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New genomic regions for resistance to anthracnose (*Colletotrichum lindemuthianum*) through GBS-based genome-wide association study in common bean (*Phaseolus vulgaris*)

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Abstract

The most effective strategy to manage bean anthracnose (ANT), caused by *Colletotrichum lindemuthianum*, is the use of resistant cultivars. This study aimed to evaluate resistance reactions of common bean accessions to *C. lindemuthianum* races 2, 9 and 1545, and to perform genome-wide association study (GWAS). Hence, 89 accessions were phenotyped and genotyped through genotyping by sequencing (GBS). As a result, 48 accessions resistant to all evaluated races were identified. Moreover, single-nucleotide polymorphisms (SNP) significantly associated with resistance were identified in new regions of chromosomes Pv03, Pv05 and Pv06, and also at the beginning of Pv04 and end of Pv11, where other resistance genes have been previously found. In reference genome these regions contain model genes encoding resistance proteins as kinases, leucine-rich repeats, receptor-like protein, copper transport protein, pentatricopeptide repeats, calcium-dependent protein kinases, and ethylene-responsive transcription factors. The genomic regions associated to ANT resistance found in this study should be validated for further use in marker assisted selection and gene pyramiding. Together with new sources of ANT resistance our findings show promise for further crop improvement.

Keywords: GWAS; GBS; *Colletotrichum lindemuthianum*; Anthracnose; Resistance sources; Candidate genes

1. Introduction

Common bean is the most important legume for direct human consumption. As functional foods, beans are low in fat and high in fiber content [1]. They also provide essential proteins for human nutrition and are an important source of vitamins and minerals, such as iron, phosphorus, magnesium, manganese, zinc, copper, and calcium [2]. Common bean production can be severely affected by diseases. Anthracnose (ANT), caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara, is a serious seed borne disease. Under disease-promoting conditions, such as high humidity and low temperature, bean anthracnose can result in yield losses up to 100 percent [3].

Adoption of certified seeds, crop rotation, and seed and foliar fungicide treatment are helpful in disease management. Nevertheless, the use of resistance cultivars is the most effective and ecologically sustainable strategy to manage bean anthracnose [4]. Studies in common bean segregating populations have already allowed the identification of more than

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20 ANT resistance *loci* identified by the genetic symbol *Co*. Anthracnose resistance genes have been mapped on chromosomes Pv01, Pv02, Pv03, Pv04, Pv07, Pv08, Pv09, and Pv11 [5, 6, 7].

Genome-wide association mapping is an important approach for identifying the genetic basis of phenotypic variation [8]. This methodology, also called whole-genome scan, tests the association of marker *loci* distributed across a genome and a specific trait (phenotype), the assumption being that the marker *loci* either cause phenotypic variation or are in linkage disequilibrium with the causal *locus* [9]. Advances in sequencing technologies and variant detection algorithms have allowed the use of genomic variants in high-throughput studies such as GWAS, along with high-density single-nucleotide polymorphism (SNP), which favor the identification of small haplotype blocks that are significantly correlated with trait variation [10].

Traditional gene mapping uses populations derived from only two parents, with limited amount of genetic variation and relatively few recombination events. GWAS, however in contrast, uses large populations with wide natural variation and takes advantage of recombination events over multiple generations in high-resolution mapping [8, 11].

With the availability of a high-quality reference genome, GWAS became a powerful tool to identify resistance loci in common bean and provides the basis for further gene mapping. Genome-wide association studies aiming at the identification of anthracnose resistance *loci* have been conducted for common bean. Different ANT races were evaluated, and SNPs and quantitative resistance *loci* have been found on chromosomes Pv01, Pv02, Pv03, Pv04, Pv05, Pv06, Pv07, Pv08, Pv10 and Pv11 [12, 13, 14, 15, 16, 17]. Since an ANT resistance *locus* was also found in Pv09 in a complementary mode of action in the 'Cornell 49242' cultivar [5], all common bean chromosomes exhibit anthracnose resistance.

C. lindemuthianum is a highly variable pathogen. According to Nunes *et al*. (2021) [18], 298 different *C. lindemuthianum* races have been reported in 29 countries. Padder *et al.* (2017) [4] identified the occurrence of 182 races worldwide. Among the mechanisms that cause pathogen variability, we can highlight the co-evolution process between common bean and *C. lindemuthianum*. In general, Mesoamerican races are virulent on common bean cultivars from both Andean and Mesoamerican gene pools; however, Andean races are more virulent on common beans from the Andean pool [19]. Also, mutation, parasexual recombination, and introduction of new races into local populations are additional mechanisms that create variability [20]. As a result, resistant cultivars can become susceptible due to the appearance of new anthracnose races. Thus, the broad variability of the ANT pathogen requires the development of different cultivars with wide and durable resistance.

C. lindemuthianum race 2 is an Andean race that, across the 12 differential cultivars proposed by Pastor-Corrales (1991) [21], attacks only Michigan Dark Red Kidney (MDRK) which possesses the *Co-1* gene. Race 2 has been identified in Argentina, Brazil, Bulgaria, Colombia, Dominican Republic, Ecuador, Greece, India, Kenya, Mexico, Peru, Tanzania, Turkey, Uganda, and the United States [4, 18]. The fact that this race is widely found in several countries, including some of the largest common bean producers, requires attention and highlights the importance of the development of resistant cultivars in order to prevent its spread.

Race 9 is a Mesoamerican race that breaks the resistance of the differential cultivars Michelite (*Co-11*) and Cornell 49242 (*Co-2*). This race is also widely distributed, having already been found in Argentina, Brazil, Burundi, Colombia, Costa Rica, Dominican Republic, Ecuador, Guatemala, Honduras, Mexico, Peru, Tanzania, and the United States [4, 18].

Anthracnose pathogen race 1545 is able to infect the following differential cultivars: Michelite (*Co-11*), Cornell 49242 (*Co-2*), TU (*Co-5*), and AB 136 (*Co-6*). This race was identified in Colombia, Costa Rica, Guatemala, Honduras, Mexico, and Nicaragua [4, 18]. Given the above, seeking new anthracnose sources of resistance is a constant aim for common bean breeding programs. That effort also includes mapping new resistance genes. Molecular markers linked to resistance genes may be used in the development of new common bean cultivars by pyramiding different resistance genes. Thus, the objective of this study was to evaluate the resistance reaction of the common bean accessions and to identify chromosome regions associated with *C. lindemuthianum* races 2, 9, and 1545 through GBS-based genome-wide association approach of recently collected landraces from different parts of the bean-growing region in Brazil.

2. Material and methods

2.1. Plant material and genotyping

Plant material used in this study is part of the gene bank collection from the Núcleo de Pesquisa Aplicada à Agricultura (Nupagri), Universidade Estadual de Maringá (Brazil). A total of 89 common bean accessions, including cultivars and

landraces, were collected in the Brazilian common bean growing regions in the states of Mato Grosso, Paraíba, Paraná, Pernambuco, and Sergipe (Figure 1, S1 Table). These accessions were previously classified using the molecular marker BMd-2 for phaseolin [22], resulting in 29 Andean and 60 Mesoamerican accessions.

Figure 1 Brazilian regions where common bean accessions were collected

The accessions were genotyped using genotyping-by-sequencing (GBS) at the University of California Davis Genome Center. DNA was collected from young leaves tissues and extracted following the Pallotta *et al.* (2003) [23] extraction protocol with modifications to eliminate RNA. The DNA quality was checked using spectrometry (Nanodrop Lite, Thermo Fisher Scientific) and electrophoresis (1% agarose gel). DNA with a A260/A280 absorbance ratio higher than 1.7 and without degradation was used for library preparation. Library preparation for GBS followed Elshire *et al.* (2011) protocol [24] using the CviAII restriction enzyme (CATG recognition site) and DNA with a specific barcode adapter for each accession. CviAII was used as the most suitable enzyme for GBS given the smaller genome size of common bean and the broader genome coverage provided by this enzyme compared to *Ape*KI [25]. The ligation step was conducted using only 0.6× of the ligation buffer. PCR amplifications were performed for fragment enrichment followed by adapter dimers check with Experion DNA analysis kit (Biorad). Sequencing was performed in HiSeq 2000 flow cell using the 50 bp protocol.

