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Selection of resistance sources to common bean anthracnose by field phenotyping and DNA marker-assisted screening

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Genet. Mol. Res. 17 (3): gmr18066
Received June 25, 2018
Accepted August 07, 2018
Published August XX, 2018
DOI http://dx.doi.org/10.4238/gmr18066

ABSTRACT. The main goal of this work was to select resistance sources to common bean anthracnose by field phenotyping and DNA marker-assisted screening. Fifty-five common bean genotypes, including differential varieties, characterized resistance sources, elite lines, cultivars and controls, were evaluated in a field inoculation trial and screened with SCAR markers linked to resistance genes that are important in Brazil. The field trial was carried out in Santo Antônio de Goiás, GO, Brazil, during the fall/winter growing season of 2014, using artificial inoculation with a mixture of six races of *Colletotrichum lindemuthianum*, selected based on their high virulence and prevalence in Brazil. Amplification reactions with the SCAR markers previously identified as linked to important anthracnose resistance genes on Brazil followed standard procedures. Twenty-eight of the 58 genotypes were resistant to anthracnose (mean severity score ≤ 3.5). Ten of these 28 resistant genotypes stood out because they presented a mean anthracnose severity score of 1.0. Four of the six SCAR markers tested shown to be useful for the assisted selection of their respective target genes (SH18 and SAS13

for *Co-4*^{2}, SAB03 for *Co-5*, and SAZ20 for *Co-6*). Two carioca seeded elite lines were highlighted by the phenotypic and molecular screening: K10 (*Co-3*^{4}, *Co-4*^{2}, *Co-5* and *Co-6*) and K13 (*Co-4*^{2}). The phenotypic and molecular characterization of candidate resistance sources to common bean anthracnose based on their disease reaction in field inoculation trials and on the analysis with molecular markers linked to resistance genes has shown to be a useful strategy. These results aid in the selection of donor parents and resistant lines to be preferably explored by common bean breeding programs in Brazil.

Key words: *Colletotrichum lindemuthianum*; *Phaseolus vulgaris*; Molecular markers; Parent selection; Plant breeding.

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is one of the main sources of vegetable protein used worldwide. It is widely grown and consumed in most countries from Latin American and African, in different regions and growing seasons (Broughton et al., 2003; Food and Agriculture Organization – http://faostat.fao.org). In Brazil, it is a legume grain grown by smallholder farmers, mainly for their subsistence, and by rural companies, which use a high level of technology for bean production. However, the national mean yield is low (around 1,450 kg/ha) when compared to the yield potential of the crop, which is over 4,000 kg/ha (Del Peloso and Melo, 2005, Embrapa Arroz e Feijão, 2018). One explanation for this fact is the large number of diseases that affect the common bean crop, including anthracnose caused by the fungus *Colletotrichum lindemuthianum* (Sacc. and Magnus) Briosi and Cavara (Paula-Júnior and Zambolim, 1998). High relative humidity and moderate temperatures between 13–26°C favor the occurrence of this disease, which can cause losses of up to 100% in areas where favorable conditions for this fungus are predominant and when susceptible cultivars with infected seeds are used (Singh and Schwartz, 2010).

Among the measures for integrated management of anthracnose, the use of resistant cultivars is highlighted because it is an efficient, safe and accessible strategy for growers using various different technological levels (Kelly et al., 2003; Singh and Schwartz, 2010). However, the wide variability of *C. lindemuthianum* is a challenge for the common bean breeders. In Brazil, dozens of physiological races of the anthracnose pathogen have already been identified and described (Rava et al., 1994; Balardin et al., 1997; Carbonell et al., 1999; Alzate-Marin and Sartorato, 2004; Ribeiro et al., 2016). Genetic and physiological diversity have also been reported among *C. lindemuthianum* isolates belonging to the same race (Davide et al., 2009; Ishikawa et al., 2011). For this reason, the identification of new anthracnose resistance sources and multi-site validation of the main identified sources are frequent components of common bean breeding programs aiming to develop cultivars with broad and durable resistance to anthracnose.

