Crop Breeding and Applied Biotechnology 7:24-28, 2007
Brazilian Society of Plant Breeding. Printed in Brazil

Association between RAPD marker $OPAS13_{950C}$ and anthracnose resistance allele *Co-4*^{3} of common bean cultivar PI 207262

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Received 01 December 2005

Accepted 08 June 2006

ABSTRACT - *Cultivar PI 207262 is an important source of resistance to common bean anthracnose caused by the fungus* Colletotrichum lindemuthianum. *Previous studies identified the presence of anthracnose resistance genes* ***Co-4***^{3} *and* ***Co-9*** *in PI 207262. Markers* $OPY20_{830C}$ *and* $OPAS13_{950C}$ *have been identified as tightly linked to* ***Co-4*** *gene of cultivar TO and Co-4*^{2} *allele of cultivar G 2333 and Selection 1308, respectively. Potentially these markers could be helpful in the selection of plants harboring the* ***Co-4***^{3} *allele derived from PI 207262. Main goals of the present study were to test markers* $OPY20_{830C}$ *and* $OPAS13_{950C}$ *to identify the most promising molecular marker linked to the* ***Co-4***^{3} *allele and determine the genetic distance between each marker and the allele. We observed that the RAPD molecular marker* $OPAS13_{950C}$ *is linked at 3.5 cM from* ***Co-4***^{3}*. This is the first study that reports a molecular marker associated to* ***Co-4***^{3} *anthracnose resistance allele of the common bean cultivar PI 207262.*

Key words: *Phaseolus vulgaris*, resistance genes, *Colletotrichum lindemuthianum*, MAS, anthracnose differential cultivars.

INTRODUCTION

Anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. and Magnus) Lams.-Scrib. slashes the common bean production in Brazil and world-wide (Pastor-Corrales 1985, Rava et al. 1993, Rava et al. 1994). New resistance sources must continuously be sought in view of the high variability in the pathogen populations and the occurrence of newly evolved virulent pathotypes (Balardin and Kelly 1998, Melotto and Kelly 2000). Different anthracnose resistance genes have been identified in differential cultivars (Pastor-Corrales 1992) and new gene symbols were adopted (Bassett 2004).

The Mesoamerican common bean cultivar PI 207262 (also known as Tlalnepantla 64, Mexico 56 or G 1320) is an important anthracnose resistance source for common bean

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and one of the differential cultivars used to characterize the virulence of *C. lindemuthianum* pathotypes. This cultivar has cream-beige seeds and is resistant to 45 Brazilian *C. lindemuthianum* pathotypes (Alzate-Marin and Sartorato 2004). PI 207262 has frequently been used as parent in the development of many Brazilian elite cultivars (Alzate-Marin et al. 2003a), including the traditional cultivar EMGOPA 201-Ouro (Silva et al. 2003).

A previous study conducted by our research group showed that PI 207262 has two anthracnose resistance genes, *Co-4*^{3} and *Co-9* (Alzate-Marin et al. 2001a, Alzate-Marin et al. 2003b, Bassett 2004). *Co-4* gene is present in cultivar TO and cultivar G2333 and Selection 1308 (derived from G 2333) owns the *Co-4*^{2} allele. The molecular markers OPY20$_{830C}$ and OPAS13$_{950C}$ were identified as linked at 0.0 cM from *Co-4* gene of TO and *Co-4*^{2} allele of Selection 1308 (Arruda et al. 2000, Young et al. 1998). In a population derived from cultivar G2333, OPAS13$_{950C}$ was located at 5.6 cM from *Co-4*^{2} allele (Alzate-Marin et al. 2001b). These markers are potential candidates to back the selection of plants harboring the *Co-4*^{3} allele of PI 207262. The availability of a molecular marker tightly linked to *Co-4*^{3} allele can save a considerable amount of time, avoid laborious inoculation procedures in breeding programs and make the process of pyramiding resistance genes easier.

The main goals of the present study were: 1) to identify the most promising RAPD molecular marker between OPY20$_{830C}$ and OPAS13$_{950C}$ of the cross Rudá and PI 207262 in bulk segregant analysis, and 2) to determine the genetic distance between the selected RAPD molecular marker and the *Co-4*^{3} allele in $F_{2:3}$ families, derived from the cross between Rudá and PI 207262, segregating for only one anthracnose resistance gene.

