Resistance of Common Bean Cultivar AB 136 to Races 31 and 69 of *Colletotrichum lindemuthianum*: the *Co-6* Locus

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ABSTRACT

Genetic resistance in common bean (*Phaseolus vulgaris* L.) is the most effective control of anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. et Magnus) Lams.-Scrib. In a previous report, two genetic symbols, *Q* and *B*, were assigned to describe the gene(s) controlling the resistance of the cultivar AB 136 to races 31 and 69 of the pathogen, respectively. In the present study, progeny tests with two identical sets of $F_{2:3}$ population were used to obtain the first experimental evidence whether one single gene controls the resistance to both races 31 and 69. The absence of recombinations between the putative genes (*Q* and *B*) allowed the conclusion that a single dominant gene controls the resistance of the cultivar AB 136 to both races 31 and 69. The symbol *Co-6* was assigned to the gene. In addition, linkage analysis with RAPD marker indicated that *Co-6* also controls the resistance of AB 136 to other races of the pathogen, or that different genes are present in the same linkage block.

KEY WORDS: Disease resistance, *Breeding.*

INTRODUCTION

Anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. and Magnus) Lams.-Scrib., is one of the most widespread and economically important fungal disease of common bean (*Phaseolus vulgaris* L.). Complete yield loss can occur with susceptible genotypes when conditions favorable to the pathogen are present during the growing season (Pastor-Corrales and Tu, 1989).

The resistance of the cultivar AB 136 to anthracnose was first reported by Schwartz et al. (1982). The monogenic inheritance of AB 136 resistance was first described by Gonçalves-Vidigal (1994), who assigned the genetic symbols *A'*, *Q*, and *B* to describe the dominant gene (s) controlling the resistance to races 23 (delta), 31 (kappa), and 69 (alpha), respectively. Different symbols were assigned because these three races were tested in independent F_2 populations derived from AB 136 x Michelite cross.

A monogenic factor in AB 136 was also described by Poletine (1997) controlling the resistance to races 69 and 453. In addition, other studies with AB 136 have identified monogenic inheritance for resistance to races 89, 73, and 64 (Alzate-Marin et al.,1999). Young and Kelly (1996) also identified in Catrachita, a AB 136 derived line, a monogenic inheritance of a resistance to races 23, 64, and 73. Kelly and Young (1996) suggested the symbol *Co-6* to designate the gene present in AB 136. Despite the lack of experimental evidence to facilitate the nomenclature it is currently assumed that the same dominant gene, *Co-6*, controls the resistance to all mentioned races. The present study was performed to experimentally verify

whether the same dominant gene controls the resistance of AB 136 to both races 31 and 69. Additionally, RAPD (Random Amplified Polymorphic DNA) marker was used to compare the results with previous report involving the resistance of AB 136 to other races of the pathogen.

MATERIAL AND METHODS

Genetic material

F_1 plants were obtained by crossing the common bean cultivars AB 136 (resistant to races 31 and 69 of *C. lindemuthianum*) and Michelite (susceptible). Three F_2 populations were obtained by selfing three F_1 plants. One F_2 population (118 plants) was inoculated with a spore suspension of race 31 of *C. lindemuthianum*. A second F_2 population (132 plants) was inoculated with race 69. Inoculations with races 31 and 69 of the pathogen were also performed on the parental cultivars AB 136 (50 plants with race 31 and 52 plants with race 69) and Michelite (45 plants with race 31 and 50 plants with race 69), and on F_1 hybrids (30 plants with race 31 and 32 plants with race 69). In order to perform progeny tests, a third F_2 population (56 plants) was used to produce, by selfing, a $F_{2:3}$ population (56 $F_{2:3}$ families). Each $F_{2:3}$ family (aproximatelly 60 individuals) was randomly divided in two sub-families of approximately 30 individuals, in order to obtain two samples of the original $F_{2:3}$ population. These two sample populations (approximately 30 individuals / sub-family) were inoculated with a spore suspension of *C. lindemuthianum* (one sample with race 31 and the other sample with race 69). All populations were grown in the greenhouse.

C. lindemuthianum isolates

The isolates of the physiological races 31 and 69 were supplied by the fungi collection at the Federal University of Viçosa and Embrapa-CNPAF. Monosporic cultures of the races were transferred to test tubes containing a Mathur et al. (1950) culture medium. They were incubated at 22º C for eight to ten days. After sporulation, the pathogen cultures were kept in a refrigerator at 5ºC and used as a culture stock for later experiments.

Genetic analysis

Plants with the first trifoliate leaf completely developed (15th day) were transferred to a humid chamber at approximately 22ºC. Inoculation of the parents and the F_1, F_2, and $F_{2:3}$ generation was carried out separately for each race to prevent contamination. This process was carried out using a brush previously moistened in a spore suspension at 1.2 x 10^6 concentration, using on adaptation of the method used by Cárdenas et al. (1964).

