Inheritance of Anthracnose Resistance in Common Bean Differential Cultivar AB 136

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ABSTRACT

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Inheritance of anthracnose resistance of the common bean (*Phaseolus vulgaris* L.) differential cultivar AB 136 to races 89, 64, and 73 (binary system designation) was studied in crosses with the susceptible differential cultivars Michelite (race 89), Mexico 222 (race 64), and Cornell 49-242 (race 73). In each cross two progenitors, the F₁, F₂, and backcross-derived plants were inoculated with the respective race under environmentally controlled greenhouse conditions. The results indicate that single dominant gene(s) control resistance to races 89 and 64, giving a segregation ratio of 3:1 in the F₂, 1:0 in the backcrosses to AB 136, and 1:1 in the backcross to Michelite (race 89), and to Mexico 222 (race 64). For race 73, the following segregation ratios between resistant and susceptible plants were observed: 13:3 in the F₂, 1:0 in the backcross to AB 136, and 1:1 in the backcross to Cornell 49-242. Such results suggest that two independent genes may determine resistance of AB 136 to race 73, one dominant (*Co-6*) and one recessive that is proposed to be assigned *co-8*. Genotypes *Co-6* or *co-8 co-8* would condition resistance, whereas susceptibility would be present in genotypes *co-6 co-6* *Co-8*. Given the dominant nature of anthracnose resistance genes present in line AB 136 and its resistance to 25 races of *Colletotrichum lindemuthianum* identified in Brazil by other researchers, we included this cultivar as one of the donor parents in our molecular marker-assisted backcross breeding program, to develop common bean cultivars resistant to anthracnose and adapted to Central Brazil.

Anthracnose, caused by the fungus *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib., is one of the most destructive diseases affecting common bean (*Phaseolus vulgaris* L.) in tropical and subtropical areas of Latin America and Central and Eastern Africa (7). In such areas a complete yield loss can occur with susceptible cultivars when contaminated seeds are used and the environmental conditions are favorable for the pathogen development (12).

In susceptible cultivars anthracnose symptoms can appear on any plant part. Initial symptoms may appear on cotyledonary leaves as small, dark brown to black lesions. Hypocotyl-infected tissues manifest minute rust-colored specks. The specks gradually enlarge longitudinally and form sunken lesions or eye-spots. These enlarge on hypocotyl of the young seedling, causing it to rot off. On older stems, the eye-shaped lesion is about 5 to 7 mm in length. In the leaves, lesions appears first on

the lower surface and leaf veins as small, angular, brick-red to purple spots which become dark brown to black. Later, lesions also appear on veinlets on the upper surface of leaves (3).

Pod infections appear as flesh to rust-colored lesions. The lesions develop into sunken cankers (1 to 10 mm in diameter) which are delimited by a slightly raised black ring and surrounded by a reddish brown border (9). The fungus can invade the pod, and the mycelia and conidia infect the cotyledons or seed coat of developing seeds. Infected seeds are often discolored and may contain dark brown to black cankers (3,7).

The use of resistant cultivars is considered to be the most viable option for controlling anthracnose, particularly for small-scale growers (15,17). However, the existence of many physiological races of the pathogen in regions such as Southern and Central Brazil represents an obstacle for creating new common bean cultivars resistant to anthracnose. Knowledge about

the frequency and distribution of physiological races of the pathogen, combined with the use of different resistance genes, are needed to develop more stable resistant cultivars adapted to such regions of Brazil (13).

A number of common bean lines have been evaluated for resistance to many races of anthracnose at the Centro Internacional de Agricultura Tropical (CIAT, Cali, Colombia). Among them, line AB 136, selected as one of the 12 international differential cultivars for anthracnose, is one of the most resistant (8). In addition, it has been demonstrated that line AB 136 showed resistance to 25 races of *C. lindemuthianum* collected in several common bean growing regions in Brazil (13). The purpose of this work was to define the inheritance pattern of resistance present in the AB 136 line in segregating populations derived from crosses made with the susceptible cultivars Michelite, Mexico 222, and Cornell 49-242 to pathogen races collected in the states of Minas Gerais and Espírito Santo (Central Brazil).

MATERIALS AND METHODS

Isolates. Three isolates of *C. lindemuthianum* belonging to three different races (64, 73, and 89) used in this work are defined in Table 1 and are among the 25 races previously characterized by Rava et al. (13). The original cultures were provided by Carlos Rava and Aloisio Sartorato (Centro Nacional de Pesquisa de Arroz e Feijão/Empresa Brasileira de Pesquisa Agropecuária [CNPq/EMBRAPA], Goiânia, GO, Brazil). To obtain high sporulation levels, fungi were grown in sterilized young pods of common bean for approximately 10 days. In order to confirm their phenotypes, isolates were inoculated in the 12 international common bean differential cultivars for anthracnose (8).