Sequences were aligned to the common bean reference genome v1.0 (landrace G19833) [26] using BWA [27]. In the filtering process, only SNPs that showed minor allele frequency (MAF) > 0.05, a minimum quality >10, and a mean read depth, across all lines, ranging from 5 to 1000, were included. Base call recalibration was performed with ReQON in R software [28]. Variants were called with SAMtools and filtered with VCFtools.

2.2. Phenotypic evaluation of anthracnose

Phenotypic evaluation was conducted at Nupagri, Universidade Estadual de Maringá. A total of 10 seeds per accession were surface disinfected with 1.5% NaClO for one minute, water rinsed, then sown in trays containing substrate and maintained under greenhouse conditions. Anthracnose races 2, 9 and 1545 belonging to Nupagri's mycology collection were confirmed by inoculation into the set of 12 common bean differential cultivars [21]. During population evaluation, differential cultivar PI 207262 was used as resistant control for all races while MDRK was used as susceptible control for race 2 and Michelite was the susceptible control for races 9 and 1545. Each race was evaluated twice in a separated experimental.

Inocula of *C. lindemuthianum* were obtained following the methodology by Cárdenas *et al.* (1964) [29]. The mycelium was grown in petri dishes containing potato dextrose agar medium. Small pieces of the mycelium were transferred to

sterilized young pods of snap beans placed in test tubes and incubated at 22°C for 14 days in darkness to promote sporulation. The resulting spores were suspended in sterile and distilled water, and inoculum concentration was adjusted to 1.2×10^6 conidia mL^{-1} using a hemocytometer (Neubauer chamber) in the microscope. Fifteen-day-old seedlings (10 plants per accession) were spray-inoculated with each race separately on the underside of the leaves.

Inoculated plants were transferred to a mist chamber and maintained at >95% relative humidity, 20 ± 2°C temperature, and 12h 680 lux light and 12h darkness, for a total of 72 hours (three days). Over the following seven days, the inoculated plants were maintained in benches with the same controlled humidity, temperature, and luminosity. Anthracnose disease symptoms were visually evaluated according to Pastor-Corrales *et al.* (1995) [30] severity scale. Plants with disease reaction scores between 1 and 3 were considered resistant, whereas plants with scores from 4 to 9 were considered susceptible [30].

A resistance index was calculated for each accession, which consisted of the ratio of the number of races to which the access was resistant to the total number of races evaluated. Likewise, a pathogenicity index was calculated for each evaluated race, which consisted of the ratio of the number of accessions susceptible to that race to the total number of accessions evaluated.

2.3. Genome-wide association analysis

Association analysis was carried out in TASSEL software version 5.2.50 [31]. The population structure matrix, which describes the percent subpopulation parentage for each line in the analysis, was obtained by principal component analysis (PCA). The kinship matrix (K) was calculated to account for individual relatedness. GWAS was performed using mixed linear model (MLM) following the equation:

$$Y = X\alpha + P\beta + K\mu + \varepsilon$$

where Y is the vector of the phenotype; X is the incidence matrix of the independent vector α of SNPs fixed effect; P is the incidence matrix of the independent vector β of the population structure fixed effect; K is the incidence matrix of the independent vector μ of the relative kinship random effect; and ε is the error term assumed to be normally distributed with a zero mean. SNP p-value < 0.001 was defined as a threshold to declare a significant association with anthracnose resistance.

2.4. Functional annotation

Common bean gene models within 100 kbp upstream and downstream of the significant markers were taken into account for candidate gene searches. The position of each SNP was sought after in the reference bean genome (G19833 version 1.1.) [26] available at NCBI and www.phytozome.org. The gene functional annotation was identified in Phytozome (http://phytozome.jgi.doe.gov) to infer the possible role of the gene in conferring anthracnose resistance.

3. Results and discussion

3.1. Genetic diversity of the common bean population

Through GBS it was possible to obtain genotypic information of the 89 common bean accessions in 28,823 SNPs distributed over the 11 bean chromosomes (Table 1). The population structure obtained by Principal component analysis (PCA) data revealed that the accessions were clustered into two distinct groups, which corresponded to either the Andean (29 accessions) or Mesoamerican (60 accessions) gene pool (Figure 2). The first (PC1) principal component explained 92 % of the variation among accessions and separated the Mesoamerican and Andean accessions. The second principal component explained 1 % of the total variation. It was responsible for distinguishing the Mesoamerican accession, which were more diverse than the Andean accessions, as shown by the high dispersion of the points on the two-dimensional plane (Figure 2).

Table 1 Length in base pairs, total number of SNPs per chromosome, per Megabase in each chromosome and interval in kilobase per SNP for the 11 chromosomes of common bean accessions in Brazil genotyped through GBS

Chromosome	Length (bp)	No. SNPs	SNPs/Mb	kb/SNP
Pv01	52,035,450	3269	62.82	15.91
Pv02	48,839,311	3651	74.75	13.37
Pv03	52,058,115	3262	62.66	15.95
Pv04	44,941,012	1788	39.78	25.13
Pv05	40,643,363	2269	55.82	17.91
Pv06	31,956,823	2304	72.09	13.87
Pv07	51,437,727	2266	44.05	22.69
Pv08	59,476,018	2276	38.26	26.13
Pv09	37,392,701	3310	88.51	11.29
Pv10	42,953,733	1660	38.64	25.87
Pv11	50,209,006	2768	55.12	18.13
Total	511,943,259	28,823		
Average			57.5	18.75

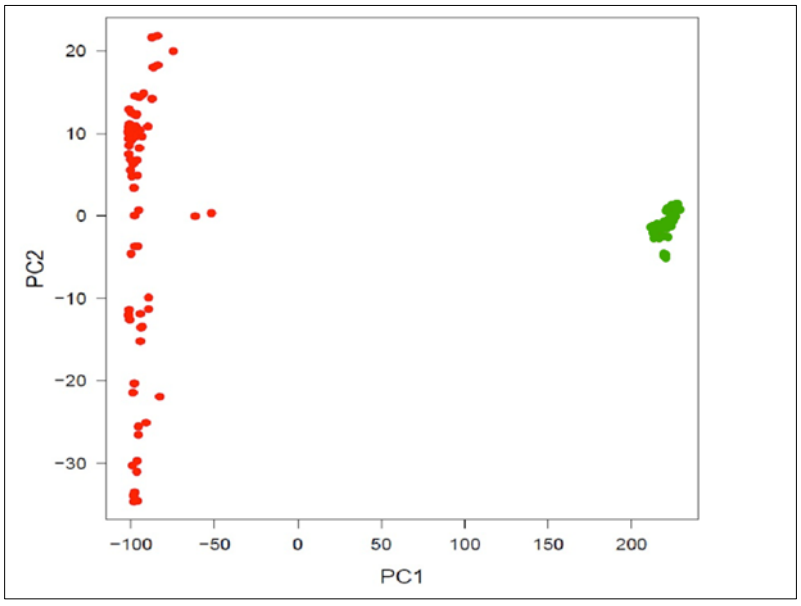

Figure 2 Plot of the genotypic variability of 89 common bean accessions through principal component analysis using 28,823 SNPs

3.2. Anthracnose resistance

Disease reaction of each accession, Resistance and Pathogenicity Index (RI and PI) are presented in Tables 2 and 3. Among Andean accessions, races 2 and 1545 both showed a 7 % PI while, in Mesoamerican accessions, those races had indexes of 50 % and 53 %, respectively. As for race 9, the PI was 3 % in Andean beans and 52 % in Mesoamericans accessions. Thus, Andean accessions presented an overall higher resistance index compared to the Mesoamerican accessions and, for that reason, they are a valuable source of anthracnose resistance.

