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Mapping of an andean gene for anthracnose resistance (*Co-13*) in common bean (*Phaseolus vulgaris* L.) Jalo Listras Pretas landrace

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Abstract

The common bean (*Phaseolus vulgaris* L.) andean Jalo Listras Pretas (JLP) landrace is an important source of resistance to anthracnose, which is a widespread disease caused by *Colletotrichum lindemuthianum* fungus. This common bean landrace carries *Co-13* gene, one of the nine ones identified andean anthracnose resistance genes. The present study set out to identify molecular markers associated with *Co-13* by evaluating the segregation patterns of 65 molecular markers, in a F_2 population derived from a cross between JLP (resistant to race 73 of *C. lindemuthianum*) and Cornell 49-242 (susceptible to race 73 of *C. lindemuthianum*) cultivars. Bulked segregant analysis was performed to identify which of these molecular markers was linked to *Co-13* in JLP. Among the analyzed molecular markers, $OV20_{680}$ marker displayed a heteromorphic band of 680 bp linked to *Co-13* resistant gene in coupling phase. Genotyping of the $OV20_{680}$ marker in F_2 population revealed a recombination of 1.7%, indicating a tight linkage between the $OV20_{680}$ marker and disease resistance gene (*Co-13*). In the recombinant inbred population BAT93/Jalo EEP558, $OV20_{680}$ segregated according to a 1:1 ratio and mapped on linkage group Pv03 at a distance of 1.8 cM from the *Co-13* locus. Jalo Listras Pretas has shown to be an important source of resistance to anthracnose, possessing a new gene that should be valuable in breeding for anthracnose resistance in common bean. Identifying molecular markers linked to andean resistance genes may facilitate the development of cultivars with broad-based resistance to anthracnose by streamlining the process of combining andean and mesoamerican resistance genes.

Keywords: *Colletotrichum lindemuthianum;* genetic mapping; molecular markers; *Phaseolus vulgaris* L.
Abbreviations: BSA_bulked segregant analysis; CTAB_Cetyl trimethylammonium bromide; RAPD_Random Amplified Polymorphic DNA; SSR_Simple Sequence Repeat; STS_Sequence Tagged Sites.

Introduction

Anthracnose, caused by *Colletotrichum lindemuthianum* fungus, is one of the most economically important diseases affecting bean crops, as it results in both crop yield reductions and depreciation of the product (Chaves, 1980). Anthracnose principally occurs in regions with moderate temperatures ranging from 18 to 24°C and high relative humidity. One of the most efficient and economic options for controlling this disease is the use of naturally resistant cultivars. Therefore, new germplasm sources and resistance genes are routinely characterized (Mahuku and Riascos, 2004). The introgression of such resistance genes into improved cultivars can be facilitated by the application of marker-assisted selection (Kelly and Vallejo, 2004). To date, a total of 19 genes and four allelic series related to anthracnose resistance have been characterized in the common bean, which are designated by the abbreviation *Co.* These genes and their allelic series are as follows: *Co-1* (McRostie, 1919), *Co-1²* (Melotto and Kelly, 2000), *Co-1³* (Melotto and Kelly, 2000), *Co-1⁴*, *Co-1⁴*/*Phg-1* (Alzate-Marin et al., 2003; Gonçalves-Vidigal et al., 2011), *Co-1⁵* (Gonçalves-Vidigal and Kelly, 2006), *Co-2* (Mastenbroek, 1960), *Co-3* (Bannerot, 1965), *Co-3²* (Fouilloux, 1976, 1979), *Co-3³* (Geffroy et al., 1999; Méndez-Vigo et al., 2005; Alzate-Marin et al., 2007; Rodrígues-Suárez et al., 2008; David et al., 2008, 2009; Geffroy et al., 2009; Campa et al., 2011), *Co-3⁴* (Gonçalves-Vidigal et al., 2013), *Co-3⁵* (Pastor-Corrales, et al., 1994; Young et al., 1998; Sousa et al., 2014), *Co-4* (Fouilloux, 1976, 1979), *Co-4²* (Young et al., 1998), *Co-4³* (Alzate-Marin et al., 2007), *Co-5* (Young and Kelly, 1996; Young et al., 1998; Alzate-Marin et al., 2007), *Co-5²* (Vallejo and Kelly, 2009), *Co-6* (Schwartz et al., 1982; Gonçalves-Vidigal, 1994; Kelly and Young, 1996; Young and Kelly, 1996), *co-8* (Alzate-Marin et al., 1997), *Co-11* (Gonçalves-Vidigal et al., 2007), *Co-12* (Gonçalves-Vidigal et al., 2008), *Co-13* (Gonçalves-Vidigal et al., 2009), *Co-14* (Gonçalves-Vidigal et al., 2012), *Co-15* (Gonçalves et al., 2010), *Co-16* (Coelho et al., 2013), *Co-u* (Geffroy, 1997; Geffroy et al., 2008), *Co-v* (Geffroy, 1997), *Co-w* and *Co- x* (Geffroy, 1997; Geffroy et al., 2008), *Co-y* and *Co-z* (Geffroy et al., 1999). On the common bean map, most of these genes are allocated in seven of 11 linkage groups (Pv01, Pv02, Pv03, Pv04, Pv07, Pv08 and Pv11) referred to as Pv groups (Ferreira et al., 2013). Among all andean anthracnose resistance genes (*Co-1*, *Co-12*, *Co-13*, *Co-14*, *Co-15*, *Co-w*, *Co-x*, *Co-y* and *Co-z*), associated molecular markers have only been found for the *Co-1* and *Co-15* loci. In the differential cultivar Michigan Dark Red Kidney, the dominant marker RAPD $OF10_{530}$ is linked in repulsion phase to the *Co-1* gene at a distance of 1.9 cM (Young and Kelly, 1997). In the differential cultivar Widusa, the $OA18_{1500}$ marker is linked in repulsion phase to the *Co-1⁵* allele at a distance of 1.2 cM (Gonçalves-Vidigal and Kelly 2006).