The seedlings were kept in the same chamber for 96 hours after inoculation, at 20ºC, under controlled light (12 hour with 689 lux illumination alternated with 12 hours of darkness) and at approximately 100% relative humidity. The plants were then transferred to tables, in a suitable environment at 22ºC with artificial light, where they stayed until the assessment began. The reaction to the physiological races were evaluated in four replications of each plant sample.

The scale proposed by Yerkes Jr. and Ortiz (1956) was used to carry out the visual assessment of the symptoms in each plant, approximately 10 days after inoculation. The first trifoliate leaves of each individual plant was scored on a 1 to 5 scale to assess the symptons induced by the physiological race. Host reactions were assessed visually eigth to 10 days after inoculation. A 1-5 scale was used, where 1 = no disease symptons; 2 = a few isolated small lesions on the mid and, occasionally, on the secondary veins of the leaf; 3= many small lesions scattered on the mid and secondary veins, with collapse of the sorrounding tissue; 4 = few to many large lesions scattered over the leaf blade; and, 5 = many large coalescing lesions accompained by tissue breakdown and chlorotic abscissed leaflets. Plants scored as 1 and 2 were considered resistant (RR or R_) and, scored as 3 to 5, susceptible (rr). This system was used

in studies carried out by Cárdenas et al. (1964), Muhalet et al. (1981), Del Peloso et al. (1989) and Gonçalves-Vidigal (1994). According to the results of the progeny tests each $F_{2:3}$ sub-family was classified as RR (only resistant plants), Rr (segregation for resistant and susceptible plants), or rr (only susceptible plants).

The nature of the resistance in AB 136 was determinated after analyzing the segregating ratios obtained from phenotypic characterization of the F_2 populations and two identical sets of $F_{2:3}$ population reaction to the disease. The chi-square (x^2) test was performed using the Genes Program (Cruz, 1997).

The two identical samples of the $F_{2:3}$ population were tested independently with races 31 and 69 and the number of recombinations between the two putative genes (*Q* and *B*) was calculated by comparing the results of each pair of $F_{2:3}$ sub-families tested with these two races. The progeny test using $F_{2:3}$ sub-families populations was performed based on the fact that each $F_{2:3}$ family genetically represent its respective F_2 ancestor and that this $F_{2:3}$ family can be randomly divided in two or more replicated samples.

RAPD linkage analysis

The RAPD marker $OPZ04_{560}$ previously presented by Alzate-Marin et al. (1999) was tested. The linkage analysis was performed in the $F_{2:3}$ generation. The linkage between the RAPD marker and the gene (s) was calculated based on the recombination ratio.

DNA from bulks of $F_{2:3}$ plants was extracted from young leaves following a modified Doyle and Doyle (1990) protocol. The DNA was then quantified by spectrophotometry, evaluated for quality in 0.8% agarose gel electrophoresis and stored at 4°C . The DNA was diluted in TE (TRIS HCl 10mM, EDTA 1 mM, pH 8.0) to a final 10 ng/ml concentration for amplification. A Perkin Elmer thermocycler 9600 was used for amplification (Williams et al., 1990) and each reaction had a total volume of 25 ml, containing the following components: 25 ng genomic DNA , 1 unit of AmpliTaq DNA polymerase, 100 mM of each of dNTP, 0.2 mM of primer, 50 mM KCl, 10 mM Tris HCl and 2 mM $MgCl_2$. The volume was completed to 25 ml with ultra pure water. The following program was used: one cycle for DNA denaturation (95°C for 1 minute), 39 cycles for amplification (15 seconds at 94°C, 30 seconds at 35°C, 60 seconds at 72°C) and seven minutes at 72°C for finalization. The products from the amplification reactions were separated in 1.4% agarose gels, stained with ethidium bromide, visualized in UV and photographed. RAPDs were registered as band presence or absence.

RESULTS AND DISCUSSION

Data from the F_1, F_2, and $F_{2:3}$ generations and from the AB 136 (resistant to races 31 and 69) and Michelite (susceptible) parents are shown in the Table 1.

Monogenic inheritance of AB 136 resistance to races 31 and 69

The chi-square test performed on the F_2 population with the race 31 supported a 3:1 expected ratio for resistant and susceptible plants (R_:rr), respectively. In addtion, the chisquare test performed with the data from the $F_{2:3}$ families confirmed a 1:2:1 (RR:Rr:rr) ratio. These results indicated that a single dominant gene present in the cultivar AB 136 is responsible for the resistance to race 31 of *C. lindemuthianum*. Similarly, the data from the F_2 and $F_{2:3}$ families inoculated with race 69 also indicated a dominant monogenic inheritance of the AB 136 resistance to this race (Table 1). These results confirm the dominant monogenic inheritance of AB 136 resistance to these races previously reported by Gonçalves-Vidigal (1994), Young and Kelly (1996), Gonçalves-Vidigal et al. 1997 and Poletine (1997).