MATERIAL AND METHODS

Common bean plant material

Seeds from cultivars PI 207262 and Rudá were provided by EMBRAPA (Goiânia, GO, Brazil) and CIAT (Tropical Agriculture International Center, Cali, Colombia), respectively. Cultivar Rudá parent, is a so-called "carioca-type" Mesoamerican bean and strongly recommended for several geographical regions in Brazil in spite of its susceptibility to most *Colletotrichum lindemuthianum* pathotypes. Crosses between cultivars Rudá and the resistant cultivar PI 207262 were performed in a greenhouse and plants of $F_{2:3}$ populations were used.

Source of *C. lindemuthianum* isolates and management procedures

Pathotype 65 used in this study is part of a group of 25 pathotypes collected in different regions of Brazil and identified by Rava et al. (1994). The original pathotype 65 was provided by Dr. Carlos A. Rava and Dr. Aloisio Sartorato (Rice & Bean Research Center - EMBRAPA, Goiânia, GO, Brazil). Inoculum was prepared by culturing the pathotype for approximately 10 days in sterile medium containing young green pods of common bean (Pio Ribeiro and Chaves 1975). The identity of pathotype 65 was confirmed by inoculation onto the 12 common bean anthracnose differential cultivars (Pastor-Corrales 1992).

Inoculation conditions

Spores (1.2 x 10^6 conidia/ml) were sprayed onto the plants with the aid of a De Vilbiss apparatus. The plants were incubated and maintained in a mist chamber (20-22 ^oC, 100% relative humidity) for seven days. After this period, each plant was scored visually for disease symptoms on a 1 to 9 scale (Rava et al. 1993). Resistant (R) phenotype was assigned to plants with no or limited symptoms (grades 1 to 3) whereas plants graded 4 or higher were considered to be susceptible (S).

Selection, validation and amplification conditions of RAPD markers

The Bulk Segregant Analysis technique (Michelmore et al. 1991) was used to select the promising RAPD markers linked to *Co-4*^{3}, an anthracnose resistance gene. Two contrasting DNA bulks of segregating populations for two resistance genes to *C. lindemuthianum* pathotype 65 were prepared. The DNA was obtained from frozen primary leaves of F_2 plants, previously collected and stored at -80 ^OC, derived from the cross of Rudá and PI 207262 (Alzate-Marin et al. 2002). For DNA amplification, the primers OPY20 and OPAS13 (OPERON technologies Inc., Alameda, CA, USA) were used.

The RAPD marker selected by bulked segregant analysis was amplified using DNA of 100 F_2 plants and used to identify the plants that possibly carried a *Co-4* allele. An average of 25 plants derived from each of 10 $F_{2:3}$ families (total of 250) that amplified the selected marker were inoculated with *C. lindemuthianum* pathotype 65, under similar conditions as described above. $F_{2:3}$ families

segregating 3:1 (resistant/susceptible) to *C. lindemuthianum* pathotype 65 were identified. Before inoculation, a healthy primary leaf of the parents and $F_{2:3}$ plants was collected and frozen at -80 °C for future DNA extraction. Populations segregating for only one gene were used to validate the RAPD markers and determine the linkage between the RAPD markers and the resistance locus.

DNA extraction was performed according to Doyle and Doyle (1990). Amplification reactions were conducted in a thermocycler model 9600 (Perkin-Elmer, Norwalk, CT, USA) according to Williams et al. (1990), following the procedures of Alzate-Marin et al. (2001b).

Linkage analyses

Chi-square analyses were used to test the phenotypic segregation of the $F_{2:3}$ populations of the cross Rudá and PI 207262 inoculated with *C. lindemuthianum* race 65. Sixty-seven $F_{2:3}$ plants were used to determine the genetic distance between the RAPD marker and the resistance gene *Co-4*^{3}. The genetic distance between the marker and the resistance gene(s) was determined using the Kosambi function with the aid of Map-maker III (Lander et al. 1987), using a LOD score minimum of 3.0.