Three AFLP markers (ECAG/MACC-1, EACA/MAGA-2 and EAGG/MAAC-8) are linked in repulsion phase to the *Co-1* gene in the A193 line (Mendoza et al., 2001). The andean *Co-1* locus was mapped (Kelly et al., 2003) to the Pv01 linkage group (Freyre et al., 1998) using the SE_{act}/M_{cca} marker in the Jalo EEP558/BAT 93 recombinant inbred lines. Sousa et al. (2013) identified the g2685 marker, linked at a distance of 5.6 cM to the *Co-15* gene in Pv04 linkage group from the Corinthiano cultivar. The andean landrace Jalo Listras Pretas (JLP) potentially represents a significant source of anthracnose resistance for common bean breeding programs. In studies performed by Vidigal Filho et al. (2007), this landrace was found to be resistant to eight of the 12 physiological races of *C. lindemuthianum* tested (races 9, 31, 65, 69, 73, 81, 89 and 95). The gene characterized in this cultivar was designated *Co-13* (Gonçalves-Vidigal et al., 2009). However, only the *Co-1* gene from mesoamerican beans has been used to broaden the resistance spectrum of common bean thus far. Therefore, studying andean landraces may prove important for discovering novel sources of resistance. Cultivars of andean origin are more resistant to mesoamerican races of *C. lindemuthianum*, and mesoamerican cultivars are more resistant to andean races of *C. lindemuthianum.* It has been suggested that incorporation of resistance genes from different groups, such as andean and mesoamerican beans, may lead to stable anthracnose resistance (Pastor-Corrales and Tu, 1989; Kelly et al., 1994; Pastor-Corrales et al., 1995). The identification of anthracnose resistance genes has been made easier by the development of associated molecular markers (Adam-Blondon et al., 1994; Freyre et al., 1998; Alzate-Marin et al., 1999; Faleiro et al., 2004; Kelly and Vallejo, 2004). Identifying such markers in both andean and mesoamerican cultivars will directly facilitate the characterization and maintenance of genetic resources available for crop improvement programs. The objective of the present study was to identify molecular markers linked to anthracnose resistance locus *Co-13*, found in andean cultivar JLP.

