FINE MAPPING OF ANTHRACNOSE RESISTANCE ALLELE *Co-1^4* IN THE COMMON BEAN CULTIVAR AND 277

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INTRODUCTION: Among the main limitations faced by the common bean, anthracnose (ANT) caused by the fungus *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi &Cavara, causes considerable losses in production and depreciation of the quality of grains when in favorable disease development conditions (Pastor-Corrales et al., 1995). The most effective strategy for anthracnose control is the use of resistant cultivars (Gonçalves-Vidigal et al., 2011). The genetic identification of more specific information about resistance genes in different cultivars provides an additional database of sources of variability, and consequently, they generate information about the use of markers for marker-assisted selection (Song et al., 2015). The Andean cultivar AND 277 is an important source of anthracnose resistance for breeding programs as it exhibits a wide spectrum of resistance, conferring resistance to both Andean and Mesoamerican races. In addition, it presents resistance to the highly virulent races 2047 and 3481. Therefore, the objectives of this study were to fine-map the anthracnose-resistance locus in AND 277 and to identify DNA markers tightly linked to the *Co-1^4* allele, previously mapped on Pv01 (Gonçalves-Vidigal et al., 2011).

MATERIALS AND METHODS: The genetic basis of ANT resistance was studied using 149 F_{12} RILs derived from the cross between Rudá and AND 277 (RILs RA). Seedlings with the first fully expanded trifoliolate were inoculated with race 3481 of *C. lindemuthianum* to determine inheritance of the disease reaction. Inoculum of ANT was produced on young green common bean pod medium incubated at 22°C for 14 days and with a final concentration of 1.2×10^6 conidia mL^{-1} used for inoculation. After inoculation, seedlings were placed in a mist chamber for 72 h at 20 ± 2°C, with a photoperiod (12 h lighting/12 h darkness), and relative humidity over 95%. The ANT symptoms were evaluated using the disease severity scale (1 to 9) proposed by Pastor-Corrales et al. (1995). Plants with disease reaction scores between 1 and 3 were considered resistant, whereas plants with scores from 4 to 9 were considered susceptible. Total genomic DNA was isolated using the DNeasy Plant Mini Kit (Qiagen, CA, USA) following the manufacturer's instructions. The DNA samples were screened using the BARCBean6K_3 Illumina Bead Chip (5,398 SNPs) at the Soybean Genomics and Improvement Laboratory, USDA-ARS-BARC-W (Beltsville, MD, USA), using the Illumina Infinium genotyping platform ® HD Assay Ultra as described by Song et al. (2015). The BeadChip was imaged using the Illumina BeadArray Reader and automatic allele calling for each locus was performed using the Genome Studio software v2.0 (Illumina, San Diego, CA, USA). All allele calls were visually inspected and any errors in allele calling due to improper cluster identification were corrected. The genetic linkage maps were created using the software MapChart (Voorrips et al., 2002). A fine linkage map was developed by adding twelve new SSR markers and two STS markers previously mapped on Pv01.

RESULTS AND DISCUSSION: Genetic mapping of AND 277 resistance allele against race 3481 using 149 RILs RA positioned the *Co-1^4* locus in a region of 1.4Mb flanked by SNP markers ss715645251 and ss715645250, at the positions of 50,301,592 bp and 51,726,047 bp, respectively on Pv01 (Figure 1A). Further, fine-mapping using an additional twelve SSRs and two STS markers

showed that the resistance allele in AND 277 was located between the markers ss715645251 (50,301,592 bp) and BARCPVSSR01356 (50,342,103 bp) (Figure 1B), spanning a region of 40.51 Kb. According to the reference genome, this genomic region contains two gene models: Phvul.001G243800, which encodes a serine/threonine-protein kinase-like protein ccr3-related and Phvul.001G243900, which encodes a double clp-n motif-containing p-loop nucleoside triphosphate hydrolases superfamily protein. Our results validate previous studies conducted by Alzate Marin et al. (2003) and Gonçalves-Vidigal et al. (2011) indicating that the ANT resistance gene in AND 277 cultivar is an allele of *Co-1* locus mapped at the end of chromosome Pv01. Furthermore, Zuiderveen et al. (2016) also identified the SNP ss715645251 (50,301,592 bp) associated with race 3481 resistance in the Andean Diversity Panel. In conclusion, the markers identified in this study by fine-mapping, ss715645251 and SSR BARCPVSSR01356, are important tools for breeding programs aiming to obtain new cultivars containing the *Co-1*^{4} allele through marker assisted selection. Therefore, it is concluded that the ss715645251 and SSR BARCPVSSR01356 markers will be important tools for breeding programs aiming to obtain cultivars with the *Co-1*^{4} allele through marker assisted selection.

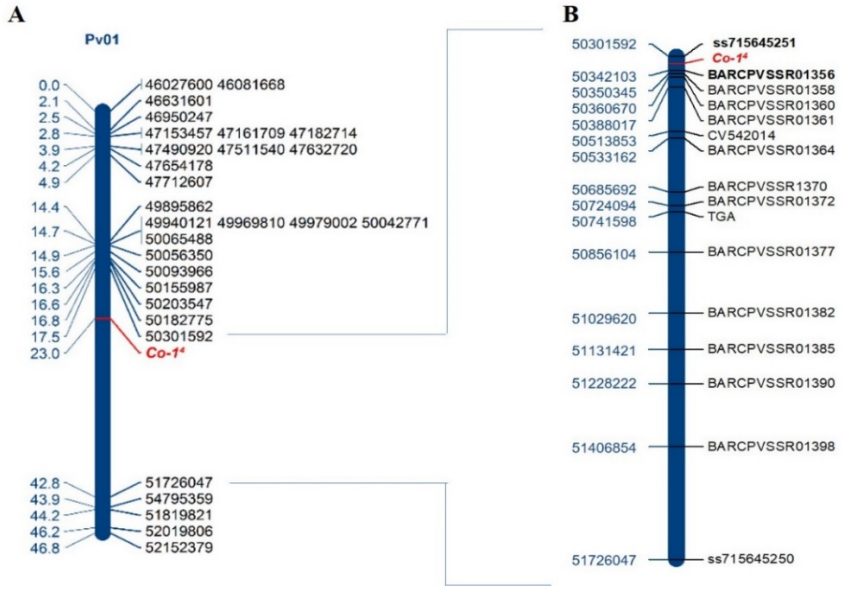

Fig 1. 1A - Genetic map of common bean linkage group Pv01 containing the anthracnose resistance allele and SNP markers used to genotype the F_{12} population Rudá × AND 277. The genetic distances in centimorgans (cM) are shown on the left side, and the physical positions (bp) on the right side. The map was built with MapChart (Voorrips et al., 2002); 1B – Fine-mapping the *Co-1*^{4} allele using SSR and STS markers positioned in region of 40.511 Kbp.

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