Resistance to races 31 and 69 controlled by the *Co-6* gene

The two samples of the $F_{2:3}$ population were tested independently with races 31 and 69 and the number of recombinations between the two putative genes (*Q* and *B*) was calculated by comparing the results of each pair of $F_{2:3}$ sub-families tested with these two races.

By comparing the progeny tests performed with the two samples of $F_{2:3}$ population, it was observed that each pair of $F_{2:3}$ sub-families showed exactly the same results when it was tested independently with races 31 and 69. Therefore, based on these progeny tests, no recombinants were found between the two putative genes *Q* (resistance to race 31) and *B* (resistance to race 69) (Table 1). It can be concluded that a single dominant gene controls the resistance of the cultivar AB 136 to both races 31 and 69. The consensus symbol *Co-6* was assigned to the gene.

Table 1. Evaluation of resistance to races 31 and 69 of *Colletotrichum lindemuthianum* in a cross of common bean genotypes Michelite x AB 136 .

Pedigree	Generation	Race	Anthracnose reaction (nº of plants)				Expected ratio	χ^2	P
			RR	Rr	R_	rr			
			Co-6Co-6	*Co-6co-6*	*Co-6_*	*co-6co-6*			
Michelite	P_1	31	0	...	...	45	...	...	...
Michelite	P_1	69	0	...	...	50	...	...	...
AB 136	P_2	31	50	...	...	0	...	...	...
AB 136	P_2	69	52	...	...	0	...	...	...
$P_1 \times P_2$	F_1	31	...	30	...	...			
$P_1 \times P_2$	F_1	69	...	32	...	...			
$P_1 \times P_2$	F_2	31	...	...	89	29	3:1	0.01	0.91
$P_1 \times P_2$	F_2	69	...	...	100	32	3:1	0.03	0.84
$P_1 \times P_2$	$F_{2:3}$	31	15	32	...	9	1:2:1	2.43	0.38
$P_1 \times P_2$	$F_{2:3}$	69	15	32	...	9	1:2:1	2.43	0.38

Linkage between the $OPZ04_{560}$ marker and the *Co-6* gene

The RAPD marker $OZ04_{560}$ (coupling phase) was observed at 1.7 cM linked to the *Co-6* gene. This marker was previously mapped at 8.5, 2.9, 2.8, and 7.5 cM of distance from the gene (s) for resistance to races 89, 73, 81, and 64, respectivelly (Alzate-Marin, et al., 1999). These linkage analysis indicate that the *Co-6* gene, which controls the resistance to races 31 and 69, might also be the same gene of resistance to races 89, 73, 81, and 64, or that it is present in the same linkage block which controls the resistance to these four races. To facilitate the nomenclature, it can be assumed that the same dominante gene, *Co-6* (as proposed by Kelly and Young, 1996), present in AB 136, controls the resistance to the different races of *C. lindemuthianum*, since there is no experimental evidence to the contrary.

CONCLUSIONS

1) Data from progeny tests showed that a single dominant gene *(Co-6)* controls the resistance of the common bean cultivar AB 136 to both races 31 and 69 of *C. lindemuthianum*.

2) Data from RAPD marker indicated that the *Co-6* gene, or a linkage block where it is present, controls the resistance of AB 136 to other races of the pathogen.

3) To facilitate the nomenclature, it can be assumed that the same dominante gene *(Co-6)* present in AB 136, controls the resistance to the different races of *C. lindemuthianum*, since there is no experimental evidence to the contrary.

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RESUMO

Resistência do feijoeiro comum cultivar AB 136 às raças 31 e 69 de *Colletotrichum lindemuthianum*: o loco *Co-6*.

Resistência genética em feijoeiro (*Phaseolus vulgaris* L.) é o mais eficiente controle para antracnose causada por *Colletotrichum lindemuthianum* (Sacc. et Magnus) Lams.-Scrib. Em um relato anterior, dois símbolos genéticos, *Q* e *B*, foram utilizados para descrever o(s) gene(s) condicionando a resistência da cultivar AB 136 às raças 31 e 69, respectivamente. No presente estudo, testes de progênies com duas amostras idênticas de população $F_{2:3}$ foram usadas para obter a primeira evidência experimental de que um único gene estaria controlando a resistência da cultivar AB 136 às raças 31 e 69. A ausência de recombinantes entre os dois genes putativos (*Q* e *B*) permitiu concluir que um único gene dominante controla a resistência da cultivar AB 136 a ambas as raças 31 e 69. O símbolo *Co-6* foi assumido para designar o gene. Adicionalmente, análise de ligação gênica com marcador RAPD indicou que *Co-6* também controla a resistência de AB 136 a outras raças do patógeno, ou que ele está presente no mesmo bloco de ligação gênica.

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