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Inheritance of anthracnose resistance to *Colletotrichum lindemuthianum* race 69 in common bean genotype PI 207262

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ABSTRACT. Common bean (*Phaseolus vulgaris* L.) cultivar PI 207262, resistant to *Colletotrichum lindemuthianum* race 69, was crossed with cultivars Michelite and Perry Marrow (susceptible to the race), Dark Red Kidney, Cornell 49242, AB 136 and G 2333 (resistant to it) and F_1 and F_2 generations were obtained under greenhouse conditions. The 15-day-old plants were inoculated using 1.2 x 10^6 spores/ml water spore suspension. The reaction of F_1 and F_2 populations showed that Dark Red Kidney, Cornell 49242 and AB 136 cultivars have respectively the dominant resistance genes *A (Co-1), Are (Co-2)* and *Q (Co-6)*. The results indicated the segregation of three dominant resistance genes, two of them present in G 2333 (*Co-5* and *Co-7*) and another present in PI 207262, which is located in *Co-4 locus*. In this experiment it was not possible to distinguish gene *Co-4*^{2} in G 2333 cultivar from gene Mexique 2 (Co-4) in PI 207262, because they are located in the same *locus*, which makes identification using traditional screening methods possible.

Key words: anthracnose, *Colletotrichum lindemuthianum, Phaseolus vulgaris*, genetic resistance.

RESUMO. Herança da resistência à raça 69 de *Colletotrichum lindemuthianum* em feijoeiro comum cultivar PI 207262. O cultivar de feijão comum (*Phaseolus vulgaris* L.) PI 207262 resistente a *Colletotrichum lindemuthianum* raça 69, foi cruzado com os cultivares Michelite e Perry Marrow (suscetíveis à raça), Dark Red Kidney, Cornell 49242, AB 136 e G 2333 (resistentes) com gerações F_1 e F_2 obtidas sob condições de casa de vegetação. Plantas com 15 dias de idade foram inoculadas usando uma suspensão de esporos com concentração de 1.2 x 10^6 esporos/ ml água. A reação de populações F_1 e F_2 mostrou que os cultivares Dark Red Kidney, Cornell 49242 e AB 136 possuem respectivamente os genes dominantes de resistência *A (Co-1)*, *Are (Co-2)* e *Q (Co-6)*. Os resultados indicaram a segregação de três genes dominantes de resistência, sendo dois presentes em G 2333 (*Co-5* e *Co-7*) e um em PI 207262, o qual está situado no *locus Co-4*. No presente trabalho, não foi possível distinguir o gene *Co-4*^{2} no cultivar G 2333 do gene *Mixique 2* (*Co-4*) em PI 207262, pelo fato de estarem presentes num mesmo *locus*, o que torna impossível a identificação por método tradicional de seleção.

Palavras-chave: antracnose, *Colletotrichum lindemuthianum, Phaseolus vulgaris*, resistência genética.

Common bean (*Phaseolus vulgaris* L.) anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. et magn.) Scrib. fungus is transmitted by infected seeds which may cause losses of 100% when these seeds are planted under humid conditions and temperatures around 13ºC and 26ºC (Del Peloso, 1992).

In the search of common bean anthracnose, the resistance is considered the most important factor in integrated control strategy, which includes cultural practices, use of mixed cultivars (Singh, 1992), quarantines and fungicides (Chaves, 1980). However, the use of genetic resistance is hindered because there are various physiological races of the agent which cause the disease, becoming important to keep an uptade knowledge to explore the genetic variability in common bean and develop new resistant cultivars (Rava *et al*., 1994).

