), the identification of markers associated with the trait is made under more rigorous criteria compared with multiclass distributions (polynomial and quantitative). The binomial transformation enhances identification of genomic regions having a major effect on the trait but underrates alleles with minor influence. Thus, the three phenotypic classes provide a complementary search for genetic factors involved in resistance to WM. Genome-wide efficient mixed model association (GEMMA) (Zhou & Stephens, 2013) was used to perform the association mapping. Principal component analysis (Price et al., 2006) was used to estimate population structure. Population relatedness was calculated using the GEMMA algorithm for centered relatedness. Bootstrapping was performed 10,000 times on the empirical distribution of P values, and the SNPs in the <0.01 and 0.1% of the distribution were considered highly significant and significant, respectively (Oladzad et al., 2019). Two models were tested using GEMMA in each phenotypic

distribution. The MM (mixed model) includes population structure and relatedness (2PCA + kinship matrix), and the EMMA (efficient mixed-model analysis) model only accounted for relatedness. The model with the lower mean square deviation (Mamidi et al., 2011) was used for further analysis. The phenotypic variation explained by the most significant markers and the cumulative effect was calculated using the likelihood-ratio-based R^2 (Sun et al., 2010) using the genABEL package available in R (Aulchenko et al., 2007; R Core Team, 2015). Finally, Manhattan plots were developed using the mhplot() function available in R (Zhao, 2007) to visualize the distribution of SNP *P* values in the genome.

2.6 | Candidate gene selection

To identify potential candidate genes associated with WM disease reaction, genes in the genomic regions ±50 Kb from the significant peak SNPs were identified using the genome annotation of the V2.1 assembly of the *P. vulgaris* reference genome (Schmutz et al., 2014; https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Pvulgaris). Genes located in those regions were further investigated by undergoing a literature and database search. Genes were selected as potential candidate genes based on their function related to disease resistance metabolic pathways.

2.7 | Parental contribution

The allele probabilities relative to each founder's genotype in the SNP pipeline generated from this MAGIC population can be useful to not only confirm the founder's diversity but also investigate their contribution across the genome for each individual. We created a model that included a subset of homozygous SNPs (4,627) in founder lines, matrices of crosses for each line, and phenotyping (scoring) data (Table 1). The physical map and the relative linkage map (Song et al., 2015) for this subset of SNPs were also added to the model. The genotype probabilities with an error probability of 0.002 were computed for all loci across the genome (Broman et al., 2019). The R/qtl2 package was used to convert these probabilities to allele probabilities relative to each founder (Broman et al., 2019). The average contribution of each parent in the MAGIC population was determined from allele probability matrices created for each chromosome, and the plots of marker allele frequency distribution in each founder as well as founder's contributions across the genome were generated.

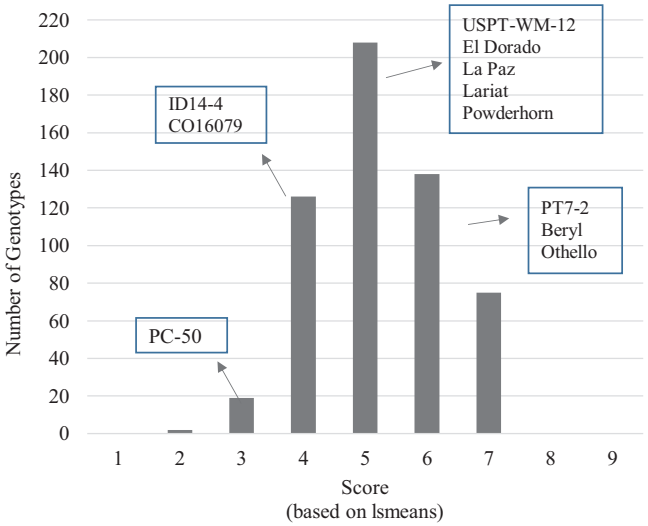

FIGURE 2 Distribution of 500 multiparent advanced generation inter-cross lines, eight founder parents, and four checks evaluated for their reaction to white mold in the greenhouse using the seedling straw test

3 | RESULTS

3.1 | Selection of resistant genotypes to white mold

The WM scoring data for the 500 lines screened from the WM-MAGIC population using the seedling straw test was normally distributed (Figure 2) as expected for traits controlled by multiple genes. Line means ranged from 2 to 7 and were statistically different (F value = 3.41; $P > F = <.0001$). Resistant check PC-50 showed strong resistance with a score of 3, while the response of USPT-WM-12 was scored as 5. The mean of both susceptible checks was 6. Broad-sense heritability (H^2) calculated from the seedling straw test (using the ANOVA table) was 0.68.