Results

Genetic resistance

A total of 116 F_2 plants derived from Jalo Listras Pretas × Cornell 49-242 cross and 20 plants from each one of the parental and F_1 generation were inoculated with race 73 of *C. lindemuthianum*. Nine days after inoculation, the segregation of the resistance phenotype was observed in the F_2 population, with 87 plants being found to be resistant and 29 susceptible to *C. lindemuthianum* ($\chi^2 = 0.00$; $P = 1.0$, Table 1). The 56 $F_{2:3}$ families segregated into the following classes: 14RR:26Rr:16rr ($\chi^2 = 0.43$; $P = 0.81$). This segregation pattern fits a model of 3R:1S (F_2) and 1RR:2RS:1SS ($F_{2:3}$), suggesting that anthracnose resistance is controlled by a monogenic inheritance, dominant locus. Therefore, resistance to race 73 of *C. lindemuthianum* in andean common bean cultivar Jalo Listras Pretas is conferred by the *Co-13* gene.

Molecular analyses

In this study, a total of 65 molecular markers distributed across all 11 genetic linkage groups were genotyped in the parents and contrasting bulks (BSA). The bulked segregant analysis showed that among the 40 RAPD markers analyzed, 16 were polymorphic between resistant and susceptible parents. Eight RAPD markers were polymorphic between resistant and susceptible bulks, all of which displayed an amplified DNA fragment in the resistant bulked DNA that was absent in susceptible bulked DNA. These polymorphic markers were regarded as candidate markers linked to *Co-13* gene. Further analysis showed that only marker $OV20_{680}$ (5'-CAGCATGGTC-3') was potentially linked to *Co-13* gene, when it was genotyped in F_2 population from Jalo Listras Pretas × Cornell 49-242 cross. An amplification product of 680 bp was present in the resistant parent and was therefore in coupling phase with the resistance gene. This product was also present in all individuals in resistant bulk but was absent from susceptible parent and all individuals in susceptible bulk (Fig 1). Segregation analysis of F_2 population (116 individuals) revealed a segregation pattern of 89(+):27(-) ($\chi^2 = 0.18$; $P = 0.67$). Genotyping of marker $OV20_{680}$ in F_2 population revealed only two recombinant individuals, corresponding to a recombination frequency of 1.72%. Segregation analysis of the $OV20_{680}$ marker in the BAT 93/Jalo EEP558 recombinant inbred population (Freyre et al., 1998) revealed a segregation ratio of 37(+):34(-) ($\chi^2 = 0.13$; $p = 0.72$ for the goodness-of-fit to a 1:1 ratio, Table 2). This result demonstrates that marker $OV20_{680}$ is linked to the resistance gene *Co-13* at a distance of 1.8 cM and that it mapped to the Pv03 linkage group of the consensus map of the Jalo EEP558/BAT 93 common bean cultivar (Freyre et al., 1998). Of the 18 microsatellite markers tested, only two (BMd-12 and BMd-42) were polymorphic among the parents and the contrasting DNA bulks. The microsatellite marker BMd-12 is located in linkage group Pv06 (Blair et al., 2003), and BMd42 marker is in linkage group Pv10 (Blair et al., 2003; 2006). However, genotyping of these two markers in F_2 population from Jalo Listras Pretas × Cornell 49-242 cross showed that these markers are not linked to *Co-13* locus. Additionally, STS markers, which are mapped on Pv03 linkage group, did not show polymorphism among the parents and bulks segregant.

Discussion

The andean *Co-13* gene for anthracnose resistance was identified by analyzing the segregation patterns occurring in a F_2 population obtained from crosses of the resistant andean bean cultivar Jalo Listras Pretas with various susceptible cultivars, including Cornell 49-242 (Gonçalves-Vidigal et al., 2009). To determine the inheritance mode of anthracnose resistance, JLP was crossed with the Cornell 49-242 cultivar, and 365 F_2 plants were inoculated with race 73 of *C. lindemuthianum*. Among these 365 plants, 275 were resistant, and 90 were susceptible. The corresponding Chi-square value for a 3:1 ratio was $\chi^2 = 0.023$ ($p = 0.88$), suggesting that the resistance is determined by a single, dominant gene at *Co-13* locus. In this study, it was applied a combination of bulked segregant analysis (BSA) (Michelmore et al., 1991) using RAPD, SSR and STS markers to identify markers linked to *Co-13* gene. BSA has proven to be a useful tool for identifying a large number of markers linked to important agronomic traits in several plant species (Paran and Michelmore, 1993; Arnedo-Andres et al., 2002; Oumouloud et al., 2008; Fondevilla et al., 2008; Gonçalves-Vidigal et al., 2011; Gonçalves-Vidigal et al., 2013). Among the 65 markers tested, RAPD $OV20_{680}$ was identified in F_2 individuals from the cross between JLP × Cornell 49-242, inoculated with race 73. The molecular analysis using the $OV20_{680}$ marker was conducted in 116 individuals F_2, revealed the existence of two recombinant, emphasizing that there is a close association between the $OV20_{680}$ marker and the *Co-13* resistance gene in Jalo Listras Pretas (Table 2). The linkage analysis showed that $OV20_{680}$ segregated in a 3:1 (P = 0.67)