In general, anthracnose resistance in common bean is monogenic and dominant, i.e., controlled by major genes with simple inheritance (Kelly and Vallejo, 2004; Gonçalves-Vidigal and Kelly, 2006, Gonçalves-Vidigal et al., 2009; Oblessuc et al., 2015). However, it can also be oligogenic, controlled by independent dominant genes (Campa et al., 2009), complementary genes with epistatic interaction (Muhalet et al., 1981; Alzate-Marin et al.,

1997), or by multiple minor genes with secondary effects (Vallejo and Kelly, 2009; Zuiderveen et al., 2016). Alzate-Marin et al. (1997) also report a simple recessive gene (*co-8*) that controls resistance to anthracnose in the common bean line AB 136. Currently, 14 anthracnose resistance loci have been characterized and reported. They are named according to the nomenclature proposed by Kelly and Young (1996): *Co-1* to *Co-17*, excluding *Co-7*, *Co-9* and *Co-10*, which have been renamed, as well as others that follow a different standard of nomenclature, such as *Co-u*, *Co-v*, *Co-w*, *Co-x*, *Co-y* and *Co-z* (Geffroy et al., 2008). Allelic series were identified for four of these loci: *Co-1*, *Co-3*, *Co-4* and *Co-5*. It has been demonstrated that the genes *Co-7* and *Co-9* (renamed as *Co-3*^{3}) and *Co-10* (renamed as *Co-3*^{4}) are indeed allelic to *Co-3* (Méndez-Vigo et al., 2005; Rodríguez-Soárez et al., 2008; Gonçalvez-Vidigal et al., 2013). Genes *Co-1*, *Co-12* and *Co-13* are from Andean gene pool, being the others of Mesoamerican origin. The mapped resistance loci are distributed in eight of the 11 common bean chromosomes: Pv01, Pv02, Pv03, Pv04, Pv07, Pv08, Pv09 and Pv11 (Bean Improvement Cooperative, 2018).

There are several molecular markers available for the assisted selection of resistance genes to common bean diseases, mainly for anthracnose. Many of them are Sequence Characterized Amplified Region (SCAR) markers (Bean Improvement Cooperative, 2018). Molecular screening of common bean genotypes with these markers has been used as a strategy for the indirect selection of potential sources of anthracnose resistance genes, i.e., molecular markers are being used as an additional tool to identify the presence or absence of anthracnose resistance alleles in common bean genotypes (Souza et al., 2005; Beraldo et al., 2009).

The main goal of the present work was to evaluate candidate common bean anthracnose resistance sources based on their reaction to the disease in a field inoculation trial and through molecular screening with SCAR markers identified as linked to resistance genes important in Brazil. This will allow selecting resistance sources to be preferably exploited as donor parents by common bean breeding programs in Brazil.

MATERIAL AND METHODS

Fifty-four candidate common bean resistance sources to anthracnose were evaluated, including international differential varieties, sources of already characterized resistance genes, elite lines and cultivars developed by Embrapa (Brazilian Agricultural Research Corporation), in addition to the susceptible control Rosinha G2. Seeds of all these genotypes were obtained from the work collection of the Embrapa common bean breeding program.

The field trial to screen the common bean genotypes for reaction to anthracnose was carried out at Embrapa Arroz e Feijão (Santo Antônio de Goiás, GO, Brazil), during the fall/winter growing season of 2014, using a randomized complete block design, with three replicates. Each plot consisted of two 3.0-m long rows, with 0.5 m between rows. Fifteen days after seedling emergence, at phenological stage V3, all plants in each plot were inoculated with a mixture of six races of *C. lindemuthianum*: 65 (isolate Cl_1614), 73 (isolate Cl_1143), 81 (isolate Cl_1164), 91 (isolate Cl_1247 isolate), 475 (isolate Cl_1322) and 1609 (isolate Cl_1294). Selection of these races was based on the following criteria: prevalence in Brazil (races 65, 73, 81 and 91) and high virulence (races 475 and 1609), in addition to sporulation capacity in a controlled environment.