Approximately 4% of the population (19 lines) was scored as resistant, with ratings equal to or less than 3. From these genotypes, four were great northern and 15 were pinto seed types (Table 2). We confirmed the distribution of the founder lines of this MAGIC population between intermediate resistance to susceptible, and some breeding lines had lower disease scores than any of the founder lines (Table 2; Figure 2), suggesting that the WM-MAGIC population was expressing transgressive segregation. Founder lines CO16079 and ID-14-4 expressed intermediate resistance with an average value of 4 while Eldorado, La Paz, Lariat, USPT-WM-12, and Powderhorn had susceptible scores of 5. Founder line PT7-2 was the most susceptible, with an average score of 6.

TABLE 2 White mold (WM) mean score (seedling straw test) of the resistant genotypes from the WMM population, its founder parents, and the WM checks with its market class

Genotype	Market class	Score ± SE[a]
WMM-68	Pinto	2.7 ± 0.7195
WMM-109	Great Northern	3.2 ± 0.6848
WMM-165	Pinto	3.5 ± 0.6315
WMM-166	Pinto	3.5 ± 0.6319
WMM-185	Pinto	3.5 ± 0.6558
WMM-190	Pinto	3.2 ± 0.6843
WMM-214	Great Northern	2.4 ± 0.7198
WMM-219	Pinto	3.3 ± 0.6856
WMM-299	Pinto	2.1 ± 0.8829
WMM-300	Pinto	3.1 ± 0.6104
WMM-483	Pinto	3.0 ± 0.6104
WMM-506	Great Northern	3.3 ± 0.6843
WMM-541	Pinto	2.8 ± 0.6559
WMM-580	Pinto	3.2 ± 0.6560
WMM-739	Great Northern	3.4 ± 0.6316
WMM-820	Pinto	3.4 ± 0.6861
WMM-851	Pinto	3.5 ± 0.7195
WMM-922	Pinto	3.1 ± 0.7194
WMM-1043	Pinto	3.3 ± 0.6560
ID14-4	Pinto	4.0 ± 0.5774
CO16079	Pinto	4.0 ± 0.5774
'El Dorado'	Pinto	5.0 ± 0.5774
'La Paz'	Pinto	4.5 ± 0.7071
'Lariat'	Pinto	4.7 ± 0.5774
'Powderhorn'	Great Northern	4.8 ± 0.5000
PT7-2	Pinto	5.5 ± 2.0817
USPT-WM-12	Pinto	4.8 ± 0.4894
'PC-50'	Cranberry	2.6 ± 0.4911
'Othello'	Pinto	6.0 ± 0.4883
'Beryl'	Great Northern	6.1 ± 0.4897

[a]White mold score based on the 1–9 visual scale from Arkwazee and Myers (2017).

Even though consistency in results is a concern with WM screening, the statistical analysis did not find significant differences in the sampling within replication (F value = 0.85; $P > F = .2231$). Also, no significant differences were observed among replications with this scoring procedure (F value = 0.68; $P > F = 1.0000$), implying the greenhouse protocol was repeatable across both samples and replications.

3.2 | Phenotypic evaluation of selected lines in the field

In the 2019 inoculated field trial at Carrington, ND, using a subset of 12 WM resistant lines listed in Table 2, disease pressure was very high and at least one line (WMM-214), showed resistance or tolerance across all replications consistently, while two other lines showed good levels of resistance in only 1 or 2 reps. In 2020, straw test results using the traditional procedure that inoculates 28-day old plants were replicated twice in greenhouses at Prosser, WA. During the first test, disease pressure was so high that none of the 19 WM-MAGIC lines evaluated obtained a score of 3 or less. However, in a second screening (Supplemental Table S1), at least two lines (WMM-214 and WMM-219) showed disease scores close to 3 (data not shown). The inoculated field trial conducted at Oakes, ND, once again exhibited very high WM pressure as evidenced by the high scores for the susceptible checks (Supplemental Figure S1). The WM-MAGIC lines 214 and 219 once again had disease scores between 3 and 4 across all reps, suggesting high levels of resistance not only in greenhouse tests but also in the field. In addition, lines WMM-483 and WMM-300 showed intermediate levels of resistance (scores of 4–5) combined with desirable upright plant architecture, suggesting a combination of physiological resistance as well as avoidance mechanisms.