Table 1. Segregation for resistance to race 73 of *Colletotrichum lindemuthianum* in common bean plants from the F_2 generation obtained from the Jalo Listras Pretas × Cornell 49-242 cross.

Parental cross	Generation	Observed ratio	Expected ratio	χ^2	*P* value
Jalo Listras Pretas (JLP)	RP	20R:0S			
Cornell 49-242	SP	0R:20S			
JLP × Cornell 49-242	F_1	20R:0S			
JLP × Cornell 49-242	F_2	87R:29S	87R:29S	0.00	1.0
JLP × Cornell 49-242	$F_{2:3}$[a]	14RR:26RS:16SS	14RR:28RS:14SS	0.43	0.81

R = resistant, *S* = susceptible, *RP* = resistant parent, *SP* = susceptible parent
[a]$F_{2:3}$ families were classified as showing all resistant individuals (RR), both resistant and susceptible individuals (RS) or all susceptible individuals (SS).

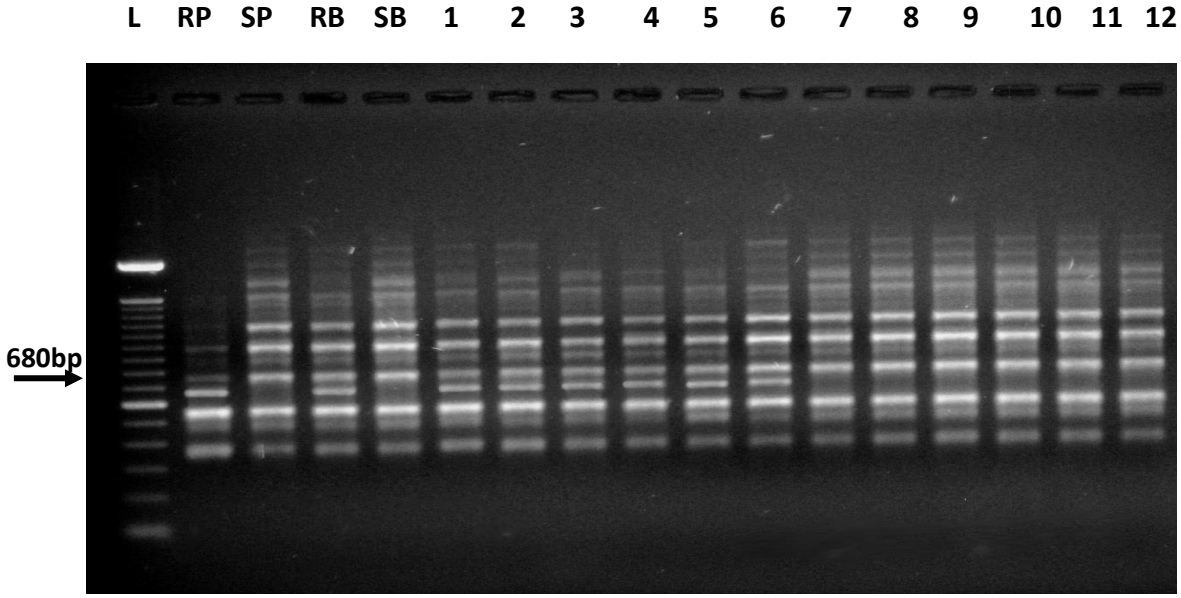

Fig 1. Electrophoretic analysis of the amplification products obtained for the $OV20_{680}$ marker. Lanes: L, 100 bp ladder; RP, Jalo Listras Pretas; SP, Cornell 49-242; RB, resistant bulk; SB, susceptible bulk; 1-6, individuals resistant to *C. lindemuthianum*; 7-12, individuals susceptible to *C. lindemuthianum*. The arrow indicates the 680 bp DNA band linked to the *Co-13* resistance gene.