Inoculum preparation was as described by Pastor-Corrales et al. (1995), but with some modifications. Inoculum production was carried out in the laboratory by streaking the fungus in test tubes containing a sterilized common bean pod partially immersed in agar-water medium. The inoculum suspension with final concentration adjusted to 1.2×10^6 conidia/mL was sprayed on all plants of each plot with the aid of a 20 L portable sprayer. About 200 mL of inoculum suspension was sprayed per plot of 1.5 m^2. Evaluation of anthracnose symptoms was performed in two stages, by two evaluators, at 15 and 30 days after inoculation, using a nine degrees severity scale, where 1 = plot with plants showing no disease symptoms, and 9 = plot with 100% of infected or dead plants (Melo, 2009). The anthracnose severity data were submitted to analysis of variance. The mean severity scores were compared by the Scott and Knott method at 5% probability using the Genes program (Cruz, 2013). Genotypes with mean scores ≤ 3.5 were considered resistant, while those with mean scores > 3.5 were considered as susceptible.

Leaf tissue samples from each tested common bean genotypes were collected in bulk, sampling 10 individual plants from each genotype, and stored in an ultra-freezer at –20ºC. DNA extraction was performed using the CTAB method, as described by Ferreira and Grattapaglia (1998). DNA samples from all genotypes were then amplified using SCAR markers identified as linked to anthracnose resistance genes important in Brazil, which are described in Table 1. Amplification reactions were performed via PCR with final volume of 15.0 µL, using the commercial kit Master Mix (Qiagen Multiplex PCR Kit). Each reaction consisted of 30 ng of DNA, 5.0 µL of Master Mix, 0.5 µL of Q-solution, and 1.0 µL of each specific primers (forward and reverse) at the concentration of 10.0 µM (Table 1). PCR reactions were performed using the following conditions: i) a first stage of 95°C for 5 minutes; ii) forty cycles of amplification including an initial denaturation step at 95°C for 15 seconds, a second annealing step of primers to the template DNA ranging from 54–to–72°C (Table 1) for 90 seconds, and a third extension step at 72°C for 1 minute; and a final extension stage of 72°C for 10 minutes.

Table 1. Sequence characterized amplified region (SCAR) markers linked to anthracnose resistance genes in common bean, with the gene identification or official symbol, resistance source, genome location, primer sequences and annealing temperature of primers.

Marker[a]	Gene	Source	Chromosome	Distance (cM)	Primer sequence	T(ºC)
SF10	*Co-3*[4]	Ouro Negro	Pv04	12.3	F: GGAAGCTTGGTGAGCAAGGA R: GGAAGCTTGGCTATGATGGT	65
SY20	*Co-4*	TO	Pv08	0.0	F: AGCCGTGGAAGGTTGTCAT R: CAGAGACCCTAGGCTTATCG	60
SAS13	*Co-4*[2]	SEL1308	Pv08	0.4	F: CACGGACCGAATAAGCCACCAACA R: CACGGACCGAGGATACAGTGAAAG	72
SH18	*Co-4*[2]	SEL1308	Pv08	4.2	F: CCAGAAGGAGCTGATAGTAGTCCACAAC R: GGTAGGCACACTGATGAATCTCATGTTGGG	60
SAB03	*Co-5*	TU	Pv07	5.9	F: TGGCGCACACATAAGTTCTCACGG R: TGGCGCACACCATCAAAAAAGGTT	54
SAZ20	*Co-6*	AB136	Pv07	7.1	F: ACCCCTCATGCAGGTTTTTA R: CATAATCCATTCATGCTCACC	60

[a] Source: Bean Improvement Cooperative (2018).

For the electrophoresis analysis, 3.0 µL of bromophenol blue dye were added to the amplification products, which were submitted to a 1.5% agarose gel electrophoresis in 1X TBE (Tris-boric acid/EDTA) at 100 volts for 2 h. For analysis of the DNA amplification products, the agarose gel was stained in ethidium bromide solution for 20 minutes and then visualized under UV light. Gel images were photo-digitalized and the amplified DNA products interpreted as present (1) or absent (0). The size of DNA fragments was measured by comparison to a 1 kb DNA ladder (Qiagen GelPilot Ladder).