3.3 | GWAS

After filtering sequencing data based on read depth, quality, heterozygosity, and minor allele frequency, 428 genotypes were used to perform GWAS with a total of 52,201 SNPs. Of the two models tested for each of the phenotypic distributions, the mixed linear model had the lowest mean square deviation value (0.0002 for quantitative, 0.0003 for polynomial, and 0.0015 for binomial) for all the phenotypic distributions evaluated. Only GWAS results using mixed linear model are presented (Figure 3).

The three phenotypic distributions identified 30 significant genomic intervals ($-\log_{10}$ (P value) ≥ 3.0) associated with resistance of dry bean to WM (Table 3). The quantitative phenotypic distribution identified 11 intervals, which accounted for 40% of the cumulative phenotypic variation, the polynomial distribution identified eight intervals that explained 41% of the cumulative phenotypic variation, and the binomial distribution identified 13 intervals that explained 57% of the phenotypic variation. Of the 30 genomic regions identified, one region was common among the three phenotypic classes, and one region was in common between polynomial and binomial distributions. Also, at least two genomic regions of the 30 identified in this research were previously reported, and these results helped to validate those regions.

Region Pv11:17.24 Mb was identified with the same SNP peak SNP (S11_17248499) for both the polynomial and binomial phenotypic data. This interval located at 8 Kb upstream from the gene model *Phvul.011G117400* annotated as an

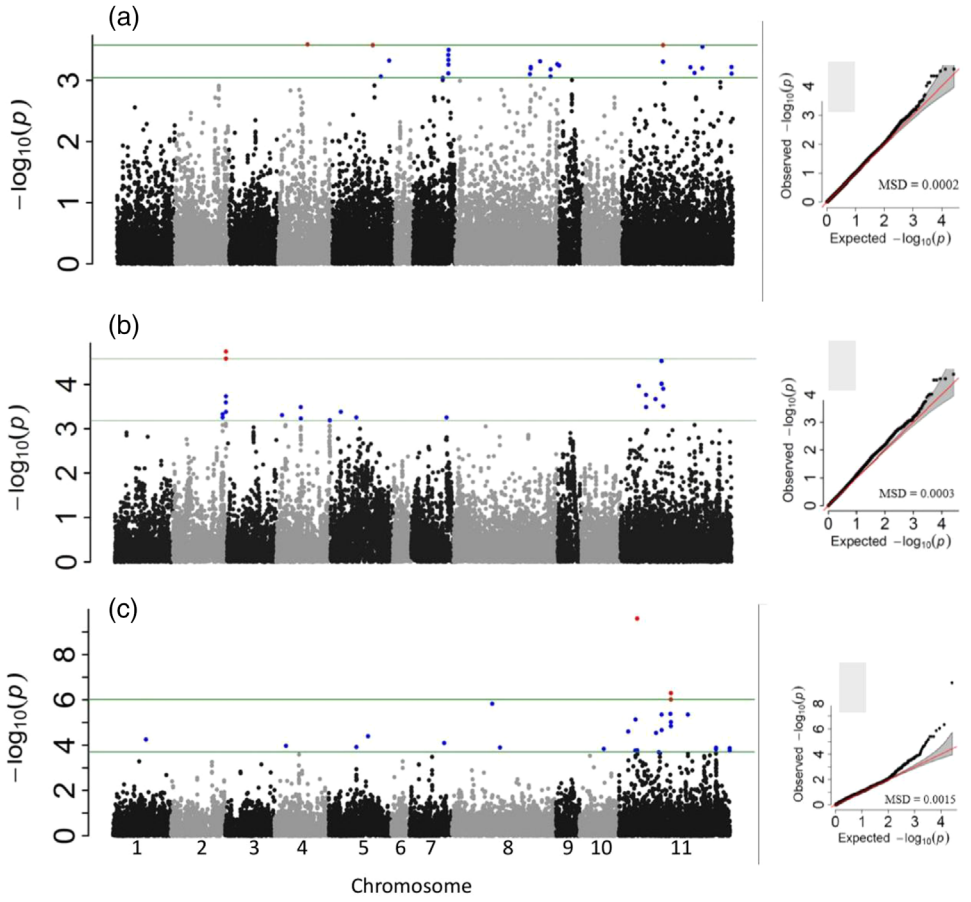

FIGURE 3 Manhattan plots and its respective quantile–quantile plots of (a) quantitative data, (b) polynomial data, and (c) binomial data for white mold multiparent advanced generation inter-cross population resistance to white mold. Green lines represent a cutoff of .01 and .1. Markers red-colored passed the cutoff value of .01, and blue-colored markers only passed the cutoff value of 0.1