Table 2 Andean common bean disease reaction (Resistant or Susceptible) against *C. lindemuthianum* races 2, 9 and 1545. Accession's resistance index (%) and races pathogenicity index (%)

Accession ID	Accession name	Origin[1]	Gene *pool*	*C. lindemuthianum* races			Resistance index (%)
				2	9	1545	
BL_2	Cocão	PE	A	R	R	R	100
BL_3	Bagajó	SE	A	R	R	R	100
BL_5	Canarinho	PE	A	R	R	R	100
BL_7	Chita Fina Verdadeiro	PE	A	S	R	S	33.33
BL_8	Jaula	PE	A	R	R	R	100
BL_9	Pintado	PE	A	R	R	R	100
BL_11	Praia	SE	A	R	R	R	100
BL_12	Camarão	PE	A	R	R	R	100
BL_13	BSF-1	PE	A	R	R	R	100
BL_15	BSF-3 Fogo na serra	PE	A	R	R	R	100
BL_27	Mulatão	PE	A	S	S	S	0
BL_74	CLPE53	PE	A	R	R	R	100
BL_75	CLPE54	PE	A	R	R	R	100
BL_77	CLPE56	PE	A	R	R	R	100
BL_78	CLPE58	PE	A	R	R	R	100
BL_79	CLPE60	PE	A	R	R	R	100
BL_93	CLPE88	PE	A	R	R	R	100
BL_94	CLPE85	PE	A	R	R	R	100
BL_165	Pitanga	PR	A	R	R	R	100
BL_166	Corinthiano	PR	A	R	R	R	100
BL_167	Perla	Argentina	A	R	R	R	100
BL_168	Jalo Vermelho	PR	A	R	R	R	100
BL_170	Jalo Listras Pretas	PR	A	R	R	R	100
BL_171	Jalo EEP 558	MG	A	R	R	R	100
BL_172	BGF 20	PR	A	R	R	R	100
BL_178	Perry Marrow	NA	A	R	R	R	100
BL_199	Enxofre	PE	A	R	R	R	100
BL_220	Jalo Pintado 2	PR	A	R	R	R	100
BL_221	AND 277	NA	A	R	R	R	100
Pathogenicity Index (%)				6.90	3.45	6.90	

[1] PE= Pernambuco, SE= Sergipe, PR= Paraná, MG= Minas Gerais, NA=not available.

Among the 89 accessions evaluated, 54% were resistant to *C. lindemuthianum* races 2, 9 and 1545, wherein 27 were from the Andean domesticated gene pool and 21 were Mesoamerican. All the Andean beans used in this study can be used to obtain resistant cultivars to anthracnose caused by the aforementioned *C. lindemuthianum* races, except the Mulatão, and Chita Fina Verdadeiro accessions.

Regarding Mesoamerican sources of resistance, Rosinha Claro, Balinha, Brilhoso, IPA 1, Mulatinho de Cacho, Flor Azul, Laje, CLPE17, CLPE32, CLPE40, CLPE41, CLPE44, CLPE45, CLPE55, CLPE68, CLPE74, Awauna UEM, UEMT 50G2, Sempre Assim, MT 55, and MT 79 can be used as donors of resistant genes against races 2, 9 and 1545. For resistance against race 2 only, the following Mesoamerican cultivars can be used: Caiaminha, CLPE80, CLPE81, CLPE87, CLPE89, CLPE94, CLPE96, Juriti, MT57G1. Mesoamerican cultivars that showed resistance to race 9 were: CLPE47, CLPE80, CLPE81, CLPE86, Juriti, Bico de Ouro, BG-18, and MT 73G1. Finally, the following Mesoamerican sources were resistant to race 1545: MT 57G1, Bico de Ouro, BG-9, BG-13, MT 73G1, CLPE3, and CLPE21.

Table 3 Mesoamerican common bean disease reaction (Resistant or Susceptible) against *C. lindemuthianum* races 2, 9 and 1545. Accession's resistance index (%) and races pathogenicity index (%)

Accession ID	Accession name	Origin[1]	Gene *pool*	*C. lindemuthianum* races			Resistance index (%)
				2	9	1545	
BL_1	Brígida	PE	M	S	S	S	0
BL_6	Rosinha Claro	PE	M	R	R	R	100
BL_10	Balinha	PE	M	R	R	R	100
BL_14	BSF-2 Pingo de Ouro	PE	M	S	S	S	0
BL_16	Brilhoso	PE	M	R	R	R	100
BL_19	IPA 1	PE	M	R	R	R	100
BL_24	Mulatinho de Cacho	PB	M	R	R	R	100
BL_30	Flor Azul	PE	M	R	R	R	100
BL_31	Bico de ouro	PE	M	S	R	R	66.67
BL_34	Laje	PB	M	R	R	R	100
BL_35	Caiaminha	PE	M	R	S	S	33.33
BL_50	CLPE17	PE	M	R	R	R	100
BL_66	CLPE40	PE	M	R	R	R	100
BL_67	CLPE41	PE	M	R	R	R	100
BL_69	CLPE44	PE	M	R	R	R	100
BL_70	CLPE45	PE	M	R	R	R	100
BL_71	CLPE47	PE	M	S	R	S	33.33
BL_76	CLPE55	PE	M	R	R	R	100
BL_80	CLPE61	PE	M	S	S	S	0
BL_81	CLPE63	PE	M	S	S	S	0
BL_82	CLPE65	PE	M	S	S	S	0
BL_83	CLPE66	PE	M	S	S	S	0
BL_84	CLPE67	PE	M	S	S	S	0
BL_85	CLPE68	PE	M	R	R	R	100
BL_86	CLPE69	PE	M	S	S	S	0
BL_87	CLPE74	PE	M	R	R	R	100
BL_88	CLPE75	PE	M	S	S	S	0
BL_90	CLPE80	PE	M	R	R	S	66.67
BL_91	CLPE81	PE	M	R	R	S	66.67

BL_92	CLPE87	PE	M	R	S	S	33.33
BL_95	CLPE90	PE	M	S	S	S	0
BL_96	CLPE91	PE	M	S	S	S	0
BL_99	CLPE92	PE	M	S	S	S	0
BL_100	CLPE89	PE	M	R	S	S	33.3
BL_102	CLPE94	PE	M	R	S	S	33.3
BL_103	CLPE96	PE	M	R	S	S	33.3
BL_104	CLPE86	PE	M	S	R	S	33.3
BL_105	CLPE83	PE	M	S	S	S	0
BL_106	BG-4	MT	M	S	S	S	0
BL_107	BG-9	MT	M	S	S	R	33.33
BL_108	BG-13	MT	M	S	S	R	33.33
BL_109	BG-17	MT	M	S	S	S	0
BL_110	BG-18	MT	M	S	R	S	33.33
BL_111	BG-23	MT	M	S	S	S	0
BL_174	Juriti	PR	M	R	R	S	66.67
BL_177	Awauna	PR	M	R	R	R	100
BL_181	MT 50G	MT	M	R	R	R	100
BL_183	MT 55	MT	M	R	R	R	100
BL_184	MT 57G1	MT	M	R	S	R	66.67
BL_186	MT 62	MT	M	S	S	S	0
BL_187	MT 73G1	MT	M	S	R	R	66.67
BL_189	MT 79	MT	M	R	R	R	100
BL_216	CLPE32	PE	M	R	R	R	100
BL_225	Sempre Assim	PE	M	R	R	R	100
BL_226	CLPE3	PE	M	S	S	R	33.33
BL_227	CLPE4	PE	M	S	S	S	0
BL_228	CLPE8	PE	M	S	S	S	0
BL_229	CLPE10	PE	M	S	S	S	0
BL_230	CLPE11	PE	M	S	S	S	0
BL_234	CLPE21	PE	M	S	S	R	33.33
Pathogenicity Index (%)				50.00	51.67	53.33	

[1] PE= Pernambuco, PB= Paraíba, MT= Mato Grosso, PR= Paraná.