RESULTS AND DISCUSSION

The results of the analysis of variance (Table 2) showed the existence of genetic variability for anthracnose severity among the common bean genotypes tested, which were grouped based on their average severity score, according to the Scott-Knott method (Table 3). Twenty-eight of the 55 genotypes tested in the field inoculation trial were resistant to anthracnose (mean severity score ≤ 3.5), represented by the genotypes that formed the "a", "b" and "c" groups in Table 3. Ten of these 28 resistant genotypes stood out because they presented a mean severity score of 1.0: BRS Esteio (*Co-?*), BRSMG Realce (*Co-?*), BRS Sublime (*Co-?*), CNFC 10729 (*Co-?*), Ouro Negro (*Co-3*^{4}), K10 (*Co-4*^{2}, *Co-3*^{4}, *Co-5* and *Co-6*), K13 (*Co-4*^{2}), SEL 1308 (*Co-4*^{2}), TO (*Co-4*) and AB 136 (*Co-6* and *co-8*) (Table 3). Among the 55 common bean genotypes that were phenotypically characterized, 27 were susceptible to anthracnose (mean severity score > 3.5), represented by the genotypes on "d", "e" and "f" groups in Table 3. These susceptible groups include the current international differential varieties for anthracnose Cornell 49-242 (*Co-2*) and Michelite (*Co-11*), in addition to known resistance sources such as BAT 93 (*Co-3*^{3}), H1 (*Co-3*^{5}), and SEL 1360 (*Co-5*^{2}). As expected, the susceptible control Rosinha G2 presented the highest mean severity score (9.0) (Table 3).

Table 2. Summary of analysis of variance for anthracnose severity in common bean genotypes tested in a field inoculation trial carried out in Santo Antônio de Goiás, GO, Brazil, during the fall/winter growing season of 2014.

Source of variation	df	Sum of squares	Mean square	*F*-value	*P*-value
Block	2	0.11	0.055	0.31	0.7367
Genotype	54	1,094.45	20.268	113.86	0.0001
Residue	108	19.22	0.178		
Total	164	1,113.78			
CV = 10.6%					
Accuracy = 99.6%					

Based on the results from this work, and as previously reported by Souza et al. (2005) and Beraldo et al. (2009), it we can see that the Brazilian common bean breeding programs have good resistance sources to anthracnose to use as donor parents. In addition, these programs are also being efficient in the development of elite lines and cultivars resistant to anthracnose. However, considering the wide variability and rapid emergence of new *C. lindemuthianum* races in Brazil (Alzate-Marin and Sartorato, 2004; Ribeiro et al., 2016), resistance to anthracnose should always be considered in the selection process of superior genotypes, since several cultivars also presented high disease mean severity scores (Table 3).

In the molecular characterization of the common bean lines and cultivars, the SCAR markers SH18 (*Co-4*^{2}), SAS 13 (*Co-4*^{2}), SAB03 (*Co-5*) and SAZ20 (*Co-6*) were specific for the loci to which they are linked, under the amplification conditions described here. However, these markers were not allele-specific. Only the SH18 marker was able to discriminate the *Co-4*^{2} allele from the *Co-4* e *Co-4*^{3} alleles (Table 3). Awale and Kelly (2001) developed the SH18 marker linked to the *Co-4*^{2} allele and had previously reported that this marker is specific for this allele.

Of the 55 common bean genotypes tested with the molecular markers, 31 presented the SF10 marker, including cultivars, elite lines and controls, independent of the presence of alleles of the locus *Co-3*, indicating the non-specificity of this marker. SF10 amplified DNA fragments for Mexico 222 (*Co-3*) and Ouro Negro (*Co-3*^{4}), as expected, but also for TU (*Co-5*) and AB136 (*Co-6* and *co-8*), as well as for other genotypes with known genes or resistance alleles, such as Kaboon (*Co-1*^{2}), Perry Marrow (*Co-1*^{3}), Cornell 49-242 (*Co-2*), H1 (*Co-3*^{5}), K23 (*Co-5*) and SEL 1360 (*Co-5*^{2}) (Table 3).