Genetic materials and crosses. Seeds of the differential cultivars Michelite, Cornell 49-242, Mexico 222, and AB 136

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Table 1. Origin of isolates of *Colletotrichum lindemuthianum*^a used in experiments

Race (binary system)	Group/race (classical nomenclature)	Origin (state - country)
64	Mexican I/ Mexican I	Espírito Santo, Brazil
73	Alpha/Alpha BR	Espírito Santo, Brazil
89	Alpha/Alpha BR	Minas Gerais, Brazil

^a Rava et al., 1994.

were provided by CNPAF/EMBRAPA. Some of their traits are shown in Table 2. Line AB 136 (male progenitor) was crossed with each of the other three cultivars. Part of the F₁ seeds was planted and the F₁ plants were backcrossed to AB 136 (BC_r) and to the corresponding susceptible progenitor (BC_s). The remaining F₁ seeds were selfed to generate F₂ seeds. All crosses were performed in the greenhouse.

Genetic analysis of AB 136 resistance to anthracnose. Spores from races 89, 64, and 73 were inoculated in plants derived from the following crosses: Michelite × AB 136, Mexico 222 × AB 136, and Cornell × AB 136, respectively (Table 2). For each cross, the following number of seeds were sowed in the greenhouse in a completely randomized design: 30 seeds from each progenitor, 30 F₁ seeds, 195 F₂ seeds, and 60 seeds from each backcross (BC_r and BC_s). Fourteen days after sowing, the first expanded trifoliate leaf from each of the 405 plants was inoculated on the lower and upper leaf surfaces with spore suspensions of *C. lindemuthianum* (1.2 × 10⁶ spores/ml). Spore suspensions were applied with a horsehair paint brush. The plants were then incubated for 7 days in a humidity chamber, which was maintained at 20 to 22°C and 100% relative humidity.

After this period, each plant was scored visually for the disease symptoms using a 1 to 9 scale adapted from Pastor-Corrales (8) where 1 = plants with no visible symptoms; 2 = few isolated small lesions on mid-veins in the lower leaf surface; 3 = more frequency of small lesions on mid-veins in the lower leaf surface; 4 = lesions present in the mid-vein and occasionally in secondary leaf veins; 5 = many small lesions scattered on mid- and secondary veins; 6 = many small lesions as described in grade 5 in the lower and upper leaf surface and in the stems and petioles; 7 = large lesions scattered over the leaf blade and many lesions in the stems and petioles; 8 = many large coalesced lesions accompanied by tissue breakdown and chlorotic or abscised leaflets, reduced plant growth, and many lesions in stems and petioles; and 9 = severely diseased or dead plants.

Resistant (R) phenotype was assigned to plants with no or limited symptoms (scores 1 to 3); whereas plants graded 4 or greater were considered to be susceptible (S). The class frequencies obtained were tested for goodness-of-fit to theoretical ratios with chi-square tests.

RESULTS AND DISCUSSION

Results of inoculations confirmed that cultivar AB 136 is resistant to *C. lindemuthianum* races 89 (Table 3), 64 (Table 4), and 73 (Table 5) under greenhouse conditions. In contrast, cultivars Michelite, Mexico 222, and Cornell 49-242 are susceptible to races 89, 64, and 73, respectively, under the same conditions. Similar results have been reported which demon-

strated that line AB 136 showed incompatible reaction when inoculated with 25 races of *C. lindemuthianum* detected in Brazil (13).

Observed and expected frequencies for resistant and susceptible reactions to race 89, for the progenitors AB 136, Michelite, and the populations derived from crosses between them, are shown in Table 3. Chi-square values revealed that a good fit was obtained for the segregation ratio of 3:1 in the F₂, 1:1 when the F₁ was backcrossed to Michelite, and 1:0 when the F₁ was backcrossed to AB 136. Similar results were observed for the cross between AB 136 and Mexico 222 inoculated with race 64 (Table 4). The segregation ratios of 3:1 in the F₂ and the good fit to the expected ratios in the backcross generations (Tables

3 and 4) indicate that resistance of AB 136 to races 89 and 64 is controlled by a single dominant gene. From these data it cannot be determined if these genes are independent or if they are in fact the same gene. However, preliminary results from our laboratory show that these two putative genes are tagged by the same PCR-based molecular marker, indicating that resistance to these two races in AB 136 is conditioned by one single dominant gene (data not shown). Most likely, this gene is the same reported previously (18) and assigned as *Co-6*.

Table 5 gives the observed and expected frequencies for resistant and susceptible reactions to race 73 for progenitors AB 136 and Cornell 49-242, the F₁ and F₂, the F₁ backcrossed to Cornell 49-242, and the F₁

Table 2. Characteristics and type of reactions to isolates of *Colletotrichum lindemuthianum* of the progenitors Michelite, Mexico 222, Cornell 49-242, and AB 136

Progenitor	Growth habit	Seed size	Seed color	Race	Reaction to anthracnose
Michelite	Indeterminate weak-stemmed	Small	White	Meso American	Susceptible to race 89
Mexico 222	Determinate erect bush	Medium	White	Durango	Susceptible to race 64
Cornell 49-242	Indeterminate weak-stemmed	Small	Black	Meso American	Susceptible to race 73
AB 136	Indeterminate	Small	Red	Meso American	Resistant to all three races