3.3. GBS-based genome-wide association analyses

Association mapping of the 89 common bean accessions with *C. lindemuthianum* race 2 resulted in the identification of three SNPs on chromosome Pv04, one SNP on Pv06, and five on Pv11. The relationship of the positions of the anthracnose resistance *loci* found in the present study and the *loci* already described in the literature are illustrated in Figure 3, using the common bean reference genome version 2.1. GWAS for anthracnose race 9 resistance resulted in the identification of three SNPs on chromosome Pv04. Association mapping of race 1545 resistance allowed the identification of one SNP on chromosome Pv03 and five SNPs on Pv05 (Table 4; Figures 4, 5, and 6). One hundred and

eleven model genes were found in the 100kb region upstream and downstream of the physical position of the SNPs associated with races 2, 9 and 1545 resistance in the common bean reference genome v1.0. Out of them, 20 were annotated with any function related to disease response and, thus, are candidate genes for anthracnose resistance (Table 5).

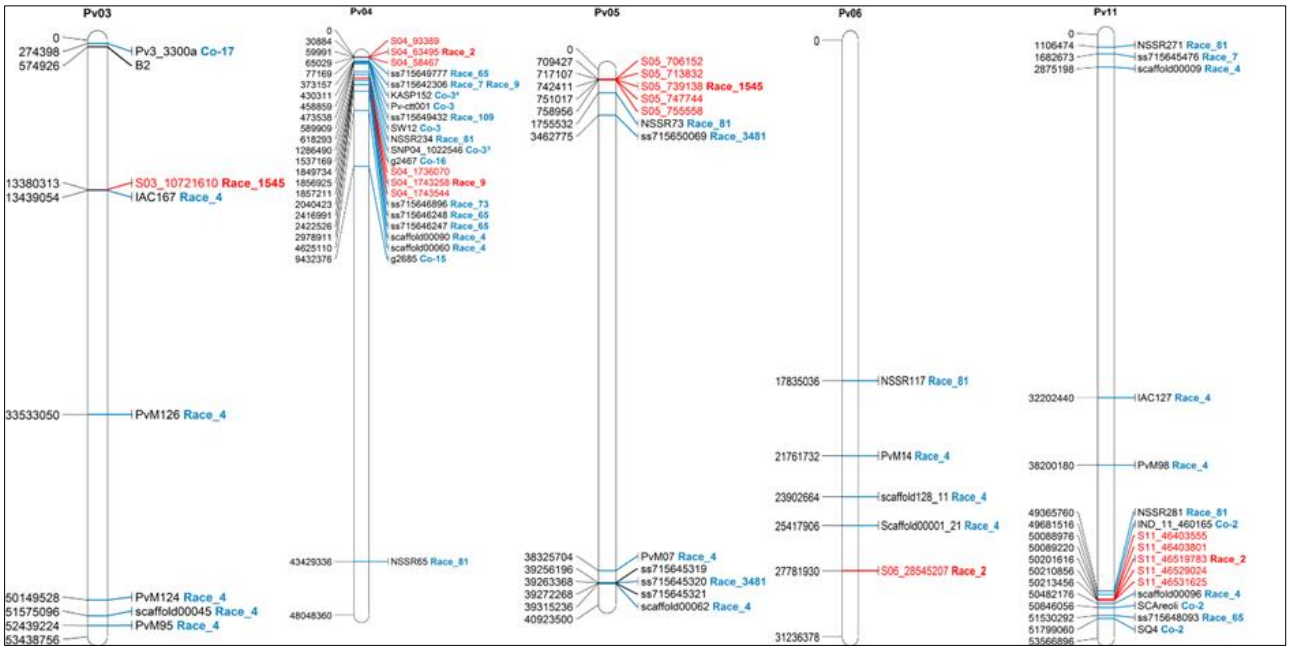

Figure 3 Common bean chromosomes Pv03, Pv04, Pv05, Pv06, and Pv11 showing anthracnose resistance loci found in this study colored in red, loci already described in the literature colored in blue, molecular markers tagging the resistance loci colored in black with physical position based on the *Phaseolus vulgaris* reference genome v2.1.

Table 4 Associations between SNP and anthracnose resistance for races 2, 9, and 1545 of *C. lindemuthianum* determined by mixed linear models (MLM)

Race	SNP	Pv[1]	Position[2]	p-value [3]	SNP R^2 (%)[4]
2	S04_58467	04	58,467	3.84E-05	21.81
2	S04_63495	04	63,495	9.12E-06	27.16
2	S04_93389	04	93,389	1.27E-04	18.64
2	S06_28545207	06	28,545,207	4.89E-04	19.23
2	S11_46403555	11	46,403,555	2.11E-04	17.28
2	S11_46403801	11	46,403,801	6.75E-04	14.34
2	S11_46519783	11	46,519,783	2.21E-04	17.23
2	S11_46529024	11	46,529,024	8.74E-04	14.32
2	S11_46531625	11	46,531,625	9.73E-04	17.45
9	S04_1736070	04	1,736,070	6.21E-04	15.18
9	S04_1743258	04	1,743,258	6.21E-04	15.18
9	S04_1743544	04	1,743,544	6.21E-04	15.18
1545	S03_13038972	03	13,038,972	6.18E-04	15.62
1545	S05_706152	05	706,152	6.88E-04	14.87
1545	S05_713832	05	713,832	6.88E-04	14.87
1545	S05_739138	05	739,138	6.88E-04	14.87
1545	S05_747744	05	747,744	6.88E-04	14.87
1545	S05_755558	05	755,558	6.88E-04	14.87

[1]Chromosomes (Pv); [2] SNP position in reference genome v1.0; [3] significance level; [4]phenotipic variation explained by the SNP (R^2).

Table 5 Candidate genes for ANT races 2, 9, and 1545 resistance within 100 Kb region at either side of the significant SNP or interval in reference genome v1.0 with function annotation related to disease response

Race	Candidate gene	Functional annotation
2	*Phvul.004G000500*	Sphingosine kinase
2	*Phvul.004G000800*	Pyruvate kinase-related
2	*Phvul.004G001200*	Copper transport protein ATOX1-related
2	*Phvul.006G174100*	Receptor like protein 55
2	*Phvul.006G174200*	PPR repeat (PPR) // PPR repeat family (PPR_2) // DYW family of nucleic acid deaminases
2	*Phvul.006G174400*	Protein kinase domain (Pkinase) // Leucine rich repeat N-terminal domain (LRRNT_2)
2	*Phvul.006G174700*	Leucine-rich repeat receptor-like protein kinase
2	*Phvul.006G174800*	Cbl-interacting serine/threonine-protein kinase 12-related
2	*Phvul.006G174900*	Cbl-interacting serine/threonine-protein kinase 2
2	*Phvul.006G175100*	LRR receptor-like serine/threonine-protein kinase mrh1-related
2	*Phvul.011G186900*	Serine/threonine-protein kinase cg17528
2	*Phvul.011G187400*	Ethylene-responsive transcription factor wri1
9	*Phvul.004G016300*	F-box domain (F-box) // Leucine Rich Repeat (LRR_2) // FBD (FBD)
9	*Phvul.004G016400*	F-box domain (F-box) // Leucine Rich Repeat (LRR_2) // FBD (FBD)
9	*Phvul.004G016600*	F-box domain (F-box) // Leucine Rich Repeat (LRR_2) // FBD (FBD)
9	*Phvul.004G016900*	Serine-threonine protein kinase
1545	*Phvul.003G080900*	Wall-associated receptor kinase galacturonan-binding
1545	*Phvul.005G008100*	Ppr repeat (ppr) // ppr repeat family (ppr_2)
1545	*Phvul.005G008500*	F-box and leucine-rich repeat protein 2/20 (fbxl2_20)
1545	*Phvul.005G009000*	Ceramide kinase / Acylsphingosine kinase

3.3.1. Genome-wide association for race 2

Association mapping of the 89 common bean accessions with *C. lindemuthianum* race 2 resulted in the identification of potential QTLs on chromosomes Pv04, Pv06, and Pv11. The significance region of the QTL on Pv04 was located in a 34,922bp-interval starting at 58,467 bp and ending at 93,389 bp in the top region of chromosome Pv04. Three SNPs in this interval - S04_58467, S04_63495, and S04_93389 - were significantly associated with the resistance and explained, respectively, 22 %, 27 % and 19 % of the phenotypic variation (Table 4, Figure 4).