The SY20 marker was specific for the *Co-4* locus, however, this marker did not discriminate the different alleles of this locus, amplifying DNA fragments only for TO (*Co-4*), SEL 1308 (*Co-4*^{2}), K10 (*Co-3*^{4}, *Co-4*^{2}, *Co-5* and *Co-6*), K13 (*Co-4*^{2}), PI 207262 (*Co-3*^{3} and *Co-4*^{3}) and G 2333 (*Co-3*^{5}, *Co-4*^{2} and *Co-5*) (Table 3). Beraldo et al. (2009) also reported the specificity of the SY20 marker for the *Co-4* locus. When screening 42 parents and 76 elite lines developed by the common bean breeding program of the *Instituto Agronômico de Campinas* (IAC, Campinas, SP, Brazil) with SY20, those authors reported that only the genotype G 2333 (*Co-3*^{5}, *Co-4*^{2} and *Co-5*) showed the expected DNA product associated with the anthracnose resistance allele *Co-4*^{2}.

The SAS13 marker, identified as linked to *Co-4*^{2} allele (Young et al., 1998; Kelly et al., 2003), was specific for the *Co-4* locus since it amplified only DNA samples from SEL 1308 (*Co-4*^{2}), K13 (*Co-4*^{2}), K10 (*Co-3*^{4}, *Co-4*^{2}, *Co-5* and *Co-6*), G 2333 (*Co-3*^{5}, *Co-4*^{2} and *Co-5*) and PI 207262 (*Co-3*^{3} and *Co-4*^{2}). However, it was shown to be non-specific to *Co-4*^{2} allele when amplifying a DNA product in PI 207262 harboring the *Co-4*^{3} allele (Table 3). Awale and Kelly (2001) and Alzate-Marin et al. (2007) already reported that the SAS13 marker amplifies different alleles of the *Co-4* locus.

DNA samples from eight genotypes were amplified by the SAB03 marker linked to the *Co-5* gene (Vallejo and Kelly, 2001; Campa et al., 2005): BRS Campeiro, BRS Esplendor, BRS Supremo, and CNFC 15875, whose the anthracnose resistance genes have not been characterized yet, and K10 (*Co-3*^{4}, *Co-4*^{2}, *Co-5* and *Co-6*), K23 (*Co-5*), G 2333 (*Co-3*^{5}, *Co-4*^{2} and *Co-5*), TU (*Co-5*) and SEL 1360 (*Co-5*^{2}), confirming the presence of the *Co-5* gene. In this case, SAB03 did not discriminate different alleles of the *Co-5* locus (Table 3).

In addition to AB136, the original source of *Co-6* gene, only line K10 (*Co-3*^{4}, *Co-4*^{2}, *Co-5* and *Co-6*) presented DNA products amplified with the SAZ20 marker linked to the *Co-6* gene (Kelly et al., 2003; Queiroz et al., 2004) (Table 3). Three elite lines were highlighted by the molecular characterization: K10 (*Co-3*^{4}, *Co-4*^{2}, *Co-5* and *Co-6*), presenting the markers SF10, SY20, SAS13, SAB03 and SAZ20, K13 (*Co-4*^{2}), presenting the markers SY20 and SAS13, and K23 (*Co-5*), presenting the markers SF10 and SAB03. In the field inoculation screening, these three genotypes were also resistant to anthracnose, with emphasis on K10 and K13, since both had a mean severity score of 1.0 (Table 3).

Table 3. Anthracnose severity for common bean genotypes tested in a field inoculation trial (Santo Antônio de Goiás, GO, Brazil) and molecular screening of those genotypes with SCAR markers linked to anthracnose resistance genes important in Brazil.