RESULTS AND DISCUSSION

The DNA of PI 207262 is amplified by OPY20 and OPAS13 primers (Costa et al. 2001). These primers amplify two molecular markers that have been identified as linked to *Co-4* of cultivar TO ($OPY20_{830C}$) and to *Co-4*^{2} ($OPAS13_{950C}$) of cultivars G2333 and Selection 1308 (Arruda et al. 2000, Alzate-Marin et al. 2001b, Young et al. 1998). DNA amplification of PI 207262 with these molecular markers linked to *Co-4* reinforces the finding that this cultivar carries an allele of this gene. Posterior studies have reported the presence of two independent dominant anthracnose resistance genes in PI 207262, the *Co-4*^{3} and *Co-9* (Alzate-Marin et al. 2001a, Alzate-Marin et al. 2003b).

However, our analyses of $OPY20_{830C}$ and $OPAS13_{950C}$ in contrasting segregating bulks for resistance to *C. lindemuthianum* pathotype 65 showed that only $OPAS13_{950C}$ could be linked to *Co-4*^{3}. Therefore, $OPAS13_{950C}$ was used to identify F_2 plants derived from the cross Rudá and PI 207262 that possibly carried allele *Co-4*^{3}. Two hundred and fifty $F_{2:3}$ plants derived from 10 F_2 plants that amplified marker $OPAS13_{950C}$ were inoculated with *C. lindemuthianum* pathotype 65 aiming to select families segregating for one anthracnose resistance gene only (data not shown). The inoculation results showed that three out of the 10 $F_{2:3}$ families segregated for one gene (segregation ratio of 3:1) (Table 1). DNA from 67 plants of these three families was tested positive for marker $OPAS13_{950C}$, confirming that this marker was indeed linked to the *Co-4*^{3} locus and located at 3.5 cM (Table 1 and Figure 1).

Our data demonstrated that the RAPD $OPAS13_{950C}$ marker, besides *Co-4*^{2} of cultivar Selection 1308, can be used to follow the allele *Co-4*^{3} from PI 207262. This is the first study that reports on a molecular marker associated to the anthracnose resistance allele *Co-4*^{3} of common bean. Since PI 207262 is a Mesoamerican parent commonly used in the development of new common bean cultivars, this molecular marker will be very useful for detecting *Co-4*^{3} in lines that are not derived from cultivars G2333 or Selection 1308. In our breeding program at BIOAGRO/UFV, this marker can be used to identify lines with *Co-4*^{3} derived from the cross Rudá and PI 207262 or to indirectly select lines carrying the second gene (*Co-9*) present in cultivar PI 207262. However when PI 207262 is crossed with another cultivar of another background (Andean), it would be interesting to confirm the genetic distance between the marker and the *Co-4*^{3} allele. Finally, the identification of the $OPAS13_{950C}$ marker linked to anthracnose resistance allele *Co-4*^{3} of PI 207262 should increase the availability of the markers that can be used for marker-assisted selection (MAS) in breeding programs.

Table 1. Linkage analysis between molecular marker $OPAS13_{950C}$ and the resistance allele *Co-4*^{3} to *Colletotrichum lindemuthianum* pathotype 65 present in PI 207262

Cross	Locus tested	Expected ratio[a]	Observed ratio[a]	χ^2	P value	CM[b]
Rudá x PI 207262	*Co-4*^{3}	3:1	55:12	1.796	70.54	-
Rudá x PI 207262	*Co-4*^{3}/$OPAS13_{950C}$	9:3:3:1	53:0:2:12	42.24	0.00	3.5

[a]Three $F_{2:3}$ families were tested
[b]Distance in centimorgans between molecular marker and resistance allele