There are various sources of resistance such as Dark Red Kidney, *A (Co-1)* gene, (McRostie, 1919); Cornell 49242, *Are (Co-2)* gene (Mastenbroek, 1960). Fouilloux (1979) identified the three *Mexique*

genes *(Co-3) (Co-4) (Co-5)* in Mexican germoplasm. Kelly and Young (1996) verified the presence of *Co-6* gene in AB 136, and G 2333 carries three different genes, *Co-4*^{2}, *Co-5* and *Co*-7 (Young *et al.*, 1998), which are well known to European and American researchers. The *Are (Co-2)* gene confers resistance to race 69 (epsilon), and has been widely used in common bean breeding programs to obtain resistant cultivars. The dominant resistance gene present in the AB 136 cultivar was first described by Schwartz *et al*. (1982). Inheritance studies showed that only the *Q (Co-6)* gene, which is independent of others previously characterized, is present in this cultivar (Young and Kelly, 1996; Gonçalves-Vidigal *et al*., 1997). Pastor-Corrales *et al*. (1994) showed that only the G 2333 line was resistant to 380 isolates of *C. lindemuthianum*. This line was resistant to all Brazilian isolates and all European and North American races (Pastor-Corrales and Tu, 1989; Balardin and Pastor-Corrales, 1990; Balardin *et al*., 1990; CIAT, 1990). Resistance in G 2333 is controlled by two independent dominant genes with equivalent effects (Pastor-Corrales *et al*. 1994). In addition, Young *et al.* (1998) detected in G 2333 three different dominant resistance genes, *Co-4*^{2}, *Co-5* and *Co*-7.

Menezes (1985) considered PI 207262 cultivar similar to TO, in relation to the susceptibility to zeta race. Based on this affirmation, Gonçalves-Vidigal (1994) considered the first cultivar mentioned carriyng dominant gene of resistance present in *locus Co-4*.

To make the use of PI 207262 as resistance source to anthracnose easy, its inheritance resistance to the physiological race 69 (epsilon) was investigated, in crosses using the Michelite and Perry Marrow cultivars acting as susceptible in this study.

Material and methods

Genetic Plant Material. The following cultivars were used as differentials of *C. lindemuthianum* races: Michelite, Dark Red Kidney, Perry Marrow, Cornell 49242, AB 136 and G2333, all of them crossed with PI 207262. The choice of these cultivars is justified because they are widely used in breeding programs. The seeds of the differential cultivars were supplied by Embrapa-CNPAF (Brazilian Agricultural Research Corporation - National Research Center for Bean and Rice at Goiânia).

Six F_1 hybrids derived from cross between PI 207262 line and the other cultivars: Michelite, Perry Marrow, Dark Red Kidney, AB 136, Cornell 49242 and G 2333 were obtained and F_1 plants were cultivated in the greenhouse to obtain the F_2 generation. Seedlings of the parents and the F_1 and F_2 generations, approximately 15 days old, were tested and assessed for their resistance or susceptibility reaction to the physiological race 69 (epsilon) of *C. lindemuthianum*.

Preparation of *C. lindemuthianum isolates.* The physiological race 69 (epsilon) was supplied by the fungi collection at the Federal University of Viçosa and Embrapa- CNPAF. There was a preference for this race because it showed high index of occurrence in the state of Paraná, besides being poorly studied and more information on the inheritance of resistance is needed.

The numeric designation of the race followed the binary system proposed by Habgoods specifications (1970). In the case of *C. lindemuthianum,* 12 differentiating cultivars were recommended by the Centro Internacional de Agricultura Tropical (Ciat), in 1990. Each cultivar received a value, 2^n, where 2 is the number of classes of reactions considered (resistant or susceptible) and n is the function of the order of the differentiate. The resistance reaction or susceptibility shown by the cultivars received values zero and one respectively. The number 69 referred to epsilon race was formed from the following susceptible cultivars: Michelite (1), Perry Marrow (4) and México 222 (64).

Following Cárdenas *et al*. (1964), the spores bound for inoculation were transferred to test tubes containing Mathur *et al*. (1950) culture medium and incubated at 22 ^oC for a period of eight to ten days. After sporulation starts, monosporic culture was kept in a refrigerator at 5^oC and used as a culture stock for this study. The isolate was inoculated in the set of 12 differential cultivars for anthracnose to confirm their phenotypes (Pastor-Corrales, 1988).

