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
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 from different parts of the bean-growing region in Brazil.
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.

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 an 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 ApeKI [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 $1.2 \times 10^6$ 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:

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

where $Y$ is the vector of the phenotype; $X$ is the incidence matrix of the independent vector $\alpha$ of SNPs fixed effect; $P$ is the incidence matrix of the independent vector $\beta$ of the population structure fixed effect; $K$ is the incidence matrix of the independent vector $\mu$ of the relative kinship random effect; and $\epsilon$ 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 | Gene pool | *C. lindemuthianum* races | Resistance index (%) |
|--------------|----------------------|--------|-----------|---------------------------|----------------------|
| BL_2         | Cocão               | PE     | A         | R R R R                   | 100                  |
| BL_3         | Bagajó              | SE     | A         | R R R R                   | 100                  |
| BL_5         | Canarinho           | PE     | A         | R R R R                   | 100                  |
| BL_7         | Chita Fina Verdadeiro | PE   | A         | S R S S                   | 33.33                |
| BL_8         | Jaula               | PE     | A         | R R R R                   | 100                  |
| BL_9         | Pintado             | PE     | A         | R R R R                   | 100                  |
| BL_11        | Praia               | SE     | A         | R R R R                   | 100                  |
| BL_12        | Camarão             | PE     | A         | R R R R                   | 100                  |
| BL_13        | BSF-1               | PE     | A         | R R R R                   | 100                  |
| BL_15        | BSF-3 Fogo na serra | PE   | A         | R R R R                   | 100                  |
| BL_27        | Mulatão             | PE     | A         | S S S S                   | 0                    |
| BL_74        | CLPE53              | PE     | A         | R R R R                   | 100                  |
| BL_75        | CLPE54              | PE     | A         | R R R R                   | 100                  |
| BL_77        | CLPE56              | PE     | A         | R R R R                   | 100                  |
| BL_78        | CLPE58              | PE     | A         | R R R R                   | 100                  |
| BL_79        | CLPE60              | PE     | A         | R R R R                   | 100                  |
| BL_93        | CLPE88              | PE     | A         | R R R R                   | 100                  |
| BL_94        | CLPE85              | PE     | A         | R R R R                   | 100                  |
| BL_165       | Pitanga             | PR     | A         | R R R R                   | 100                  |
| BL_166       | Corinthiano         | PR     | A         | R R R R                   | 100                  |
| BL_167       | Perla               | Argentina | A       | R R R R                   | 100                  |
| BL_168       | Jalo Vermelho       | PR     | A         | R R R R                   | 100                  |
| BL_170       | Jalo Listas Pretas  | PR     | A         | R R R R                   | 100                  |
| BL_171       | Jalo EEP 558        | MG     | A         | R R R R                   | 100                  |
| BL_172       | BGF 20              | PR     | A         | R R R R                   | 100                  |
| BL_178       | Perry Marrow        | NA     | A         | R R R R                   | 100                  |
| BL_199       | Enxofre             | PE     | A         | R R R R                   | 100                  |
| BL_220       | Jalo Pintado 2      | PR     | A         | R R R R                   | 100                  |
| BL_221       | AND 277             | NA     | A         | R 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, Mulatino de Cacho, Flor Azul, Laje, CLPE17, CLPE32, CLPE40, CLPE41, CLPE44, CLPE45, CLPE55, CLPE68, CLPE74, Awaua 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       | Origin¹ | Gene pool | C. lindemuthianum races | Resistance index (%) |
|--------------|----------------------|---------|-----------|-------------------------|----------------------|
|              |                      |         |           | 2 | 9 | 1545      |                      |
| BL_1         | Brigida              | 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      | R         | R | R | R | 100           |
| BL_24        | Mulatino 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         |
Table 2. Association mapping of B. vulgaris accessions with C. lindemuthianum races 2, 9, and 1545 resulted in the identification of SNPs on chromosomes Pv03, Pv04, and Pv05 (Table 4; Figures 4, 5, and 6).

### 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](image-url) 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 | Pv¹ | Position² | p-value³ | SNP R² (%)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_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       |

¹Chromosomes (Pv); ²SNP position in reference genome v1.0; ³significance level; ⁴phenotypic variation explained by the SNP (R²).
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 wr1 |
| 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*⁵) from the Mesoamerican breeding line BAT93 [35].