ratio and was linked at a distance of 1.8 cM from the *Co-13* locus. The marker $OV20_{680}$ has previously been mapped to linkage group Pv03 (Kelly and Vallejo, 2004), confirming that the resistance gene found in JLP is located in that linkage group (Fig 2). The updated genetic mapping analysis performed for the common bean suggests that a possible cluster of genes conferring resistance to anthracnose (ANT_{BJ}), common bacterial blight (CBB_{S95}) and bacterial brown spot (BBS_{BA}) is located in this linkage group (Gepts et al., 1993; Ariyarathne et al., 1999; Tar'an et al., 2001; Kelly et al., 2003). Studies have demonstrated the existence of several genomic regions containing genes conferring resistance to common bean. These studies have revealed the presence of families of analogous genes (RGAs) and/or resistance genes organized in cluster (Geffroy et al., 1998; Creusot et al., 1999; Geffroy et al., 1999; Melotto and Kelly, 2001; Melotto et al., 2004; Ferrier-Cana et al., 2003, 2005). Gepts (1999) and Kelly et al. (2003) reported the map locations of major resistance genes related to bean rust, anthracnose, common bacterial blight and white mold in gene clusters on Pv01, Pv04, Pv07 and Pv11 chromosomes in the common bean. The linkage group shown in Fig 2 corresponds to the version of core map reported by Freyre et al. (1998) based on original map (Nodari et al., 1993) and resembles maps previously presented by Kelly et al. (2003), Kelly and Vallejo (2004) and Blair et al. (2003, 2006). The identification of genes clusters involved in resistance to different pathotypes could aid in marker-assisted selection. Pyramiding of monogenic resistance genes in common bean breeding programs has been proposed as an effective and economic strategy for developing cultivars that show a stable, broad resistance spectrum to anthracnose (Park et al., 2003). *Co-13* gene is an ideal candidate to be included in such breeding programs. The OV20 marker was found linked to the above-mentioned genes clusters (*Co-13/BBS_{BA}/CBB_{S95}/ANT_{BJ}*) and it should be recommended to breeding programs. The OV20 marker, in particular, will reduce the time and cost of pyramiding the *Co-13* gene into commercial common bean cultivars. Similarly, mapping analyses conducted by Gonçalves-Vidigal et al. (2013) demonstrated the efficiency the only molecular marker, g2303, was effective to found markers linked gene clusters (*Co-3/Co-10/Phg-ON*). The common bean cultivar JLP represents an important source of anthracnose resistance because it possesses andean *Co-13* gene, which confers resistance to the predominant *C. lindemuthianum* race occurring in Brazil. The $OV20_{680}$ molecular marker provides a valuable tool for common bean breeding programs aimed at generating cultivars with a more durable resistance spectrum using a combination of andean and mesoamerican resistance genes.

Materials and Methods

Plant material

The experiments were conducted in the greenhouse of the Laboratório de Melhoramento do Feijão Comum e de

Table 2. Reaction of F_2 plants from the Jalo Listras Pretas × Cornell 49-242 cross inoculated with race 73 of *Colletotrichum lindemuthianum* and the presence (+) or absence (-)[c] of the OV 20 molecular marker.

Generation	Observed ratio			Expected ratio			χ^2	*P* value	Linkage distance (cM)[b]
	RR[a]	RS	SS	RR	RS	SS			*Co-13*/OV20
F_2	87	0	29	87	0	29	0.00	1.00	
OV20	89(+)	0	27(-)	87	0	29	0.18	0.67	1.8

[a] R = resistant plants; S = susceptible plants [b] The linkage distance between the *Co-13*/OV20 marker indicates two recombinants were observed for the reaction to pathogens.[c] Marker present (+); absent (-).