Ankyrin repeat family protein. This SNP is the most significant in the binomial distribution, as it alone explains 17.7% of the phenotypic variation. The second Pv11 region is shared between the three phenotypic distributions and is located at Pv11:25.67 Mb. Gene model *Phvul.011G123500* was found in that region and is annotated as a receptor-like-protein-kinase HAESA.

Two regions were associated with resistance-related gene clusters: one with the quantitative data distribution that was already reported (Miklas et al., 2013) and one in the binomial distribution. For the quantitative distribution, QTL WM 7.4 was confirmed with peak SNP S07_30821924 [$-\log_{10}$ (*P* value) = 3.50] harboring gene models *Phvul.007G187700, Phvul.007G188100, Phvul.007G188300*, and *Phvul.007G188900* annotated as pathogenesis-related-4, prenylated RAB acceptor 1.E, Malectin/receptor-like protein kinase family protein, and Pentatricopeptide repeat (PPR) superfamily protein, respectively. For the binomial data, the Pv11:52.88 region with peak SNP S11_52882970 [$-\log_{10}$ (*P* value) = 3.87] was near gene models *Phvul.011G209000, Phvul.011G208900, Phvul.011G208800, Phvul.011G208700, Phvul.011G208400, Phvul.011G208100*, and *Phvul.011G208000* annotated as Eukaryotic aspartyl protease family proteins.

3.4 | Parent's genetic contribution

Since the development of a MAGIC population includes eight founder parents, we should expect each parent's genetic contribution to be around 12.5% absent any significant relatedness among any of the parents in a genomic region. For this WM-MAGIC population, intermating did not include every possible cross, and the nonequal inclusion of gametes from each founder parent might be a concern. Thus, we calculated the average parental contribution and confirmed it to be around 12.5% (Figure 4) with parts of chromosomes Pv02, Pv07, Pv10, and Pv11 showing some deviations.

4 | DISCUSSION

The discovery of genetic factors controlling quantitatively inherited disease resistance requires high quality phenotypic

TABLE 3 Peak single-nucleotide polymorphisms (SNPs) (0.1%) associated with white mold resistance in the white mold multiparent advanced generation inter-cross population in quantitative, polynomial, and binomial phenotypic distributions

Type of phenotype	Interval		SNP	Peak SNP			Cumulative variation
	Chromosome	Genomic interval or position	Base	$-\text{Log}_{10}(P)$	Variation		
						—%—	
Quantitative	4	26.44	S04_26441542	G/C	3.59	6.2	40
	5	27.36	S05_27362293	C/T	3.06	1.7	
	7	24.05	S07_24051914	G/A	3.04	4.7	
		30.81–30.82	S07_30821924	G/A	3.50	6	
	8	40.19–50.52	S08_40526382	C/T	3.20	4.8	
		63.04	S08_63046675	C/A	3.20	5.1	
	11	25.67[a]	S11_25670846	G/A	3.50	6.6	
		34.17	S11_34170894	C/T	3.22	5	
		36.35	S11_36353491	C/T	3.12	4.8	
		39.84–39.90	S11_39903426	T/A	3.55	6.3	
		52.4	S11_52401988	C/T	3.22	6.1	
Polynomial	2	47.80–47.91	S02_47914415	T/C	4.5	7.8	41
	4	22.62	S04_22625095	C/T	3.44	5.9	
		47.08	S04_47083551	C/G	3.28	5.3	
	11	17.24[†]	S11_17248499	C/T	4.11	7.2	
		23.53	S11_23535005	G/A	3.65	6.3	
		25.67[a]	S11_25670803	G/A	4.57	7.81	
		37.03	S11_37039553	C/T	3.44	5.5	
		44.61	S11_44618477	A/G	3.13	4.9	
Binomial	1	24.24	S01_24248170	T/G	4.25	7.8	57
	4	13.33	S04_13332737	T/A	3.97	6.3	
	5	18.11	S05_18115593	A/G	3.92	7.8	
		22.99	S05_22999183	C/T	4.4	8.3	
	7	29.3	S07_29300748	T/C	4.09	6	
	8	27.8	S08_27805286	G/C	5.83	10.2	
		31.13	S08_31137455	A/C	3.89	7.3	
	10	24.58	S10_24587949	G/A	3.84	7.18	
	11	17.24[b]	S11_17248499	C/T	9.6	17.7	
		25.67[a]	S11_25671010	C/T	3.63	7.1	
		34.38	S11_34384862	T/C	5.35	10.4	
		48.13	S11_48139011	A/G	3.89	5.8	
		52.88	S11_52882970	T/C	3.87	7.6	