Genotype	Resistance gene	Gene pool[a]	Market class	Mean severity[b]	SCAR marker[c]					
					SF10/ *Co-3*^{4}	SY20/ *Co-4*	SH18/ *Co-4*^{2}	SAS13/ *Co-4*^{2}	SAB03/ *Co-5*	SAZ20/ *Co-6*
Ouro Negro	*Co-3*^{4}	MA	Black	1.00 a	1	0	0	0	0	0
TO	*Co-4*	MA	*Carioca*	1.00 a	0	1	0	0	0	0
SEL 1308	*Co-4*^{2}	MA	Black	1.00 a	0	1	1	1	0	0
K13	*Co-4*^{2}	MA	*Carioca*	1.00 a	0	1	1	1	0	0
K10	*Co-3*^{4}, *Co-4*^{2}, *Co-5*, *Co-6*	MA	*Carioca*	1.00 a	1	1	0	1	1	1
AB 136	*Co-6*, *co-8*	MA	Red	1.00 a	1	0	0	0	0	1
BRS Esteio		MA	Black	1.00 a	1	0	0	0	0	0
BRSMG Realce		A	*Rajado*	1.00 a	1	0	0	0	0	0
BRS Sublime		MA	*Carioca*	1.00 a	1	0	0	0	0	0
CNFC 10729		MA	*Carioca*	1.00 a	1	0	0	0	0	0
Kaboon	*Co-1*^{2}	A	Brown	1.33 a	1	0	0	0	0	0
G 2333	*Co-3*^{5}, *Co-4*^{2}, *Co-5*	MA	Red	1.33 a	1	1	0	1	1	0
Jalo Vermelho	*Co-12*	A	Jalo	1.33 a	0	0	0	0	0	0
BRS Embaixador		A	DRK	1.33 a	0	0	0	0	0	0
BRS Estilo		MA	*Carioca*	1.33 a	1	0	0	0	0	0
PI 207262	*Co-3*^{3}, *Co-4*^{3}	MA	Light brown	1.67 b	1	1	0	1	0	0
Jalo Listras Pretas	*Co-13*	A	*Jalo*	1.67 b	0	0	0	0	0	0
BRS Radiante		A	*Rajado*	1.67 b	1	0	0	0	0	0
MDRK	*Co-1*	A	Red	2.00 b	0	0	0	0	0	0
Perry Marrow	*Co-1*^{3}	A	Brown	2.00 b	1	0	0	0	0	0
AND 277	*Co-1*^{4}	A	Red	2.00 b	0	0	0	0	0	0
Widusa	*Co-1*^{5}	MA	Calima	2.00 b	0	0	0	0	0	0
TU	*Co-5*	MA	Black	2.00 b	1	0	0	0	1	0
BRS Supremo		MA	Black	2.00 b	1	0	0	0	1	0
BRS Executivo		A	Sugar bean	2.33 b	0	0	0	0	0	0
BRS Notável		MA	*Carioca*	2.33 b	0	0	0	0	0	0
Mexico 222	*Co-3*	MA	White	3.00 c	1	0	0	0	0	0
K23	*Co-5*	MA	*Carioca*	3.33 c	1	0	0	0	1	0
BRS Esplendor		MA	Black	4.67 d	1	0	0	0	1	0
CNFP 10120		MA	Black	4.67 d	1	0	0	0	0	0
IAC Alvorada		MA	*Carioca*	4.67 d	1	0	0	0	0	0
Pérola		MA	*Carioca*	5.00 d	0	0	0	0	0	0
CNFP 15330		MA	Black	5.33 d	0	0	0	0	0	0
Jalo Precoce		A	*Jalo*	5.33 d	0	0	0	0	0	0
SEL 1360	*Co-5*^{2}	MA	Black	5.67 d	1	0	0	0	1	0