Table 3. Segregation for resistance to race 89 of *Colletotrichum lindemuthianum* in the cross Michelite × AB 136

Pedigree	Generation	No. of plants		Expected ratio	χ^2	P
		Resistant	Susceptible			
Michelite	P1	0	27	...	...	...
AB 136	P2	30	0	...	...	...
Michelite × AB 136	F ₁	17	0	...	...	...
Michelite × AB 136	F ₂	129	42	3:1	0.031	0.9
F ₁ × Michelite	BC _s	29	30	1:1	0.017	0.9
F ₁ × AB 136	BC _r	56	0	1:0	0.000	1.0

Table 4. Segregation for resistance to race 64 of *Colletotrichum lindemuthianum* in the cross Mexico 222 × AB 136

Pedigree	Generation	No. of plants		Expected ratio	χ^2	P
		Resistant	Susceptible			
Mexico 222	P1	0	30	...	...	...
AB 136	P2	30	0	...	...	...
Mexico 222 × AB 136	F ₁	22	0	...	...	...
Mexico 222 × AB 136	F ₂	145	45	3:1	0.175	0.7
F ₁ × Mexico 222	BC _s	28	31	1:1	0.152	0.7
F ₁ × AB 136	BC _r	56	0	1:0	0.000	1.0

Table 5. Segregation for resistance to race 73 of *Colletotrichum lindemuthianum* in the cross Cornell 49-242 × AB 136

Pedigree	Generation	No. of plants		Expected ratio	χ^2	P
		Resistant	Susceptible			
Cornell 49-242	P1	0	30	...	...	...
AB 136	P2	27	0	...	...	...
Cornell 49-242 × AB 136	F ₁	22	0	...	...	...
Cornell 49-242 × AB 136	F ₂	155	35	13:3	0.013	0.9
F ₁ × Cornell 49-242	BC _s	27	31	1:1	0.275	0.7
F ₁ × AB 136	BC _r	58	0	1:0	0.000	1.0

backcrossed to AB 136. Chi-square values revealed that a good fit was obtained for the segregation ratio of 13:3 in the F_2 , 1:1 when the F_1 was backcrossed to Cornell 49-242, and 1:0 when the F_1 was backcrossed to AB 136. Such results suggest the action of two independent genes for resistance of AB 136 to pathogen race 73: one dominant (*Co-6*) (18) and one recessive, which is proposed to be assigned the genetic symbol *co-8*. Genotypes *Co-6_* or *co-8 co-8* would condition resistance, whereas susceptibility would be present in genotypes *co-6 co-6 Co-8_*.

Anthrachnose resistance in common bean has been reported to be controlled by a single dominant gene (1,5). However, it has been demonstrated that two independent dominant genes are responsible for resistance of cultivar G 2333 (10). A third independent dominant gene (*Mexique 3*) has also been assigned to condition resistance of cultivar G 2333 (18). In addition, the presence of more than one gene pair in complex interactions has also been observed. For instance, it has been reported that resistance to races alpha, gamma, and delta are conditioned by two recessive genes, and for race beta one recessive gene (14). Besides, resistance to race beta has been assigned to complementarity of dominant or recessive genes (2), and yet by a multiple allelic series of a recessive gene (6). Resistance to race BA-5 (Brazil group I) has been shown to be conditioned by the action of a dominant and a duplicated recessive gene (11).

Using the cross Michelite $\times$ AB 136, it was concluded that single dominant genes in AB 136 are responsible for resistance to races alpha and delta and that the action of a dominant and complementary genes are responsible for resistance to race kappa (16). A single dominant gene present in the cultivar Catrachita, a breeding line derived from the cross BAT 1225 $\times$ AB 136, has been shown to be responsible for resistance to races 7 and 23 (delta), 64 (alpha), and 73 (alpha-Brazil) (4,18).

The data presented in this work and that of others (16,18) confirm that at least one single dominant gene in cultivar AB 136 is conditioning resistance to races delta, alpha, alpha-Brazil, kappa, and Mexican I.

On the other hand, this is the first observation of a second gene (in this case, recessive) conditioning resistance in cultivar AB

136 to anthracnose race 73 (alpha-Brazil). This gene has not been observed in cultivar Catrachita, an improved line derived from AB 136, probably because it has not been transmitted during the breeding process, or else in view of not using the susceptible cultivar Cornell 49-242 (18). Also, this gene has not been previously detected in cultivar AB 136, most likely because race 73 (alpha-Brazil) was not used in that study (16). Based on these data and on that of others (10,17), it was concluded that the genetic basis for inheritance of anthracnose resistance in common bean appears to be dependent, on the tester genotype used as the susceptible parent, and on the pathogen physiological race used for inoculation.

Given the dominant nature of anthracnose resistance gene(s) present in the AB 136 cultivar observed in this study against important pathogen physiological races that occur in Brazil, and the broad spectrum of resistance present in this cultivar (13), we selected it as one of the donor parents in our molecular marker-assisted backcross breeding program, to create common bean cultivars resistant to anthracnose and adapted to Central Brazil.

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