DEVELOPMENT OF SCAR MARKERS LINKED TO COMMON BEAN ANTHRACNOSE RESISTANCE GENES *Co-4* and *Co-6*

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Anthracnose, caused by fungus *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scrib., is among the main diseases of the common bean (*Phaseolus vulgaris* L.) in Brazil an in other bean-growing areas around the world. This disease may cause total yield loss when susceptible cultivars are grown under environmental conditions appropriate for the pathogen proliferation. The development of resistant cultivars is an alternative strategy to control of the disease. New cultivars have to be developed along the years because of the high pathogenic variability of the fungus. Several RAPD markers have been identified and used to facilitate the development of resistant cultivars. Because of the reproducibility problems associated with the RAPD technique these markers can be used to derive SCAR markers which are more specific and reproducible. The objective of this work was to develop SCAR markers from RAPD markers linked to anthracnose resistance genes *Co-4* (RAPD markers OPY20 and OPC08) and *Co-6* (RAPD markers OPAZ20 and OPZ04) previously identified in our laboratory (Alzate-Marin et al., 1999; Alzate-Marin et al., 2000; Arruda et al., 2000). The RAPD bands of interest were excised from 1.5% agarose gels and purified with the Gel Extraction Kit (QIAgen). The DNA fragments were cloned into the pGEM-T Easy vector (Promega), which was used to transform competent DH5α *Escherichia coli* cells. The positive clones were sequenced in an automated sequencer (ABI Prism 377, Perkin Elmer), and the SCAR primers were designed, synthesized and tested in appropriate populations segregating for resistance genes *Co-4* or *Co-6* (Table 1). The sequences of the SCAR primers and the amplification conditions are depicted on Table 2.

These SCAR markers are now being used in the common bean breeding program developed at the BIOAGRO/UFV, which aims to pyramid resistance genes in different commercial bean cultivars grown in Brazil.

Table 1. Segregation analysis and genetic distances (centiMorgans - cM) between SCAR markers and anthracnose resistance genes *Co-4* or *Co-6*.

Locus tested	*Parents*			Expected ratio[a]	Observed ratio	χ^2	*Prob. (%)*	cM
	Resistant		*Susceptible*					
SCARY20/*Co-4*	Rudá	x	TO	9:3:3:1	115:3:0:44	177.56	0.00	1.2
SCARC08/*Co-4*	Ouro Negro	x	TO	9:3:3:1	69:5:4:47	227.21	0.00	7.8
SCARAZ20/*Co-6*	***Rudá***	***x***	***AB 136***	9:3:3:1	176:13:2:48	149.57	0.00	7.1
SCARZ04/*Co-6*	Michelite	x	AB 136	9:3:3:1	63:0:1:17	63.23	0.00	2.9

[a] **Considering independence between the loci.**

Table 2. Sequences and amplification conditions for SCAR markers linked to anthracnose resistance genes *Co-4* and *Co-6*.

Marker	Primer sequence (5' – 3')[a]	PCR profile				Size (bp)
		Cycles	Denaturation	Annealing	Extension	
SCARAZ20	F: ACCCCTCATGCAGGTTTTTA R: CATAATCCATTCATGCTCACC	35	94 °C/30 s	60 °C/1 min	72 °C/90 s	845[b]
SCARZ04	F: GGCTGTGCTGATTAATTCTGG R: TGCTCATTTTATAATGGAGAAAAA	45	94 °C/30 s	45 °C/2 min	72 °C/90 s	567[c]
SCARY20	F: AGCCGTGGAAGGTTGTCAT R: CCGTGGAAACAACACACAAT	35	94 °C/30 s	65 °C/1 min	72 °C/90 s	830[b]
SCARC08	F: AGAATGCCTTTAGCTGTTGG R: CAGAGAGGCTAGGCTTATCG	35	94 °C/30 s	65 °C/1 min	72 °C/90 s	910[b]

[a] **F = forward; R = reverse**
[b] **Coupling phase;** [c] **Repulsion phase**

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