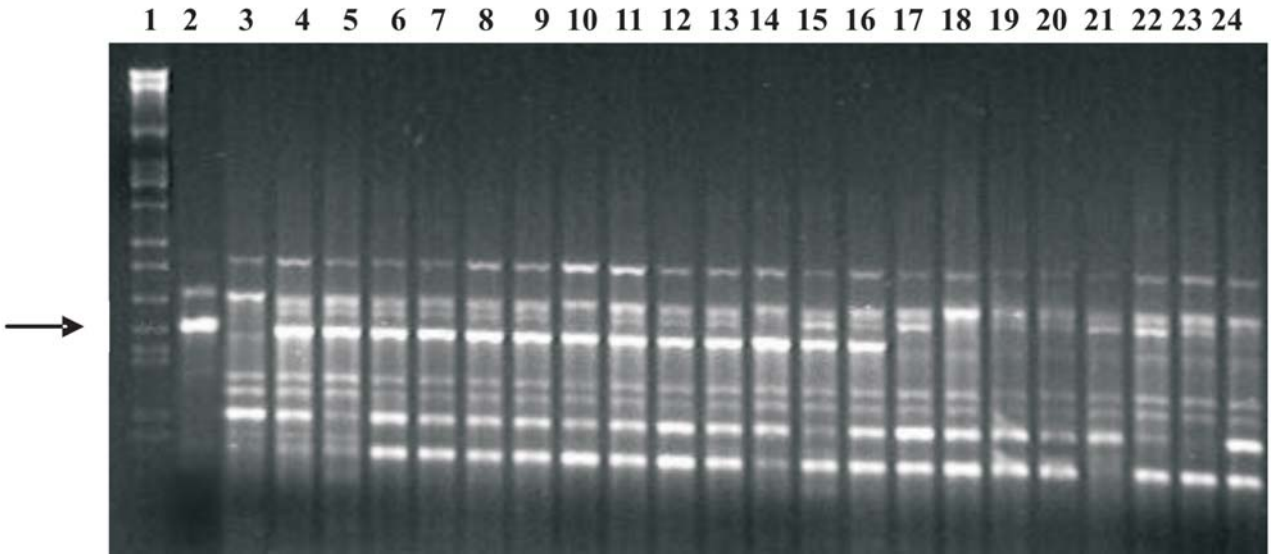

Figure 1. Electrophoretic analysis of amplification products obtained with RAPD primer OPAS13. Lanes are as follows: 1, lambda DNA digested with *Eco*RI, *Bam*HI and *Hin*dIII (size markers); 2, PI 207262; 3, Rudá; 4-16, $F_{2:3}$ plants resistant to *C. lindemuthianum* pathotype 65; 17-24 $F_{2:3}$ plants susceptible to *C. lindemuthianum* pathotype 65. The arrow indicates marker OPAS13$_{950C}$ linked in coupling phase to the *Co-4*^{3} gene

Associação entre o marcador RAPD OPAS13$_{950C}$ e o alelo de resistência a antracnose *Co-4*^{3} do cultivar de feijoeiro PI 207262

RESUMO - *O cultivar de feijoeiro PI 207262, possuidor dos genes de resistência à antracnose* (Colletotrichum lindemuthianum) ***Co-4*^{3}** *e* ***Co-9****, é uma importante fonte de resistência a esta doença. Marcadores RAPD foram previamente identificados como ligados ao gene* ***Co-4*** *do cultivar TO (OPY20$_{830C}$) e ao alelo* ***Co-4*^{2}** *(OPAS13$_{950C}$) dos cultivares G 2333 e Seleção 1308. Tais marcadores são candidatos potenciais para auxiliar na seleção de plantas que possuam o alelo* ***Co-4*^{3}** *derivado de PI 207262. O principal objetivo deste trabalho foi testar os marcadores RAPD OPY20$_{830C}$ e OPAS13$_{950C}$ visando identificar sua ligação ao alelo* ***Co-4*^{3}** *e posteriormente determinar a distância genética entre cada marcador e o alelo. Observou-se que o marcador RAPD OPAS13$_{950C}$ está ligado a 3.5 cM do alelo* ***Co-4*^{3}** *e que o marcador OPY20$_{830C}$ não apresenta ligação com* ***Co-4*^{3}***. Este é o primeiro trabalho que relata um marcador molecular associado ao alelo de resistência à antracnose* ***Co-4*^{3}** *do cultivar do feijoeiro PI 207262.*

Palavras-chave: *Phaseolus vulgaris* L., genes de resistência, *Colletotrichum lindemuthianum*, SAM, Cultivares diferenciadores para antracnose.

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