Anthracnose resistance genes have been mapped in this same region at the beginning of chromosome Pv04 in several distinct cultivars. This includes mainly the ANT resistance *locus Co-3* and its allelic series *Co-3*^{2}, *Co-3*^{3}, *Co-3*^{4}, and *Co-3*^{5} [32, 33, 34, 35, 36]. In addition, *Co-y* of the Andean cultivar Jalo EEP558 has been mapped at the same location as *Co-9* (later renamed *Co-3*^{3}) from the Mesoamerican breeding line BAT93 [35].

The BAT93 resistance gene against race 73 (*Co-3*^{B}) is flanked by markers SNP04_027 (552,092 bp) and 254-G15 (1,618,118 bp). However, the resistance against race 38 (*Co-3*^{B}) was fine-mapped in two regions; the first is flanked by SNPs SNP04_1022546 (1,286,490 bp) and SNP04_1308175 (1,419,089 bp), and the second region is delimited by markers IND04_10936 (1,908,814 bp) and SNP04_1231633 (2,047,754 bp) [37].

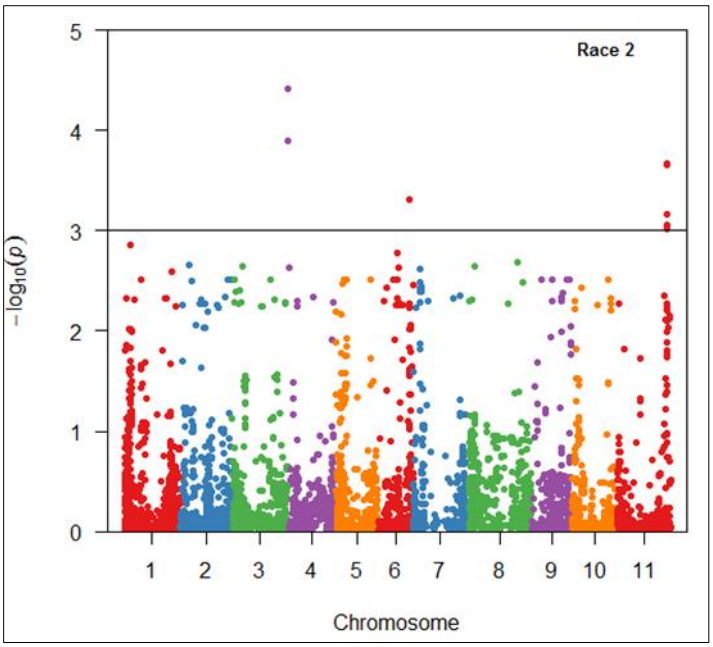

Figure 4 Manhattan plot showing SNPs and *p*-values from GWAS for anthracnose resistance against race 2. Significance threshold p<0.001

The resistance gene *Co-3*[4] in the Ouro Negro cultivar was first mapped at the position 3,356,300 bp of Pv04, linked at 0.0 cM to the STS marker g2303 [36]. Later, Valentini *et al.* (2017) [38], studying co-segregation analysis for rust and anthracnose diseases in the population Ouro Negro × Rudá, mapped *Co-3*[4] at a distance of 0.1 and 0.3 cM from KASP152 (487,659 bp) and KASP153 (575,006 bp), respectively. Additional resistance alleles were found in the *Co-3* cluster in the Andean cultivars Widusa, Kaboon, Xana, and MDRK, as well as in the Mesoamerican A252 line [5, 39, 40, 41, 42].

The *Co-16* resistance gene present in the cultivar Crioulo 159 mapped in a different position from *Co-3* on Pv04 at 1,428,279 bp linked to the marker g2467 at 5 cM [43]. Moreover, resistance gene *Co-15* present in the cultivar Corinthiano was mapped on chromosome Pv04 linked at 5.6 cM to the marker g2686 located at 9,078,200 bp [44].

GWAS for anthracnose resistance using different races also identified regions in Pv04 conferring resistance. Zuiderveen *et al.* (2016) [12] found associations on Pv04 for resistance to races 7 and 109. The SNP ss715642306 at the position 447,165 bp was associated with race 7. Resistance to race 109 was associated with the SNP ss715649432 located at 532,194 bp. The authors suggested that this resistance could be associated with the *Co-3 locus.* Perseguini *et al.* (2016) [13] observed associations with resistance to race 4 on Pv04. The associated markers were scaffold00090_802505 and scaffold00060_874577 located at 2,701,631 bp and 4,395,872 bp, respectively. Wu *et al.* (2017) [14] found two SSR markers associated with race 81 resistance on Pv04: NSS234 marker located at 673,367 bp and NSSR65 marker located at 41,368,421 bp. The first marker may be in the same genomic region of *Co-3*. Vidigal Filho *et al.* (2020) [16] found associations on Pv04 for resistance to races 9, 65 and 73. The SNP ss715649432 at the position 532,254 bp was associated with race 9. Resistance to race 65 was associated with the SNP ss715646248 located at 2,142,289 bp. The SNP ss715646896 at the position 1,224,240 bp was associated with race 73. Through linkage and genome-wide association analyses different loci controlling resistance to different isolates of race 65 of *Colletotrichum lindemuthianum* were identified by Costa *et al.* (2021) [17]. In chromosome Pv04 the identified SNPs are located from 46,027 bp (ss715649777) until 2,147,821 bp (ss715646247). Therefore, the region identified in the present study for resistance to race 2 corroborates the region identified using different isolates of race 65 by Costa *et al.* (2021) [17].

In the reference genome, 16 candidate genes are located close to the region from the SNPs S04_58467 and S04_93389, associated with race 2 resistance in this study. Three of these genes encode proteins with functional annotation potentially related to disease reaction (Table 5). Among them, *Phvul.004G000500* and *Phvul.004G000800* encode kinases, which act as pattern-recognition receptors by recognizing pathogen-associated molecular patterns (PAMPs) [45, 46] (Jones and Dangl, 2006; Zipfel, 2014). Also, *Phvul.004G001200* encodes a Copper transport protein ATOX1-related protein. The ATOX1 protein is a candidate gene for resistance to soybean mosaic virus strain SC5 and was differentially expressed in resistant and susceptible cultivars under infection [47]. Previous studies in Arabidopsis revealed upregulation at the transcription level of ATOX1 encoding-gene in response to cytokinin that acts as key

signaling molecule inducing resistance actions against pathogen infection [48]. Thus, the beginning of Pv04 contains a large cluster or multiple clusters of phenotypic resistance genes. It may not be surprising, therefore, that more than 40 nucleotide-binding leucine-rich repeat (NBS-LRR) encoding-genes and other genes involved in host-pathogen interactions have been identified in this region [26].

The SNP S06_28545207 was significantly associated with race 2 resistance on chromosome Pv06. In the reference genome v1.0, this SNP is located at 28,545,207 bp and explains 19 % of the phenotypic variation (Table 4, Figure 4). Other GWAS for anthracnose resistance found associations with resistance to ANT on Pv06. Perseguini *et al.* (2016) [13] identified one SSR and four SNP markers associated with resistance to race 4 on Pv06, namely: PvM14 (22,466,054 bp), scaffold00128_112577 (24,577,146 bp), scaffold00128_197955 (24,659,226 bp), scaffold00001_2118513 (26,202,771 bp), and scaffold00001_1947432 (26,390,866 bp). Also, Wu *et al.* (2017) found one marker associated with resistance against race 81 on Pv06 (NSSR117, at 18,546,221 bp).

In the common bean reference genome, 23 candidate genes are found in the 100 kb region upstream and downstream of the physical position of the SNP S06_28545207. Seven genes encode proteins related to disease response (Table 5). The gene model *Phvul.006G174100* encodes a Receptor-like protein (RLP) that is a pattern recognition receptor (PRR) that mediate pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) to allow recognition of a broad range of pathogens [49]. Pentatricopeptide repeats (PPRs) were also found in this study (*Phvul.006G174200)*. PPRs are involved in plant defense; they translocate to chloroplast and mitochondria to perform post-transcriptional processing such as RNA editing, splicing and translation modification [50]. The gene models *Phvul.006G174400, Phvul.006G174700,* and *Phvul.006G175100* encode leucine-rich repeat receptor-like protein kinase (LRR-RLK) that is known to actively participate in the regulation of the growth, development, signal transduction, immunity, and stress responses of plants [51]. This study also found the model genes *Phvul.006G174800* and *Phvul.006G174900* that encode Calcium-dependent protein kinases (CDPKs). They act in hormone and stress signaling and pathogen response [52, 53].