[a]Region was found in common in the three different phenotypic classes.
[b]Region was found in common in binomial and polynomial phenotypic distributions.

data and high-resolution genotypic data. The response to the WM pathogen is highly affected by the environment, making it a challenging disease to evaluate phenotypically. Among the commonly used protocols to evaluate the resistance of common bean to WM, the conventional straw test method (Petzoldt & Dickson, 1996) has been the most important. The disadvantage of this protocol is the age of the plant required for screening, increasing the demand for both space and time. The seedling straw test method (Arkwazee & Myers, 2017) overcomes these limitations and provided repeatable WM infection response data in this study. This seedling protocol takes 21 d from the day of sowing seed to the day of scor-

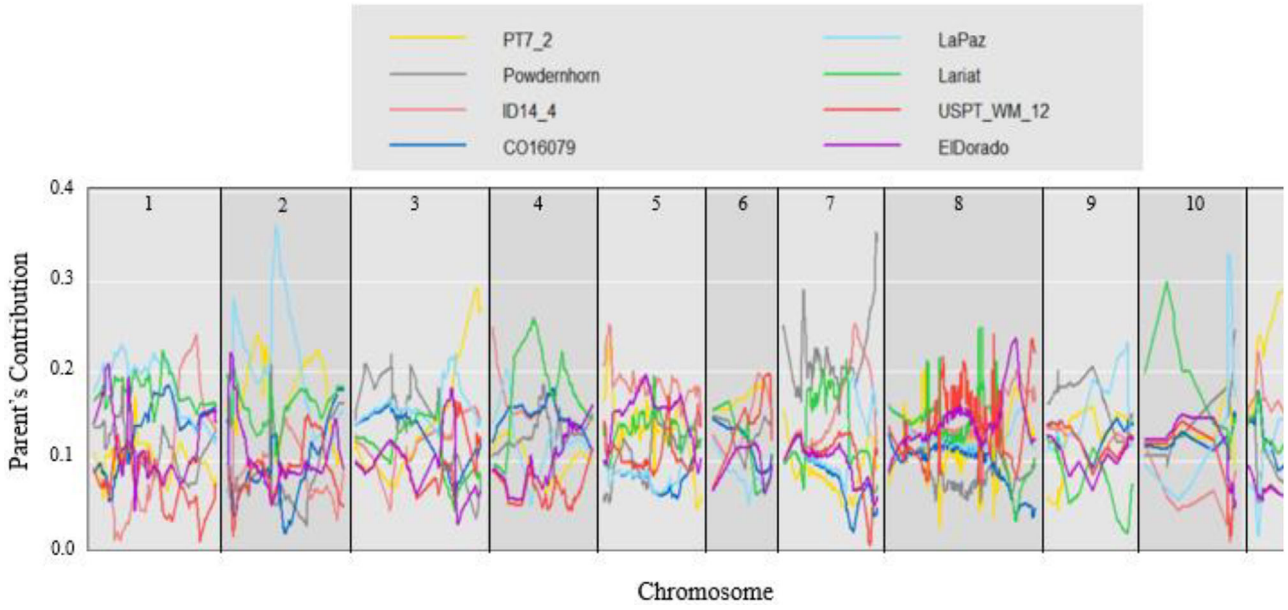

FIGURE 4 Founder's genetic contribution to the white mold multiparent advanced generation inter-cross population