Genotype	Resistance gene	Gene pool[a]	Market class	Mean severity[b]	SCAR marker[c]					
					SF10/ *Co-3*^{4}	SY20/ *Co-4*	SH18/ *Co-4*^{2}	SAS13/ *Co-4*^{2}	SAB03/ *Co-5*	SAZ20/ *Co-6*
CNFP 10794		MA	Black	5.67 d	0	0	0	0	0	0
Rudá		MA	*Carioca*	5.67 d	0	0	0	0	0	0
BRS Requinte		MA	*Carioca*	6.00 d	0	0	0	0	0	0
BRSMG Majestoso		MA	*Carioca*	6.00 d	1	0	0	0	0	0
BRS Grafite		MA	Black	6.33 e	1	0	0	0	0	0
IPR Uirapuru		MA	Black	6.33 e	1	0	0	0	0	0
BRS Pitanga		MA	Purple	6.67 e	1	0	0	0	0	0
BRS Agreste		MA	*Mulatinho*	6.67 e	1	0	0	0	0	0
BRS Valente		MA	Black	7.00 e	1	0	0	0	0	0
BRSMG Talismã		MA	*Carioca*	7.00 e	1	0	0	0	0	0
BRS FC104		MA	*Carioca*	7.00 e	0	0	0	0	0	0
Cornell 49-242	*Co-2*	MA	Black	7.33 e	1	0	0	0	0	0
Michelite	*Co-11*	MA	White	7.33 e	0	0	0	0	0	0
BRS Campeiro		MA	Black	7.33 e	1	0	0	0	1	0
CNFC 15873		MA	*Carioca*	7.33 e	0	0	0	0	0	0
BAT 93	*Co-3*^{3}	MA	*Mulatinho*	7.67 f	1	0	0	0	0	0
BRSMG Madrepérola		MA	*Carioca*	8.00 f	0	0	0	0	0	0
CNFC 15875		MA	*Carioca*	8.00 f	0	0	0	0	1	0
Rosinha G2[d]		MA	Small Pink	9.00 f	0	0	0	0	0	0

[a] Andean (A) and Mesoamerican (MA) gene pools. [b] Mean scores of disease severity based on a 1-to-9 scale, where resistance reaction ≤ 3.5 (1 = no symptoms and 9 = dead plants); all plots were inoculated with a mix of the *C. lindemuthianum* races 65 (isolate Cl_1614), 73 (isolate Cl_1143), 81 (isolate Cl_1164), 91 (isolate Cl_1247), 475 (isolate Cl_1322), and 1609 (isolate Cl_1294). [c] Presence (1) or absence (0) of SCAR marker.
[d] Susceptible control variety.

According to Kelly et al. (2003), the greater the number of anthracnose resistance alleles present in the same common bean genotype, the greater will be the durability and the efficiency of this resistance. Souza et al. (2014) reported that cultivars or elite lines harboring the alleles *Co-4*^{2}, *Co-5* and *Co-6*, individually or in combination (pyramiding), are those with the highest resistance spectrum to anthracnose in Brazil.

Phenotypic and molecular characterization of candidate resistance sources to common bean anthracnose based on their disease reaction in field inoculation trials and on analysis with molecular markers linked to resistance genes has shown to be a useful strategy. It supports the selection of donor parents and resistant lines to be preferably explored by common bean breeding programs in Brazil.

As previously suggested by Souza et al. (2014), the use of the resistant alleles from Mesoamerican gene pool *Co-3*^{4} (Ouro Negro), *Co-4*^{2} (SEL 1308 and K13), *Co-5* (TU and K23), and *Co-6* (AB 136), in combination, as in K10 (*Co-3*^{4}, *Co-4*^{2}, *Co-5* and *Co-6*), but preferably with alleles from the Andean gene pool, such as *Co-1*, *Co-13* and others not characterized yet, such as that present in BRSMG Realce (*Co-?*), should be prioritized by the common bean breeding programs aiming to develop cultivars and elite lines with high

spectrum and durable resistance to anthracnose. Four of the six SCAR markers tested in the present work (SH18 and SAS13 for *Co-4*^{2}, SAB03 for *Co-5*, and SAZ20 for *Co-6*), have shown to be useful for assisted selection of the target resistant genes. This is because these markers were specific for the loci to which they are linked, although they did not discriminate alleles from the same locus, except for SH18, which was shown to be specifically linked to *Co-4*^{2}.

ACKNOWLEDGMENTS

The authors are thankful to the *Universidade Federal de Minas Gerais* (UFMG, Montes Claros, MG, Brazil), *Universidade Federal de Goiás* (UFG, Goiânia, GO, Brazil) and *Embrapa Arroz e Feijão* (Santo Antônio de Goiás, GO, Brazil) for the opportunity to develop this research, in addition to their technical and logistic support. This work was financially supported by Embrapa (Brazilian Agricultural Research Corporation – Grant No. 02.14.01.003.00) and by CNPq (Brazilian Council for Scientific and Technological Development – Grant No. 474534/2013-8). L.C. Melo, H.S. Pereira and T.L.P.O. Souza were supported by CNPq.

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