Inoculation and incubation. Plants with their first trifoliate leaf completely developed were transferred to a humid chamber at approximately 22°C ± 2°C. The inoculation of the parents, F_1 and F_2 generations from the six crosses, was performed by the use of a brush previously moistened in a spore suspension, at de 1.2 x 10^6 concentration, from an adaptation oh the method used by Cárdenas *et al*. (1964).

After the inoculation, the seedlings were kept in the same humid chamber for 96 hours at 20^oC ± 2°C, controlled light (12 hours with 680 lux illumination alternated with 12 hours of darkness) and approximately 100% relative humidity. Four replications of the parents and their F_1 and F_2 generations from the six crosses were evaluated for the referred physiological race.

Symptoms evaluation. The system proposed by Yerkes Jr. and Ortiz (1956) was used, after approximately 10 days of the inoculation. The scores were assigned to the first trifoliate leaves, whose values were on a 1-to-5 scale, in individual plants to assess the symptoms induced by the physiological race. Plants scored as 1 and 2 were considered resistant and scored as 3 to 5, susceptible. This system was used in studies carried out by Cárdenas *et al*. (1964), Muhalet *et al*. (1981), Fukuda (1982), Del Peloso *et al*. (1989) and Gonçalves-Vidigal (1994).

Results and discussion

The observed phenotypes of the cultivars based on their reaction to race 69 and characterization is shown in Table 1.

Two of the six crosses belong to the R x S combination and four to the R x R combination. All the F_1 plants behaved as resistant. (Table 2).

In the case of R x S combination, one of the crosses, PI 207262 x Michelite, showed segregation which fitted the ratio of 57R:7S, indicating the segregation of the dominant resistance gene, *Mexique 2 (Co-4)*, present in PI 207262 and TO, and cited by Fouilloux (1976) and Bannerot *et al*. (1971), in addition to the segregation of the complementary dominant genes: *X*, also present in PI 207262, and *Y*, in the susceptible cultivar Michelite (Del Peloso *et al.* (1989). According to this author, this genes acting as complementary factors, with gene *X* also present in the cultivar Perry Marrow and Y in Michelite, as confirmed by the present work. The 3R:1S segregation, involving the crosses between PI 207262 x Perry Marrow, resistant and susceptible cultivars respectively, indicated that there was only the action of the *Mexique 2 (Co-4)* gene, as previously cited. The three *Mexique* genes are independent from the *Are (Co-2)* gene (Fouilloux, 1979; Pastor-Corrales and Tu, 1989) and, with exception of the allele present in México 227, are frequently used in the characterization of the races.

Within the combinations involving two resistant cultivars (R x R), three crosses showed data that fitted the ratio 15R:1S in F_2 generation, indicating the segregation of two dominant resistance genes, acting as independent factors, with equivalent effects. In the cross between the cultivars PI 207262 and Dark Red Kidney, the segregation data obtained are explained by the action of the dominant gene *A (Co-1),* presented in this last cultivar and first related by McRostie (1919). Other references to this gene are also cited by Cárdenas *et al*. (1964), Fouiloux (1979), Del Peloso *et al*. (1989), Gonçalves-Vidigal (1994) and Kelly *et al*. (1994). According by Menezes (1985), this gene confers resistance to alpha, epsilon, zeta and eta races, however its resistance is "broken" by the virulence of delta, teta, capa, lambda and mu races. But recent studies have shown a more complex spectrum resistance to *A (Co-1)* gene (Kelly *et al*., 1994; Tu, 1994), because it confers resistance to most virulent races of anthracnose present in Central America (Pastor-Corrales *et al*., 1995). The second gene is present in PI 207262 cultivar, the gene already cited, *Mexique 2 (Co-4)*.