Five SNPs were found significantly associated with resistance to race 2 on chromosome Pv11. The SNPs S11_46403555, S11_46403801, S11_46519783, S11_46529024, and S11_46531625 explained, respectively, 17 % each of the phenotypic variation (Table 4, Figure 4). These SNPs were located at the end of the chromosome in a 128,070 bp interval, from 46,403,555 bp to 46,531,625 bp. The *Co-2 locus,* a major anthracnose resistance gene reported in the Mesoamerican differential cultivar Cornell 49242 was mapped in the same region of chromosome, linked to the markers SCAreoli located at 47,134,388 bp and SQ4 located at 48,063,823 bp [5].

A 252 cultivar carries a resistance gene against race 31 linked to the marker SCAreoli at 14.0 cM on chromosome Pv11 [42]. A resistance *locus* was mapped in the AB 136 cultivar at the end of Pv11, in the same region of *Co-2*, between markers IND11_460165 (46,0Mb) and IND11_477711 (47,7Mb) [40].

Other GWAS for races 4, 7, and 81 found associations with resistance to ANT located on chromosome Pv11. Three SNPs and two SSR markers were found associated with resistance against race 4: Scaffold00009_1366067 (2,695,661 bp), scaffold00009_825782 (3,270,820 pb), IAC127 (28,334,236 bp), PvM98 (38,007,419 bp) and Scaffold00096_204246 (46,792,860 bp). The latter markers cover part of the *Co-2* region, the other markers are located in other regions of Pv11 [13]. The SNP ss715645476 (1.69 Mb) was found associated with resistance to race 7 on Pv11 and seems to be a distinct resistance gene, which may play a complementary role to the resistance gene found for race 7 on Pv04 [12]. For race 81, two clusters of resistance genes were found associated with ANT on Pv11. The first cluster is located at 1,111,792 bp (relatively close to the SNP found for race 7), while the second cluster is located at 45,753,810 bp, and is composed of 34 NBS-LRR genes from *Phvul.011G181400* to *Phvul.011G198400* [14] and might be located in the *Co-2* cluster. Costa *et al.* (2021) [17] identified the marker ss715648093 located at 47,800,050 associated to the resistance of the isolate Cl1532 of race 65 in Pv11, also in the *Co-2* cluster.

In the reference genome, 21 gene models are found between SNPs S11_46403555 and S11_46531625, and the 100kb boundaries of these SNPs. Two genes encode proteins that could be related to disease response (Table 5). The *Phvul.011G186900* gene model encodes a serine/threonine-protein kinase cg17528. Moreover, *Phvul.011G187400* encodes an ethylene-responsive transcription factor wri1, which is known to be involved in the regulation of gene expression by stress and in signal transduction pathways. Sessa *et al.* (1995) [54] stated that pathogenesis-related protein activation at transcriptional level can happen by the plant hormone ethylene. The accumulation of pathogenesis-related protein also can occur in response to ethylene in the presence of calcium.

3.3.2. Genome-wide association for race 9

Genome-wide association for race 9 resistance resulted in the identification of three SNPs on chromosome Pv04 accounting for 15 % of the total phenotypic variation, for each marker (Table 4, Figure 5). The SNPs S04_1736070, S04_1743258, and S04_1743544 are positioned in the beginning of the chromosome in a genomic region encompassing 7,474 bp interval from 1,736,070 bp to 1,743,544 bp. This region has been mapped for disease resistance in different bean cultivars corresponding to the *Co-3* cluster. The importance of Pv04 in conferring resistance to anthracnose has been discussed and addressed in the previous section of this study.

In the reference genome, 16 gene models are found close to the three SNPs. Three of them, *Phvul.004G016300*, *Phvul.004G016400*, and *Phvul.004G016600*, encode F-box domain (F-box) // Leucine Rich Repeat (LRR_2) // FBD (FBD) (Table 5). The LRR domain provides a versatile structural framework for the formation of protein–protein interactions. This protein belongs to the NBS-LRR gene family, which has been recruited to detect intracellular interference by diverse pathogen effectors and initiate effector-triggered immunity (ETI) [55, 56]. *Phvul.004G016900* encodes a serine-threonine protein kinase, a type of protein known to act in the plant immune system. Kinases operate as pattern-recognition receptors (PRRs) that recognize hormones, PAMPs, and pathogens effectors, and activate immune responses [57].

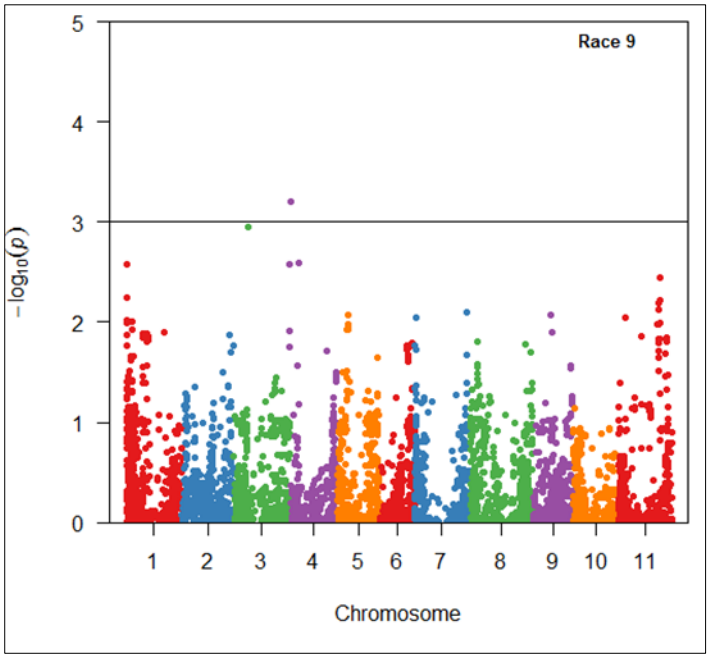

Figure 5 Manhattan plot showing SNPs, and *p*-values from GWAS for anthracnose resistance against race 9. Significance threshold p<0.001

3.3.3. Genome-wide association for race 1545

Genome-wide association analyses for race 1545 resistance led to the identification of one SNP on chromosome Pv03 and five SNPs on Pv05 (Table 4, Figure 6). The SNP S03_13038972 located at 13,038,972 bp on Pv03 explained 15 % of the phenotypic variation. Currently, there are two resistance genes mapped on chromosome Pv03. The first mapped gene was *Co-13*, reported in the Andean Jalo Listras Pretas landrace and linked to marker $OV20^{680}$ [58]. Remarkably, the *Co-13* gene also confers resistance to race 1545 [59]. The second gene is *Co-17*, which was described in the Mesoamerican SEL 1308 cultivar [6].

Previous GWAS for race 4 also identified six markers associated with resistance on Pv03. The markers and their respective positions were: IAC167 (13,097,396 bp), PVEST236 (32,935,150 bp), PvM126 (32,935,183 bp), PvM124 (48,995,346 bp), scaffold00045 (50,422,102 bp), and PvM95 (51,280,966 bp) [13]. The region found associated with race 1545 in this work is located at the position 13,038,972 bp, which is 58kb distant from the marker IAC167 (13,097,396 bp) associated with race 4 resistance. In the reference genome v1.0, 10 model genes are found close to SNP S03_13038972 on Pv03. Only the gene *Phvul.003G080900* encodes a protein kinase, which acts in defense response.