The BAT93 resistance gene against race 73 (*Co-3⁵*) is flanked by markers SNP04_027 (552,092 bp) and 254-G15 (1,618,118 bp). However, the resistance against race 38 (*Co-3⁶*) 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 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 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- 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 bp), IAC127 (28,334,236 bp), PvM98 (38,007,419 bp) and Scaffold00009_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 CI1532 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 wr1, 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](image.png)

**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 Llistras Pretas landrace and linked to marker OV20680 [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 | Cacão       | PE    | Recife         | -8.058880 | -34.880833| 4           | Others           | A         |
| BL_3 | Bagajó      | SE    | Poço Verde     | -10.707777| -38.182777| 268         | Others           | A         |
| BL_4 | Canarinho   | PE    | Lajedo         | -8.663888 | -36.336666| 661         | Others           | A         |
| BL_5 | Rosinha Claro| PE  | Calçado        | -8.741944 | -36.333888| 643         | Rosinha          | M         |
| BL_6 | Chita fina  | PE    | São João       | -8.875833 | -36.366944| 716         | Others           | A         |
| BL_7 | Jaula       | PE    | São João       | -8.890277 | -36.492770| 842         | Others           | A         |
| BL_8 | Pintado     | PE    | Ibimirim       | -8.540555 | -37.690277| 401         | Others           | A         |
| BL_9 | Bolinha     | PE    | Lajedo         | -8.540555 | -36.320000| 532         | Mulatinho        | M         |
| BL_10| Praia       | SE    | Poço Verde     | -8.053888 | -34.880833| 268         | Others           | A         |
| BL_11| 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 | PB | 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 | -36.488833 | 820 | Mulatinho | M |
| BL_27 | Mulatão | PE | Bezerros | -8.889999 | -36.492777 | 470 | Mulatinho | A |
| BL_30 | Flor Azul | PE | Águas Belas | -9.110833 | -36.492777 | 376 | Mulatinho | M |
| BL_34 | Feijão Laje | PB | São Miguel de Iraí | -7.250000 | -35.210000 | 45 | Mulatinho | M |
| BL_35 | Caiaminha | PE | Caçado | -8.741944 | -36.333888 | 643 | Rosinha | M |
| BL_50 | CLPE17 | PE | Lajedo | -8.663888 | -36.366666 | 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 | -36.366666 | 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 | Fabita | PE | Lajedo | -8.663888 | -36.336666 | 661 | Others | A |
| BL_95 | Feijão Preto | PE | Caçado | -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 | Caçado | -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 | | | | | | | |
| BL_168 | JaloVermelho | PR | Capitã 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 | | | | | | | |
| 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 |
| Code     | Name          | State | PL Latitude  | PL Longitude | Size | Sex |
|----------|---------------|-------|--------------|--------------|------|-----|
| BL_183   | MT 55         | MT    | -15.505158   | -58.059069   | 172  | M   |
| BL_184   | MT 57G1       | MT    | -15.521236   | -58.049863   | 184  | M   |
| BL_186   | MT 62         | MT    | -15.505158   | -58.059069   | 172  | M   |
| BL_187   | MT 73G1       | MT    | -15.538369   | -58.047044   | 186  | M   |
| BL_189   | MT 79         | MT    | -15.538369   | -58.047044   | 186  | M   |
| BL_199   | Enxofre       | PE    | -8.663888    | -36.336666   | 661  | A   |
| BL_216   | Feijão Carioca| PE    | -7.840000    | -35.901944   | 494  | M   |
| BL_220   | Jalo Pintado 2| PR    | -25.484708   | -53.583041   | 380  | A   |
| BL_221   | AND277        |       |              |              |      |     |
| BL_225   | Sempre Assim Branco | PE | Águas Belas | -9.110833 | -37.122777 | 376 | M |
| BL_226   | CLPE3         | PE    | -8.875833    | -36.366944   | 716  | M   |
| BL_227   | CLPE4         | PE    | -8.875833    | -36.366944   | 716  | M   |
| BL_228   | CLPE8         | PE    | -8.875833    | -36.366944   | 716  | M   |
| BL_229   | CLPE10        | PE    | -8.772777    | -36.622777   | 849  | M   |
| BL_230   | CLPE11        | PE    | -8.705833    | -36.488888   | 820  | M   |
| BL_234   | FeijãoPreto   | PE    | -8.741944    | -36.333888   | 643  | M   |

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