ing, representing a reduction of 15 to 22 d compared with the conventional straw test method (Petzoldt & Dickson, 1996). This allows for efficient use of time, space, and experimental resources. While this protocol is suitable to identify resistant genotypes, it is more challenging to identify intermediate resistance because the defense response of young seedlings may not be robust enough to combat the aggressive growth of the pathogen. For this reason, we considered genotypes as resistant when they present values from 1 to 3. Genotypes with these scores were able to continue growing despite infection since primary leaves were still healthy. Genotypes with a score of 4 were considered to have an intermediate resistance since they had low chance to survive, and genotypes with values from 5 to 9 were considered susceptible since they never survived. Intermediate resistant genotypes may need to be evaluated using the conventional straw test method to better differentiate their reaction to WM. Broad-sense heritability (H^2) calculated from the seedling straw test data (using the ANOVA table) was 0.68. Previous studies have shown similar values ranging between 0.40 and 0.80 (Kolkmann & Kelly, 2003; Miklas & Grafton, 1992; Miklas et al., 2001, 2004). However, since this value is estimated from only one greenhouse experiment, caution needs to be exercised when interpreting this estimate considering the results presented and the demonstrated complex nature of the resistance to WM potentially is due to unknown gene interactions among multiple loci with small genetic effects. It is expected that estimates of narrow-sense heritability (h^2) would be lower since it only account for additive effects.

The complex nature of WM resistance (Miklas et al., 2001, 2006a; Soule et al., 2011) complicates the introgression of resistance from genotypes of one gene pool to another or between landraces, accessions, or market classes. Moreover, it makes it challenging to introgress and combine resistances into dry bean genotypes with desirable agronomic traits for the various bean market classes. Some WM resistant dry bean genotypes have been developed (Tu & Beversdorf, 1982; Singh et al., 2007; Saladin et al., 2000; Schwartz & Singh, 2013), but only a few pinto bean improved germplasm lines with some level of resistance have been developed thus far. USPT-WM-12 (Miklas et al., 2014) is the only pinto bean improved germplasm that has provided a mixture of intermediate physiological resistance with plant avoidance mechanisms and good agronomic traits that can be effectively exploited by breeders. The WM-MAGIC population contains at least 19 resistant lines (15 pinto and four great northern) based on the seedling straw test screening, which surpassed the physiological resistance of USPT-WM-12. The identification of lines with higher levels of seedling straw test resistance to WM than any of the founders likely results from combining different major- and minor-effect resistance alleles from the founders via recombination during the development of the WM-MAGIC population. In addition, screening of the second set of 560 lines is currently underway and will allow the identification of additional resistant lines.

Screening of the 19 WM-MAGIC resistant lines using the regular straw test in 2020 plus the field evaluations made in 2019 and 2020 allowed the identification of few lines with either high levels of resistance (WMM-214 and WMM-219) or intermediate resistance combined with upright plant architecture (WMM-300 and WMM-483) that showed consistent levels of resistance across different tests and environments. These consistent results are of special importance given that greenhouse evaluations were made with isolate 1980, but field

evaluations in North Dakota were made with local isolates, which shows that the high levels of resistance are maintained even with potential differences among strains and isolates; however, this needs to be investigated further. Line WMM-214 showed the highest levels of resistance; however, it also exhibited very late maturity and stay-green traits, which may be both correlated to WM resistance (Miklas et al., 2004) but are undesirable for cultivar releases. Additional crosses with this WMM-214 will be required to transfer the high levels of WM resistance into genotypes with better agronomic traits. Nonetheless, WMM-214, WMM-219, WMM-300, and WMM-483 represent new resistance sources for breeding programs especially for the pinto and great northern market classes. Powderhorn is the only reported great northern genotype with good field avoidance to WM (Kelly et al., 2014). Field yield trials are currently underway during the 2021 growing season with selected WMM resistant lines and checks to identify lines that combine either high or intermediate levels of WM resistance with good agronomic performance.

Association mapping using the seedling straw test phenotypic data and the 52,000 SNP HapMap identified new genetic factors associated with WM resistance in dry bean. The quantitative 1–9 scale may compromise the GWAS detection of alleles conferring a major effect on resistance. Thus, we classified the phenotypic data as three different phenotypic distributions (quantitative, polynomial, and binomial) for the GWAS analysis. The GEMMA software (Zhou & Stephens, 2013) is especially useful when data is viewed as different distributions because it first determines the type of phenotypic distribution and then selects the appropriate statistic method to protect the model against errors (Zhou & Stephens, 2013).