Table 1. Common bean (*Phaseolus vulgaris* L.) cultivars used in this study

Cultivar	Reaction to anthracnose race 69	Characteristics		
		Growth habit ⋆	Seed size	Seed color
Dark Red Kidney	Resistant	I	Large	Red
Perry Marrow	Susceptible	II	Medium	White
Michelite	Susceptible	III	Small	White
PI 207262	Resistant	III	Small	Brown
Cornell 49242	Resistant	III	Small	Black
AB 136	Resistant	IV	Small	Red
G 2333	Resistant	IV	Small	Red

⋆I = determinate; II = indeterminate, erect bush; III = indeterminate, weak-stemmed, semi-climber; IV = indeterminate, weak-stemmed, climber (Singh, 1982)

Table 2. Segregation for resistance to race 69 (epsilon) of *Colletotrichum lindemuthianum* (*) in common bean

Crosses		F_1	F_2				
			Nº of Plants		Expected ratio	X^2	Probability
			R	S	R:S		
PI 207262 x Michelite	(R x S)	R	272	30	57:7	0.3123	0.58
PI 207262 x Perry Marrow	(R x S)	R	153	54	3:1	0.1304	0.72
PI 207262 x Dark Red Kidney	(R x R)	R	199	15	15:1	0.2105	0.64
PI 207262 x Cornell 49242	(R x R)	R	91	7	15:1	0.1333	0.72
PI 207262 x AB 136	(R x R)	R	161	12	15:1	0.1391	0.71
PI 207262 x G 2333	(Rx R)	R	242	4	63:1	0.0064	0.93

⋆ R = Resistance S = Susceptibility

For 15R:1S segregation, involving now the cross PI 207262 x Cornell 49242, data indicated the presence of the resistance gene present in the first cultivar as already mentioned, besides *Are (Co-2)* gene, previously cited by Mastenbroek (1960) and Tu (1984). Nowadays, this gene has been widely used by breeders as resistance source and really related in literature (Bannerot *et al*., 1971; Fouilloux, 1979; Muhalet *et al*., 1981; Menezes and Dianese, 1988; Tu, 1992; Adam-Blondon *et al*., 1994; Young and Kelly, 1996).

In relation to the last cross involving the 15R:1S ratio, PI 207262 x AB 136, it was observed that the same preceding resistance mechanism occurred with the *Mexique 2 (Co-4)* and *Q (Co-6)* gene, with the last one related by Gonçalves-Vidigal (1994). The resistance of the cultivar AB 136 was described by Schwartz *et al*. (1982). Such gene has been related as independent of all other previously characterized.

The last assessed cross involved two resistant cultivars: PI 207262 and G 2333, whose data in F_2 generation fitted 63R:1S ratio, indicating that the resistance was controlled by the action of three dominant genes. As one of them was already mentioned and described, *Mexique 2 (Co-4)* gene, it was observed that the other involved genes are dominant and independent. Such genes are present in G 2333. One of them was named *Co-5* according to the latest nomenclature proposing symbols to resistance genes, (Kelly and Young, 1996), while the second one received temporarily *Co*-7 denomination, until a complex characterization with other genes resistance may be conducted (Young *et al*., 1998). In all these crosses, the resistance genes were located in independent loci, and each of them can give full resistance, even in the presence of the recessive allele of the other two, behaving, therefore, as triplicate dominance resistance factors. The allele tests carried out for race 69 (epsilon) indicate that all the genes present in the resistant cultivars are independent from each other.

To explain the obtained results for differential cultivar PI 207262, in relation to race 69 (epsilon), the presence of just one resistance dominant gene: *Co-4*, besides the presence of other dominant one, *X*, that acts as resistance complementary factor became evident. In relation to the other involved cultivars, an independent action to five resistance dominant genes: *A(Co-1)*, present in Dark Red Kidney; *Are (Co-2)*, present in Cornell 49242; *Q (Co-6)*, present in AB 136 and, finally, *Co-5* and *Co*-7, present in G 2333 was proposed.

The segregation data obtained from cross PI 207262 x G2333, indicated that G 2333 has the dominant genes *Co-5* and *Co*-7 and PI possesses one dominant gene in *locus Co-4*. Besides these genes, a third one named *Co-4*^{2} in G 2333 did not show segregation when the referred cultivar was crossed with PI 207262, because it is considered an allele of the same *locus*. Each of these genes acts as independent dominant factors.

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