

# SNP DISCOVERY IN GENOMIC REGIONS FLANKING THE COMMON BEAN ANTHRACNOSE RESISTANCE LOCUS *Co-4*

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## INTRODUCTION

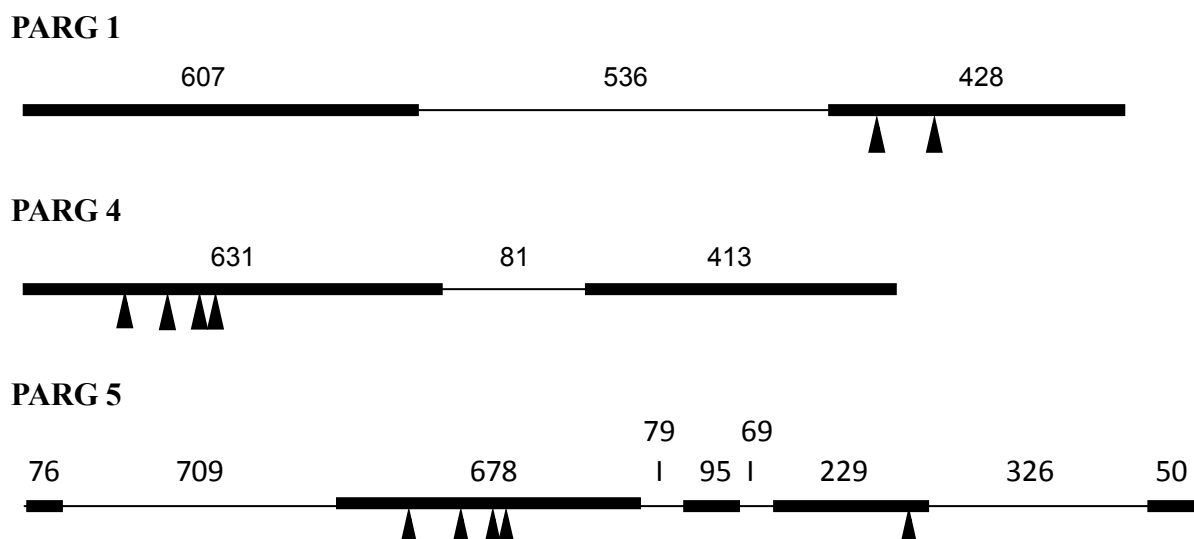
Anthrachnose, caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara, is one of the major fungal diseases that affect the common bean (*Phaseolus vulgaris* L.) crop in tropical and subtropical areas. Long periods of moderate temperatures (13-27°C) and high humidity during the crop cycle may cause losses up to 100%. A efficient and cost-effective strategy available for the bean anthracnose control is the use of resistant cultivars. In Brazil as well as in other parts of the world, an important resistant allele explored by the common bean breeding programs is the *Co-4*<sup>2</sup> (Souza *et al.* 2014). Molecular markers linked to resistance genes have been used as an important auxiliary tool by common bean breeding programs worldwide. The main goal of this work was to characterize the genetic variability of genomic regions flanking the anthracnose resistance locus *Co-4* aiming to develop SNP markers for specific identification of the different alleles presented by this locus, mainly of the *Co-4*<sup>2</sup>.

## MATERIAL AND METHODS

DNA sequences from amplified products obtained using the SCAR marker SAS13, linked to the locus *Co-4*, and DNA samples from 15 common bean genotypes were used to mining conserved domains of plant resistance genes (putative genes) using the BLAST tool and the *P. vulgaris* reference genome sequence (<http://phytozome.jgi.doe.gov/pz/portal.html#>). Among those 15 bean genotypes were 12 anthracnose resistant lines, including TO (*Co-4*), SEL1308 (*Co-4*<sup>2</sup>) and PI207262 (*Co-4*<sup>3</sup>), and three susceptible lines. For each identified putative gene, PCR primers were designed and used to amplify DNA samples from the same 15 common bean genotypes used before. After sequencing, all DNA fragment sequences were aligned and used for SNP discovery. The total nucleotide diversity ( $\theta$ ) in these genomic regions associated with the anthracnose resistance locus *Co-4* was calculated according to Halushka *et al.* (1999).

## RESULTS AND DISCUSSION

Five putative genes were identified and signed as PARG (Putative Anthracnose Resistance Gene) 1-to-5. All these genes were annotated as harboring the domain STK (Serine Threonine Kinase), a conserved domain in plant disease resistance genes. Three putative genes were selected based on their DNA sequence quality for SNP discovery. A total of 60 SNPs were identified in CDS (Coding DNA Sequence) regions. Out of these SNPs, 20 were classified as transitions and 40 as transversions. The total nucleotide diversity was  $\theta = 0.006151$ . Twenty-two SNPs were classified as synonymous mutations and 38 as non-synonymous. Eleven SNPs were selected as potential markers for specific identification of the locus *Co-4* and of the different alleles presented by this locus (Figure 1 and Table 1). These selected SNP markers are being tested in co-segregation studies using an F<sub>2:3</sub> population derived from a test cross that used SEL 1308 (*Co-4*<sup>2</sup>) as the resistant parent.



**Fig. 1.** Representation of three putative genes identified in genomic regions flanking the common bean anthracnose resistance locus *Co-4*. Exons (dark bars), introns (lines), and their respective lengths in bp, in addition to the position of each one of the 11 selected SNPs, are showed.

**Table 1.** SNPs selected as potential markers for specific identification of the common bean anthracnose resistance locus *Co-4* and of the different alleles presented by this locus.

Putative Gene	<i>Co-4</i> Allele	-	<i>Co-4</i>	<i>Co-4</i> <sup>2</sup>	<i>Co-4</i> <sup>3</sup>
	SNP Position	REF*	TO	SEL1308	PI207262
PARG 1	817	G	A	A	A
	990	G	T	T	T
PARG 4	157	A	G	G	A
	178	G	C	C	G
	192	G	C	C	G
	194	G	T	T	G
PARG 5	269	A	T	A	A
	315	G	C	G	G
	340	G	T	G	G
	357	A	T	A	A
	934	A	A	G	A

\**P. vulgaris* reference genome sequence.

## REFERENCES

- Halushka MK *et al.* (1999) Patterns of single-nucleotide polymorphisms in candidate genes for blood-pressure homeostasis. *Nature Genetics* 22(3):239–47.
- Souza TLPO *et al.* (2014) Anthracnose resistance sources to be explored by the common bean breeding programs in Brazil. *Annual Report of the Bean Improvement Cooperative* 57:217–218.