By applying the three phenotypic distribution approaches, Oladzad et al. (2019) identified SNPs and intervals with major and minor effects for quantitative resistance to *Rhizoctonia solani* in common bean. The significant SNPs identified with the binomial data had the highest phenotypic variation, and the peak SNPs were the most significant based on the *P* values when compared with the other phenotypic distributions. The binary distribution data also identified more regions associated with WM resistance. As suggested by Oladzad et al. (2019), we found the binary distribution data to identify genes conferring major effects on the disease reaction because of the strict classification of the disease response as either resistant or susceptible. The advantage of the aggressive seedling straw test method is that it easily distinguishes genotypes with a stronger resistance response from those with an intermediate resistance response that appear to be active at a later growth stage. Thus, the young seedling phenotypic data set is probably ideal for the binomial GWAS methodology.

The GWAS detected new genotype–phenotype associations for all three phenotypic distributions on chromosome Pv11. The Pv11:25.67 Mb peak was common across all the phenotypic distributions, and gene model *Phvul.011G123500* was identified as a potential candidate gene. This gene is an ortholog of gene *HSL1*, a leucine-rich-repeat receptor kinase (Jinn et al., 2000) in *Arabidopsis* (www.uniprot.org). Leucine-rich-repeat receptor kinases have various functions in plant immunity including acting as a detection system for the presence of pathogens in external layers of the plant (Tang et al., 2017). The percentage of variation explained by this genomic region was the highest in the polynomial and quantitative approaches (7.8 and 6.6%, respectively).

A second Pv11 region at Pv11:17.24 Mb was identified with both binomial and polynomial data. Gene model *Phvul.011G117400*, annotated as an ankyrin repeat protein family, was located 8 Kb downstream from the most significant SNP. This protein family is well studied in many crops and has a confirmed role in plant resistance against pathogens. Researchers demonstrated that one ankyrin repeat-containing protein in rice serves as a positive regulator in basal defense against rice blast (*Magnaphorte oryzae*) and is activated by the jasmonate and salicylic acid-signaling pathways (Mou et al., 2013). In *Arabidopsis*, a gene related to an ankyrin repeat protein plays an essential role as a signaling element of receptor-like proteins (RLP) gene-regulating immunity against bacterial pathogens (Yang et al., 2012). Other ankyrin repeat genes were identified in past studies as candidate genes for salt and drought stress in soybean (*Glycine max* L.) and common bean (Zhang et al., 2016; Cortés & Blair, 2018). The peak SNP used for detecting this region in the binomial distribution explains 17.7% of the variation and has the highest *P* value in this study. This could be an allele conferring major effect on the resistance as its effect was greatest with the binomial data.

Besides the shared regions, we also identified one cluster of genes in Pv11 with the binomial approach at Pv11:52.88 Mb. A family of seven genes, annotated as members of the eukaryotic aspartyl protease protein family, were identified within ±50 Kb of the peak SNP. Overall, proteases play a role in host–parasite interactions. They are found in both the plants and their pathogens and are involved in defending plants from pathogens and in camouflaging pathogens from plant proteases and receptors of pathogens effectors (Hou et al., 2018). Gene models identified in this cluster are orthologs to the *CDR1* gene from *Arabidopsis*. The *CDR1* gene is involved in the induction of local and systemic defense responses (Xia et al., 2004). Another study found an aspartyl protease to be an important factor in promoting the activation of *BAG6*-mediated basal resistance gene (Li & Dickman, 2016). The *BAG6* gene in *Arabidopsis* helps enhance the resistance response against *Botrytis cinerea* (de Bary) (Li & Dickman, 2016), a pathogen that shares features with *S. sclerotiorum* because of its necrotrophic lifestyle.

This study identified three major novel QTL regions involved in common bean resistance to WM, and there-

fore, we are proposing Pv11:17.24 Mb, Pv11:25.67 Mb, and Pv11:52.88 Mb to be named as WM11.2, WM11.3, and WM11.4, respectively. Besides the identification of novel regions, this research also validated QTL identified previously. With the peak at SNP S07_24051914, this study confirmed WM7.4 previously identified in three different biparental populations (Perez-Vega et al., 2012; Miklas et al., 2013). One of those three populations share USPT-WM-12 (Vasconcellos et al., 2017) as a common parent with this WM-MAGIC population. Another region, previously reported as WM8.3 (Miklas & Delorme et al., 2003; Maxwell et al., 2007; Soule et al., 2011), was fine mapped and pyramided with WM7.1 (Mamidi et al., 2016). Our research found marker S08_40526382 to be significant and close to this region.

