Validation of a RAPD marker linked to the anthracnose resistant gene *Co-5* in the common bean

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ABSTRACT

Cultivar TU, one of the differential cultivars for anthracnose, carries the resistance gene *Co-5* that expresses resistance to a wide range of pathotypes in Brazil. The main goals of this study were to analyze the inheritance of resistance to anthracnose in the cultivar TU in segregating populations derived from crosses with cultivar Rudá, and to validate the random amplified polymorphic DNA (RAPD) marker $OPB03_{450C}$, previously identified as being linked to resistance gene *Co-5* in cultivar Selection 1360. Results revealed that one single gene present in cultivar TU, *Co-5* is responsible for resistance, and that the band of 450 bp of primer OPB03 was linked to it at 15.4 cM. As $OPB03_{450}$ is not close enough from the *Co-5* gene of TU, it will not be useful during the pyramidation programs. However, this molecular marker can be useful for identification of the *Co-5* allele in other cultivars that carry this gene.

KEY WORDS: *Colletotrichum lindemuthianum*, *Phaseolus vulgaris* L., molecular markers, MAS, cultivar TU.

INTRODUCTION

The common bean (*Phaseolus vulgaris L.*) is the main source of vegetable protein in most Latin American and African countries. The Brazilian population is the world largest consumer, with an annual average intake of 22-23 kg/person (Ventura and Costa, 1992; Borém and Carneiro, 1998). Fungal diseases are one of the main problems affecting common bean productivity, and anthracnose, caused by the fungus *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scrib., is among the main diseases of the common bean in Brazil and in other growing regions of the world (Pastor-Corrales, 1985).

New breeding strategies, such as MAS (marker-aided selection) and pyramidation of resistance genes assisted by molecular markers, have been proposed as alternative solutions for development of varieties with long-lasting resistance (Haley et al., 1993; 1994; Michelmore, 1995; Johnson and Gepts, 1994; Kelly, 1995). In Brazil, the gene Co-5 of cultivar TU has proven to be effective against 26 out of 27 C. *lindemuthianum* races identified (Rava et al., 1994, Thomazella et al., 2000). Common bean breeding programs aiming at pyramiding resistance genes could use this gene. Using allelism tests, it was possible to confirm allelic interrelations between gene Co-5 from cultivar TU and one of the three resistance genes present in cultivar G 2333, the one that is present in Selection 1360 (Young and Kelly, 1996). The cultivars TU and G 2333 are part of 12 international differentials common bean cultivars for anthracnose (Pastor-Corrales, 1992). The RAPD molecular marker $OPB03_{450C}$ was found to be linked to Co-5 gene in a population derived from Selection 1360 (Young and Kelly, 1997). However, the distance between $OPB03_{450C}$ and Co-5 in cultivar TU has not yet been determined.

The main goal of this study was to define the resistance inheritance pattern to C. *lindemuthianum* pathotype 89 in segregating populations derived from crosses between cultivar TU (resistant) and cultivar Rudá (susceptible to most C. *lindemuthianum* races) and to validate the RAPD marker $OPB03_{450C}$ previously identified as being linked to the resistance gene Co-5 in cultivar Selection 1360.

MATERIAL AND METHODS

Source of C. *lindemuthianum* isolates and culture conditions

The isolate of pathotype 89 of C. *lindemuthianum* used in this work was colleted in the Viçosa region (state of Minas Gerais, Brazil) and identified in our bean-breeding program (BIOAGRO/UFV). To

increase the amount of spores, the isolates were cultivated for approximately 10 days in a sterile medium containing common bean green pods. To confirm the identity of the isolate, it was inoculated in a 12-common-bean differential series according to Pastor-Corrales (1992).

Common bean genetic material, crosses and seed production

Seeds from the differential cultivar TU and the susceptible cultivar Rudá were provided by CIAT (Tropical Agriculture International Center, Cali, Colombia) and EMBRAPA (Goiânia, GO, Brazil) respectively. Cultivar TU is a Mesoamerican, indeterminate prostrate with small black seeds, and Rudá is also a Mesoamerican, indeterminate commercial cultivar with "carioca type-seed". TU was used as male parent and crossed with Rudá under greenhouse conditions. All populations were kept in the greenhouse.

Genetic analyses and evaluation of disease symptoms

The F_2 seeds of cross Rudá vs. TU and those of their respective parents were sown in the greenhouse. Primary leaves from each individual were collected, identified and stored at –80°C. Spores (1.2×10^6 conidia/ml) of pathotype 89 of C. *lindemuthianum* were sprayed with a De Vilbiss No. 15 apparatus to one primary leaf on 10-day-old plants. The plants were incubated in a mist chamber (20-22° C, 100% relative humidity) for seven days and then the disease symptoms were visually scored using a 1-to-9 scale. 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) (Rava et al., 1993).

DNA extraction and amplification

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

Validation of RAPD markers

The Bulked Segregant Analysis technique (Michelmore et al., 1991) was used to validate the RAPD marker linked to resistance gene Co-5. Two contrasting bulks were prepared, each containing DNA from eight resistant or susceptible F_2 individuals. For DNA amplification, the primer OPB03 was used (Operon Technologies Inc., Alameda, CA, USA).

Linkage analyses

Chi-square analyses were used to test the phenotypic segregation of the F_2 population originated from the cross Rudá/TU and to determine possible linkages between the RAPD marker $OPB03_{450C}$ and the resistance gene(s). Genetic distances between the markers and the resistance gene(s) were determined with the aid of the program MAP-MAKER III (Lander et al., 1987), using a LOD score minimum of 3.0.