Biologia Molecular of Núcleo de Pesquisa Aplicada à Agricultura (Nupagri) at the Universidade Estadual de Maringá, Paraná, Brazil, using a F_2 population of 116 individuals derived from a cross between Jalo Listras Pretas (resistant) × Cornell 49-242 (susceptible). The andean cultivar Jalo Listras Pretas presents large seeds that are cream colored with black stripes. This cultivar exhibits a band corresponding to "T" phaseolin that is characteristic of beans of andean origin (Gonçalves-Vidigal et al., 2009) and is resistant to race 73 of *C. lindemuthianum.* Cornell 49-242 is one of the 12 differential cultivars of mesoamerican origin. This cultivar displays small black seeds and is susceptible to race 73 of *C. lindemuthianum*. The $F_{2:3}$ families (obtained by selfing F_2 plants) were used to identify F_2 plants that were homozygous resistant for use in bulked segregant analysis (BSA; Michelmore et al. 1991). A total of 56 families (approximately 15 plants per family) were sown in plastic trays and inoculated with the same race of the pathogen used in F_2 population.

Inoculation and disease evaluation

Race 73 of *Colletotrichum lindemuthianum* was obtained from the mycology collection of the Núcleo de Pesquisa Aplicada à Agricultura at the Universidade Estadual de Maringá. This physiological race was cultured in Petri dishes containing Mathur's medium (Mathur et al., 1950). The identification of race 73 was confirmed by inoculating a set of 12 common bean differential cultivars used to characterize the virulence spectra of anthracnose (Pastor-Corrales 1991). The initial inoculum of race 73 of *C. lindemuthianum* was obtained from monosporic cultures prepared in young green common bean pod medium, transferred to test tubes containing 5 mL of water agar and kept upright at 22ºC for 14 days (Cárdenas et al., 1964). After confirmation of the race, seedlings with fully developed first trifoliate leaves from the parents and F_1, F_2 and $F_{2:3}$ families were inoculated with a *C. lindemuthianum* spore suspension. The spore suspension containing 1.2×10^6 spores mL^{-1} was spray-inoculated onto seedlings by using a De Vilbiss number 15 atomizer powered by an electric air compressor (Schulz, SA, Joinville, Santa Catarina, Brazil). Following inoculation, the plants were placed in a mist chamber for 72 h at 20 ± 2ºC under a light cycle of 12 h of daylight and 12 h of darkness (light intensity of 300 µmol m^{-2} s^{-1} at 1 m height) and relative humidity over 95%. After incubation period, the inoculated plants were transferred to open-air benches and maintained at 22ºC under artificial light for seven days before visual assessment of symptoms was conducted. Anthracnose disease reactions were rated visually using a scale of 1-9 (Pastor-Corrales, 1991). Plants exhibiting disease reaction scores between 1 and 3 were considered resistant, whereas plants showing scores of 4 to 9 were considered susceptible.

DNA extraction

DNA was extracted from the central leaflet of the first trifoliate leaf of 116 F_2 individuals from resistant (Jalo Listras Pretas) and susceptible parental (Cornell 49-242) genotypes.

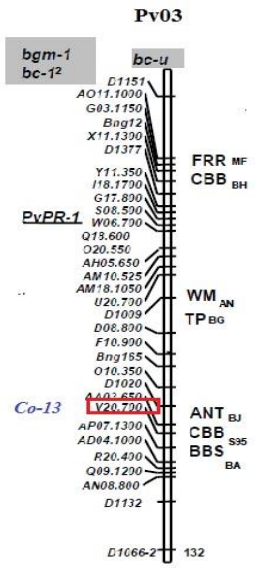

Fig 2. Location of the Co-13 gene for resistance to common bean anthracnose, and the OV20 molecular marker on linkage group Pv03 of Phaseolus vulgaris L. using the population from the Jalo Listras Pretas × Cornell 49-242 cross. The map was obtained of the http://bic.css.msu.edu/ pdf/Bean Core map 2009.pdf.

DNA extraction was performed according to the method described by Afanador et al. (1993), with the following modification: DNA was extracted from the central leaflet of the first trifoliate leaf utilizing 400 µL of CTAB extraction buffer. The extracted DNA was employed as a template for amplification of the molecular markers.