The peak markers S11_17248499, S11_25670846, S11_52882970, S07_24051914, and S07_30821924 for WM11.2, WM11.3, WM11.4, and WM7.4 QTL have potential for breeding purposes. Those markers presented significant *P* values, and at least one gene related to biotic stress was found in a window of 50 Kb upstream and downstream. Regarding new resistant germplasm, dry bean breeding programs around the United States are already using some of the 19 resistance lines from the WM-MAGIC population in crosses geared toward future development of pinto bean cultivars with improved resistance to WM.

We were able to confirm the equal contribution of each parent in this population following the procedures in Dell'Acqua et al. (2015). Hybridization in common bean is laborious and inefficient as each cross provides only two to four hybrid seeds. For that reason, we could not perform intermating for each possible cross combination, so instead we followed a one-way funnel cross scheme. Despite this scheme and its limitations, Figure 4 shows that gametes from each founder were essentially equally represented, even though F_1 plants were not intermated among them, and this equal representation of gametes may be one of the reasons for the normal phenotypic distribution obtained (Figure 2). However, the minimal variation in parental contribution in some chromosomes might be due to bias on sampling generated during the crosses, SNP location and sampling, or cross-over deviations caused by linkage disequilibrium. In addition, one of the founders of the WM-MAGIC population, CO16079, is the product of interspecific crosses with scarlet runner bean and, therefore, we speculate that this could explain in part the lack of recombination observed in some portions of specific chromosomes. Similar results are reported by Dell'Acqua et al. (2015), in which some founders had higher contribution within some sections of specific maize chromosomes. In conclusion, and despite its limitations, the one-way funnel method used in this study still allowed for good representation of all potential gametic combinations perhaps at a higher rate than initially thought. This crossing scheme could be used in other crops with similar limitations regarding crosses and amount of hybrid seed produced and still allowing a good gametic representation and allele recombination.

The WM-MAGIC population can be used to identify new sources of variation and new genomic regions associated with other traits of interest if the founder's parents present polymorphism for the phenotype of interest. For example, preliminary evaluations found the eight founder parents are polymorphic for resistance to race 20-3 of *Uromyces appendiculatus*, the causal agent of bean rust (Ixcotoyac-Cabrera, 2021).

Future research is planned for testing the 19 resistant lines for agronomic traits in the field and in the WM nurseries available across the United States so the resistance lines can be challenged with additional strains and isolates of the pathogen across other regions. In addition, this will help to further select among the 19 lines for those with the best WM avoidance characteristics via field evaluation. The genotypic and phenotypic data obtained from this research will be further used to perform genomic prediction studies, and the remaining genotypes from this WM-MAGIC population ($n = 560$) will be used for validation. Both phenotypic and genotypic datasets are available at an online repository

DATA AVAILABILITY STATEMENT

Both phenotypic and genotypic datasets used in this study can be found at the following link: https://hdl.handle.net/10365/32231. Authors should be notified and properly acknowledged if any of these datasets is used for further analyses.

ACKNOWLEDGMENTS

Funding for this study was provided by USDA, Agricultural Research Service (USDA–ARS) through the National Sclerotinia Initiative, Agreement No. 58-3060-9-037, and Northarvest Bean Growers Association. Dr. Jawahar Jyoti for providing advice for some of the statistical analyses. We also want to thank all the research support personnel at USDA-Prosser-WA and NDSU, including research assistants, postdocs, technicians, students, and interns for their help at different stages of this research.

AUTHOR CONTRIBUTIONS

Edgar Escobar: Data curation; Formal analysis; Investigation; Methodology; Validation; Writing-original draft. Kristin Simons: Data curation; Formal analysis; Investigation; Methodology; Software; Supervision; Validation; Writing-review & editing. Phillip Miklas: Conceptualization; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Writing-review & editing. Rian K. Lee: Data curation; Formal analysis; Investigation; Methodology; Software; Supervision; Validation; Visualization. Stephan Schroder: Data curation; Investigation; Methodology; Writing-review & editing. Nonoy Bandillo: Conceptualization; Formal analysis; Investigation; Methodology; Software; Supervision; Validation; Writing-review &

editing. Michael Wunsch: Methodology; Supervision; Validation; Writing-review & editing. Juan M. Osorno: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing-review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding this research.

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How to cite this article: Escobar, E., Oladzad, A., Simons, K., Miklas, P., Lee, R. K., Schroder, S., Bandillo, N., Wunsch, M., McClean, P. E., & Osorno, J. M. (2022). New genomic regions associated with white mold resistance in dry bean using a MAGIC population. *Plant Genome*, e20190. https://doi.org/10.1002/tpg2.20190