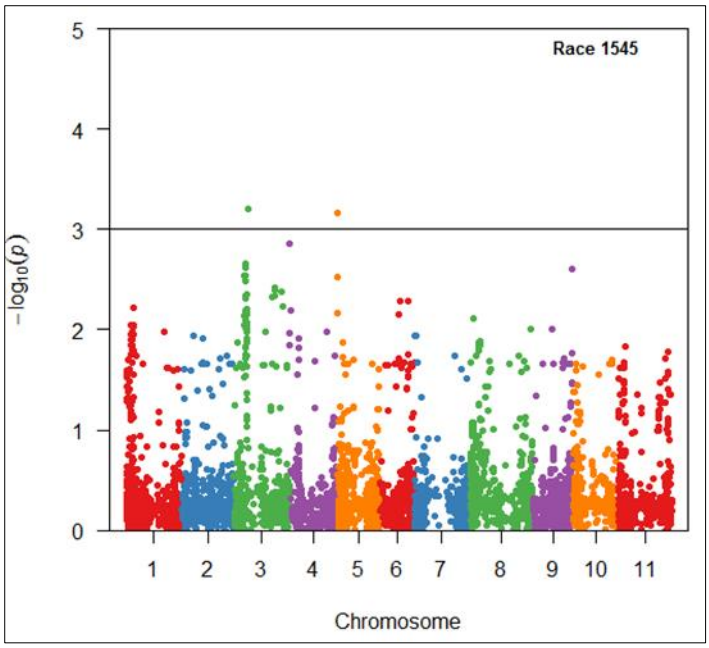

Figure 6 Manhattan plot showing SNPs, and *p*-values from GWAS for anthracnose resistance against race 1545. Significance threshold $p<0.001$.

The five SNPs identified on Pv05 - S05_706152, S05_713832, S05_739138, S05_747744, and S05_755558 (Table 4, Figure 6) - were located in a genomic region of 49,406 bp at the beginning of the chromosome, between 706,152 bp and 755,558 bp. Each SNP explained 15 % of the phenotypic variation. Previous GWAS for race 4 identified two markers associated with the resistance on chromosome Pv05: PvM07 (38,024,011 bp) and Scaffold00062 (39,080,673 bp) [13]. Vidigal Filho *et al.* (2020) [16] identified three SNPs associated with race 3481 in Pv05: ss715645319 (39,020,188 bp), ss715645320 (39,027,362 bp) and ss715645321 (39,035,656 bp). Through the same type of study, resistance against race 81 was associated with marker NSSR73 at the position of 1,746,532 bp on Pv05 [14]. Costa *et al.* (2021) [17] identified the marker ss715650069 located at 3,452,977 bp associated to the resistance of the isolate Cl1532 of race 65 in Pv05. Therefore, the resistance *loci* against race 1545 found in this study were located in a different position from the region found for races 4 and 3481. Moreover, it is located in a distance of 1.0 Mb and 2,7 Mb from the genomic region identified for resistance to race 81 and 65, respectively.

A total of 25 genes were found close to the SNPs associated with resistance to race 1545 on chromosome Pv05. Three genes encode proteins that might function in resistance. Gene *Phvul.005G008100* encodes a PPR (pentatricopeptide) repeat, which is a type of protein that is modular RNA-binding and mediates gene expression in organelles and nucleus [60]. Gene *Phvul.005G008500* encodes an F-box and leucine-rich repeat protein 2/20. F-box proteins regulate various cellular processes such as cell cycle transition, transcriptional regulation, and signal transduction, and LRR domain are involved in protein-protein interaction [61]. Finally, gene *Phvul.005G009000* encodes a protein kinase (Table 5).

In summary, the results show that both Andean and Mesoamerican bean accessions evaluated in this study are genetically distinct in response to races 2, 9, and 1545 of *C. lindemuthianum*. Some of this genetic material could be valuable in future bean breeding programs as new sources of resistance to anthracnose. Genome-wide association for *C. lindemuthianum* race 2 resulted in the identification of SNPs on chromosomes 4, 6, and 11 associated with resistance to ANT. The SNPs found on Pv04 and Pv11 may be located in the *Co-3* and *Co*-2 clusters, respectively. GWAS for race 9 showed that SNPs at the beginning of Pv04 are associated with resistance to ANT. These SNPs are located close to the *Co-3* cluster, a genomic region where other ANT resistance genes have been mapped previously. Genome-wide association against race 1545 was found in previously unreported genomic regions on Pv03 and Pv05.

4. Conclusion

The present study delivers valuable results as we identified new Andean and Mesoamerican common bean anthracnose resistance sources and 18 SNPs significantly associated with resistance to races 2, 9, and 1545. Furthermore, we found 20 candidate genes for ANT resistance that encoded proteins with functions previously related to disease resistance. These proteins are kinases, leucine-rich repeats, receptor- like protein, copper transport protein, pentatricopeptide

repeats, calcium-dependent protein kinases, and ethylene-responsive transcription factor. The genomic regions associated to ANT resistance found in this study should be validated for further use in marker assisted selection and gene pyramiding. Together with new sources of ANT resistance our findings show promise for further crop improvement.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that No conflict of interest.

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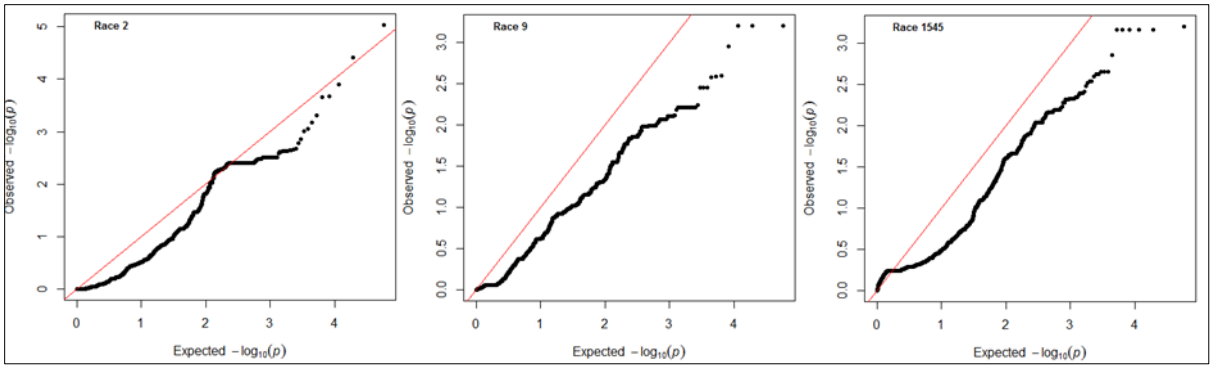
5. Supporting information

Supplemental material is available online for this article.



S1 Figure QQ plot showing SNPs and p-values from GWAS for anthracnose resistance against race 2, 9 and 1545, respectively.

S1 Table Identification of the 89 common bean accessions from Brazil evaluated in this study.

Code	Common name	State	City	Latitude	Longitude	Height (m)	Commercial group	Gene pool
BL_1	Brigida	PE	Recife	-8.058880	-34.880833	4	Carioca	M
BL_2	Cocão	PE	Recife	-8.058880	-34.880833	4	Others	A
BL_3	Bagajó	SE	Poço Verde	-10.707777	-38.182777	268	Others	A
BL_5	Canarinho	PE	Lajedo	-8.663888	-36.336666	661	Others	A
BL_6	Rosinha Claro	PE	Calçado	-8.741944	-36.333888	643	Rosinha	M
BL_7	Chita fina	PE	São João	-8.875833	-36.366944	716	Others	A
BL_8	Jaula	PE	São João	-8.890277	-36.492770	842	Others	A
BL_9	Pintado	PE	Ibimirim	-8.540555	-37.690277	401	Others	A
BL_10	Bolinha	PE	Lajeado	-8.540555	-36.320000	532	Mulatinho	M
BL_11	Praia	SE	Poço Verde	-8.053888	-34.880833	268	Others	A
BL_12	Camarão	PE	Calçado	-8.741944	-36.333888	643	Others	A