RESULTS AND DISCUSSION

The segregation analyses in the F_2 population derived from cross Rudá vs. TU revealed a 3:1 ratio

Table 1. Segregation analyses of RAPD marker $OPB03_{450C}$ and Co-5 gene in an F_2 population from the cross Rudá/TU inoculated with pathotype 89 of C. *lindemuthianum*.

Locus Tested	Generation	Expected ratio	Observed ratio	χ^2	P	cM [1/]
Co-5	F_2	3:1	61:23	0.25	61.70	...
$OPB03_{450C}$ [2/]	F_2	3:1	60(+):24(-) [3/]	0.57	45.00	...
Co-5/$OPB03_{450C}$ [2/]	F_2	9:3:3:1 [4/]	55:6:5:18	45.59	0.00	15.4

[1/] Distance in centimorgans in relation to Co-5 (resistance gene); [2/] Molecular marker linked in coupling phase (c) to Co-5; [3/] (+) Band present; (-) Band absent; [4/] Ratio expected (9R+:3R-:3r+:1r-) if the resistance gene Co-5 and one marker ($OPB03_{(450)}$) were independently inherited. The symbol + represents band present and the symbol –, band absent.

suggesting the involvement of one dominant gene (Co-5) determining resistance to pathotype 89 of C. *lindemuthianum* (Table 1). In resistant and susceptible bulks of this segregating population, one RAPD molecular marker of 450 bp linked in coupling (c) and generated by primer OPB03 was verified (Figure 1). Co-segregation analyses in the F_2 population revealed that marker $OPB03_{450C}$ was located 15.4 cM from the resistance gene (Table 1).

The RAPD molecular marker $OPB03_{450C}$, linked to Co-5, was identified and validated in populations derived from cultivar Selection 1360 and BC_1F_2 lines carrying the Co-5 gene derived from cultivar G 2333, respectively. In segregating populations derived from Selection 1360 in crosses with Black Magic and Blackhawk, distances of 7.2 and 4.4 cM were observed, respectively (Young and Kelly, 1997). However, in BC_1F_2 lines derived from crosses between Rudá and G 2333, the distance was 16.4 cM (Alzate-Marin et al., 2001). These differences must have been caused by new recombination events when different female progenitors were used. In the present study, cultivar TU was used as an alternative resistance source, and a distance of 15.4 cM was observed. This distance was found to be similar to that observed in BC_1F_2 lines derived from crosses between Rudá and G 2333.

As Co-5 has proven to be effective against a large number of Brazilian isolates of C. lindemuthianum, the validation of marker $OPB03_{450C}$ linked to the major gene Co-5 is a contribution for breeding programs which aim at pyramiding resistance genes to anthracnose in common beans. However, as $OPB03_{450}$ is 15.4 cM distant from the Co-5 allele of TU, it will not be useful during the pyramidation of anthracnose resistance genes that make use of the progenitors analyzed in this study. However, this molecular marker can be useful for the identification of the Co-5 allele in other populations derived from crosses with G 2333, Selection 1360 and TU.

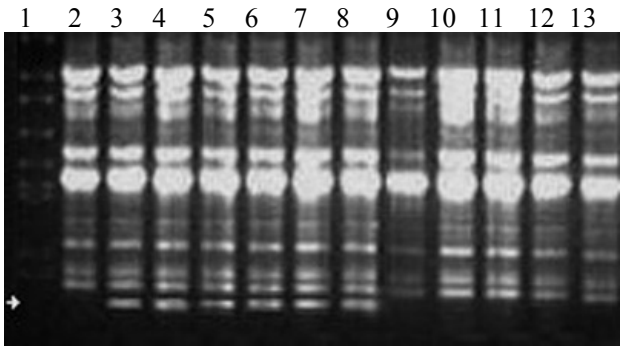

Figure 1. Electrophoresis analysis of amplification products obtained with primer OPB03. Lanes are as follows: 1, lambda DNA cut with EcoRI, BamHI and HindIII (size markers), 2, Rudá, 3, TU, 4-8, F_2 plants resistant to C. lindemuthianum pathotype 89, 9-13, F_2 plants susceptible to pathotype 89. The arrow indicates a DNA band of 450 bp linked in coupling phase to the resistance gene.

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RESUMO

Validação de um marcador RAPD ligado ao gene *Co-5* de resistência à antracnose do feijoeiro comum

O cultivar TU, um dos cultivares diferenciadores para antracnose do feijoeiro, possui o gene Co-5 o qual confere resistência a um amplo número de patótipos no Brasil. Os principais objetivos deste trabalho foram estudar a herança da resistência a antracnose do cultivar TU em populações segregantes derivadas do cruzamento com o cultivar Rudá, e validar o marcador molecular RAPD $OPB03_{450C}$, previamente identificado como ligado ao gene Co-5 no cultivar Seleção 1360. Os resultados revelaram que um único gene do cultivar TU, o Co-5, é responsável pela resistência, e que a banda de 450 pb do primer OPB03 está ligada ao gene a uma distância de 15,4 cM. Como $OPB03_{450C}$ esta distante do gene Co-5 do cultivar TU, não poderá ser utilizado em programas de piramidação de genes. No entanto, este marcador molecular poderá ser utilizado na identificação do alelo Co-5 en outros cultivares que possuam este gene.

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