Molecular marker analyses

A total of 65 molecular markers, including 40 RAPD, 18 SSR and 7 STS markers, distributed across all 11 genetic linkage groups found in the common bean (Yu et al., 2000; Gaitán-Solís et al., 2002; Blair et al., 2003; McClean et al., 2010; McConnell et al., 2010) were tested in the parents and in resistant and susceptible bulks. Two contrasting bulk DNA samples were constructed by pooling equal volumes of fluorometrically standardized DNA from six homozygous resistant (RB) and six homozygous susceptible plants (SB); the plants were selected on the basis of phenotypic data on the reaction to the anthracnose pathotype in the Jalo Listras Pretas × Cornell 49-242 $F_{2:3}$ generation (Michelmore et al., 1991). All amplification reactions were performed with a thermal cycler (MJ Research Inc., Waltham, MA). The molecular markers RAPD were PCR-amplified by polymerase chain reaction (PCR) in a total volume of 25 µL containing 50 ng of total DNA, 1.25 mM each dNTP, 3.0 mM $MgCl_2$, 10 mM Tris-HCl pH 8.3, 50 mM KCl buffer, 0.4 µM primers (Operon Technologies) and 1 unit of *Taq* DNA polymerase (Invitrogen). PCR cycling program consisted 40

cycles of 15 s at 94ºC, 30 s at 35ºC, and 60 s at 72ºC, followed by a 5 min extension at 72ºC and 4 min at 4ºC (Williams et al., 1990). Following the addition of 2 µL loading buffer (30% glycerol and 0.25% bromophenol blue), the PCR products for $OV20_{680}$ were analyzed on 1.2% agarose gels stained with ethidium bromide (0.02%). PCR products were visualized under ultraviolet light (Spectroline photosystem), and images were captured with a digital camera (Canon Power Shot A620). The 100bp DNA Ladder was used to establish fragment size. All SSR and STS amplification reactions were also performed using a thermal cycler (MJ Research Inc., Waltham, MA). PCR cycling conditions consisted of 3 min at 95ºC, followed by 35 cycles of 30 s at 92ºC, 1 min at 50ºC and 60 s at 72ºC, with a final 5 min extension step at 72ºC and then cooling at 4ºC for 4 min. PCR amplification was performed in a total reaction volume of 20 µL containing 30 ng of total DNA, 0.2 mM each dNTP, 1.5 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.3, 50 mM KCl buffer, 0.2 µM forward and reverse primers (Operon Technologies) and 1 unit of *Taq* DNA polymerase (Invitrogen). Following the addition of 2 µL of loading buffer (30% glycerol and 0.25% bromophenol blue), PCR products were analyzed in 10% polyacrylamide gels stained with SYBR safe (0.02%). Obtained DNA bands were visualized under ultraviolet light, and digital images were recorded by using an L-PIX Image EX model (Loccus Biotecnologia-Loccus do Brasil, Cotia, SP, Brasil).

Molecular mapping and Statistical analyses

To map the *Co-13* gene onto the common bean consensus map, both the F_2 generation arising from the Jalo Listras Pretas × Cornell 49-242 cross and a population of recombinant inbred lines (RILs) consisting of 71 lines derived from a cross between BAT93/Jalo EEP558 (Freyre et al., 1998) were used. The primer sequence used for the OV20 marker was 5'-CAGCATGGTC-3'. The segregation of RAPD markers and resistance to anthracnose in the F_2 population were analyzed using a Chi-square test (χ^2). Estimates of recombination frequencies and the genetic distance between markers and the *Co-13* resistance gene were calculated using Mapmaker/EXP 3.0 (Lincoln and Lander et al., 1993). The distance between the locus and the marker was calculated using Kosambi's mapping function (Kosambi, 1944). The applied linkage group nomenclature follows Pedrosa-Harand et al. (2008).

Conclusion

In conclusion, the results obtained in this work demonstrated that the $OV20_{680}$ marker, previously been mapped to linkage group Pv03, is linked to *Co-13* resistance gene present in the Jalo Listras Pretas cultivar. The linkage between this marker and the gene will be of great importance for marker assisted introgression of this gene into commercial cultivars and elite lines, which would broaden the spectrum of resistance of bean cultivars. Jalo Listras Pretas has shown to be an important source of resistance to anthracnose, possessing a new gene that should be valuable in breeding for anthracnose resistance in common bean. In addition, genetic mapping analysis suggests that this marker is linked to a cluster of genes conferring resistance to diverse pathogens and it should be recommended to breeding programs.

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