BL_13	BSF-1 Creme	PE	Belém do São Francisco	-8.753888	-38.963888	305	Others	A
BL_14	BSF-2 Pingo de Ouro	PE	Belém do São Francisco	-8.053888	-34.880830	305	Carioca	M
BL_15	BSF-3 Fogo na serra	PE	Belém do São Francisco	-8.053888	-34.880830	305	Others	A
BL_16	Brilhoso Mulatinho	PE	São João	-8.875833	-36.366944	716	Mulatinho	M
BL_19	IPA 1 Mulatinho	PE	Recife	-8.058880	-34.880833	4	Mulatinho	M
BL_24	Mulatinho de Cacho	PB	Arara	-6.827777	-35.757777	467	Mulatinho	M
BL_25	Mulatinho	PE	Jucati	-8.705833	-36.488888	820	Mulatinho	M
BL_27	Mulatão	PE	Bezerros	-8.889999	-36.492777	470	Mulatinho	A
BL_30	Flor Azul	PE	Águas Belas	-9.110833	-37.122777	376	Mulatinho	M
BL_31	Bico de ouro	PE	Águas Belas	-9.110833	-37.122777	376	Mulatinho	M
BL_34	Feijão Laje	PB	São Miguel de Itaipu	-7.250000	-35.210000	45	Mulatinho	M
BL_35	Caiaminha	PE	Calçado	-8.741944	-36.333888	643	Rosinha	M
BL_50	CLPE17	PE	Lajedo	-8.663888	-36.336666	661	Preto	M
BL_66	Feijão Carioca	PE	Caruaru	-8.282777	-35.975833	554	Carioca	M
BL_67	Feijão Carioca	PE	Sta Maria do Cambucá	-7.840000	-35.901944	494	Carioca	M
BL_69	Feijão Mulatinho	PE	Arcoverde	-8.420833	-37.061388	663	Mulatinho	M
BL_70	Feijão Carioca	PE	Vertentes	-7.902777	-35.987777	401	Carioca	M
BL_71	Feijão Mulatinho	PE	Arcoverde	-8.420833	-37.061388	663	Mulatinho	M
BL_74	Favita	PE	São João	-8.875833	-36.366944	716	Others	A
BL_75	Favita	PE	São João	-8.875833	-36.366944	716	Others	A
BL_76	Feijão Carioca	PE	São João	-8.875833	-36.366944	716	Carioca	M
BL_77	Enxofre	PE	São João	-8.875833	-36.366944	716	Enxofre	A
BL_78	Favita	PE	São João	-8.875833	-36.366944	716	Others	A
BL_79	Favita	PE	São João	-8.875833	-36.366944	716	Others	A
BL_80	Feijão Preto	PE	São João	-8.875833	-36.366944	716	Preto	M
BL_81	Feijão Preto	PE	São João	-8.875833	-36.366944	716	Preto	M
BL_82	Feijão Carioca	PE	São João	-8.875833	-36.366944	716	Carioca	M
BL_83	Feijão Preto	PE	Lajedo	-8.663888	-36.336666	661	Preto	M
BL_84	Feijão Preto	PE	Jucati	-8.705833	-36.488888	820	Preto	M
BL_85	Feijão Carioca	PE	Jucati	-8.705833	-36.488888	820	Carioca	M
BL_86	Feijão Preto	PE	Jupi	-8.711944	-36.415000	782	Preto	M

BL_87	Feijão Carioca	PE	Arcoverde	-8.420833	-37.061388	663	Carioca	M
BL_88	Feijão Mulatinho	PE	São João	-8.875833	-36.366944	716	Mulatinho	M
BL_90	Feijão Preto	PE	São João	-8.875833	-36.366944	716	Preto	M
BL_91	Feijão Preto	PE	São João	-8.875833	-36.366944	716	Preto	M
BL_92	Feijão Mulatinho	PE	Arcoverde	-8.420833	-37.061388	663	Mulatinho	M
BL_93	Feijão Colorido	PE	Casinha	-7.741111	-35.721111	390	Others	A
BL_94	Favita	PE	Lajedo	-8.663888	-36.336666	661	Others	A
BL_95	Feijão Preto	PE	Calçado	-8.741944	-36.333888	643	Preto	M
BL_96	Feijão Mulatinho	PE	Caruaru	-8.282777	-35.975833	554	Mulatinho	M
BL_99	Feijão Preto	PE	Caruaru	-8.282777	-35.975833	554	Preto	M
BL_100	Feijão Mulatinho	PE	Calçado	-8.741944	-36.333888	643	Mulatinho	M
BL_102	Feijão Preto	PE	São Caetano	-8.325833	-36.142777	552	Preto	M
BL_103	Feijão Preto	PE	São Caetano	-8.325833	-36.142777	552	Preto	M
BL_104	Feijão Colorido	PE	Surubim	-7.831944	-35.755833	394	Others	M
BL_105	Feijão Mulatinho	PE	Sta Maria do Cambucá	-7.840000	-35.901944	494	Mulatinho	M
BL_106	BG-4	MT	Cáceres	-15.799572	-57.385088	180	Mulatinho	M
BL_107	BG-9	MT	Mirassol do Oeste	-15.583333	-57.979166	285	Mulatinho	M
BL_108	BG-13	MT	Cáceres	-15.731691	-57.351783	151	Mulatinho	M
BL_109	BG-17	MT	Cáceres	-16.261083	-58.292461	202	Mulatinho	M
BL_110	BG-18	MT	Cáceres	-16.251022	-58.294869	186	Mulatinho	M
BL_111	BG-23	MT	Cáceres	-15.998333	-57.481666	311	Mulatinho	M
BL_165	Pitanga	PR	Pitanga	-24.729000	-51.721425	829	Others	A
BL_166	Corinthiano	PR	Loanda	-22.971027	-53.106013	344	Others	A
BL_167	Perla						Others	A
BL_168	JaloVermelho	PR	Capitão Leônidas Marques	-25.484708	-53.583041	380	Others	A
BL_170	JaloListras Pretas	PR	Nova Santa Rosa	-24.428666	-53.971819	480	Others	A
BL_171	Jalo EEP 558						Others	A
BL_172	BGF 20	PR	Terra Rica	-22.688794	-52.61755	381	Others	A
BL_174	Juriti	PR	Londrina	-23.354722	-51.16472	573	Carioca	M
BL_177	Awauna UEM	PR	Maringá	-23.435833	-51.89472	565	Preto	M
BL_181	MT 50G2	MT	Mirassol do Oeste	-15.496902	-58.044955	169	Rosinha	M

BL_183	MT 55	MT	Mirassol do Oeste	-15.505158	-58.059069	172		M
BL_184	MT 57G1	MT	Mirassol do Oeste	-15.521236	-58.049863	184	Rosinha	M
BL_186	MT 62	MT	Mirassol do Oeste	-15.505158	-58.059069	172		M
BL_187	MT 73G1	MT	Mirassol do Oeste	-15.538369	-58.047044	186	Rosinha	M
BL_189	MT 79	MT	Mirassol do Oeste	-15.538369	-58.047044	186		M
BL_199	Enxofre	PE	Lajedo	-8.663888	-36.336666	661	Enxofre	A
BL_216	Feijão Carioca	PE	Santa Maria do Cambucá	-7.840000	-35.901944	494	Carioca	M
BL_220	Jalo Pintado 2	PR	Capitão Leônidas Marques	-25.484708	-53.583041	380	Others	A
BL_221	AND277						Others	A
BL_225	Sempre Assim Branco	PE	Águas Belas	-9.110833	-37.122777	376	Branco	M
BL_226	CLPE3	PE	São João	-8.875833	-36.366944	716	Preto	M
BL_227	CLPE4	PE	São João	-8.875833	-36.366944	716	Preto	M
BL_228	CLPE8	PE	São João	-8.875833	-36.366944	716	Preto	M
BL_229	CLPE10	PE	Caetés	-8.772777	-36.622777	849	Preto	M
BL_230	CLPE11	PE	Jucati	-8.705833	-36.488888	820	Preto	M
BL_234	FeijãoPreto	PE	Calçado	-8.741944	-36.333888	643	Preto	M

A - Andean, M – Mesoamerican, PE – Pernambuco, PB – Paraíba, SE – Sergipe, PR – Paraná, MT – Mato Grosso.