Characterization of *Colletotrichum lindemuthianum* races in Paraná state, Brazil

Cláudia Thomazella*; Maria Celeste Gonçalves-Vidigal; Pedro Soares Vidigal Filho; William Mário de Carvalho Nunes and João Batista Vida

Departamento de Agronomia, Universidade Estadual de Maringá, Bloco 32, Av. Colombo, 5790, CEP 87020-900, Maringá, PR, Brazil. (* Corresponding Author: E-mail: cthomazella@yahoo.com.br)

ABSTRACT

Anthracnose caused by *Colletotrichum lindemuthianum* is one of the most important diseases in common beans (*Phaseolus vulgaris* L.). This research aimed at studying the *Colletotrichum lindemuthianum* in Paraná state region, Brazil, using race identification through differential cultivars. Nine races were identified from the eighteen isolates assessed: 7, 31, 65, 69, 73, 81, 87, 89 and 95. It was further observed that the Dark Red Kidney, Cornell 49242 and Mexico 222 cultivars were susceptible to races 7, 31, 87 and 95, races 31, 73, 89 and 95 and races 65, 69, 73, 81, 87 89 and 95, respectively. Cultivars AB 136, carrier of the *Co-6* gene and cultivar G 2333, carrier of the *Co-4*^{2}, *Co-5* and *Co-7* genes, were resistant to the identified races. These results showed the pathogenic variability and the importance of monitoring *Colletotrichum lindemuthianum* and resistant bean plant cultivars.

KEY WORDS: Anthracnose, common bean, breeding.

INTRODUCTION

Anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. et Magnus) Lams.- Scrib. is one of the most important diseases in common beans. It is widespread throughout the world, affecting susceptible cultivars especially in regions with moderate to cold temperatures and relatively high humidity. According to Chaves (1980), this disease can cause yield losses of up to 100% when conditions favorable for the pathogen are present.

Pathogen variability hinders the use of genetic resistance, which is the most practical and economic method of disease control. Barrus (1911, 1918) was the first author to study *Colletotrichum lindemuthianum* variability through the identification of two physiological races (alpha and beta) based on the reaction of the differential cultivars Michelite, Perry Marrow and Dark Red Kidney to the pathogen. Later, a third race, gamma, was found by Burkholder (1918). In 1942, Andrus and Wade found the delta race, Blondet (1963) found the epsilon race, Krüger et al. (1977) the kappa race and Fouiloux (1975) the alpha-Brazil race.

Kimati studied *Colletotrichum lindemuthianum* variability in Brazil for the first time in 1966, and identified the alpha, delta and Mexican II group races in São Paulo State. Araújo reported the occurrence of the Alfa group in Paraná State in 1973. Races belonging to the Alfa, Mexican II and Brazilian I and II groups were found in Minas Gerais (Oliari et al., 1973).

Paradela Filho et al. (1991) reported that the identification of new groups of *Colletotrichum lindemuthianum* races based on the reaction of only three differential cultivars (Michelite, Perry Marrow and Dark Red Kidney) was complete, and no longer reliable, according to the description of the Brazilian I group. The need to use a greater number of differential cultivars in such studies was recommended. Therefore, the adoption of a race identification system based on the definition of a series of international differential cultivars started with the purpose of standardizing the nomenclature (Pastor-Corrales, 1991; Rava et al., 1993; Kelly et al., 1994; Pastor-Corrales et al., 1995; Sicard et al., 1997; González et al., 1998; Mesquita et al., 1998; Balardin and Kelly, 1998; Thomazella et al., 2000).

There are various sources of resistance such as Dark Red Kidney carrier of the *A (Co-1)* gene (McRostie, 1919) and Cornell 49242, *Are (Co-2)* gene (Mastenbroek, 1960). Fouilloux (1979), identified the three *Mexique* genes *(Co-3), (Co-4)* and *(Co-5)* in germplasm from Mexico. Kelly and Young (1996) observed the presence of the *Co-6* gene in AB 136 and of the three different genes, *Co-4*^{2}, *Co-5* and *Co-7*, in G 2333 (Young *et al.*, 1998), which are well known to European and American researchers.

Melotto and Kelly (1999) reported the existence of three different alleles (*Co-1, Co-1^2, Co-1^3*) in the *Co-1* locus, which were resistant to anthracnose. *Co-1^3* and *Co-1^2* were the symbols attributed to the alleles that give resistance to *Colletotrichum lindemuthianum* present in the Perry Marrow and Kaboon cultivars, respectively. The *Are* gene has extensively been used in anthracnose resistance breeding programs in Brazil since 1960. The breakdown of the *Are* gene by the Kappa and 89 races has been observed in different parts of the world, including Brazil (Menezes, 1985; Thomazella et al., 2000; Bonett et al., 2001)

In 1998, eighteen isolates of *C. lindemuthianum* were collected in bean cultivars originated from the South, Southwest, and Northwest regions of Paraná State. This study aimed at characterizing these isolates of *Colletotrichum lindemuthianum* based on their reaction concerning the differential bean cultivars.

MATERIAL AND METHODS

Colletotrichum lindemuthianum isolates were obtained from infected plant material collected in common bean producing regions of Paraná State (Table 1).

Experiments were carried out in the Phytopathology laboratory and in the Applied Agriculture Research Program laboratory (Propagri) at the State University of Maringá city, Paraná State , from 1998 to 1999.

The pathogen was isolated from pods presenting lesions with abundant sporulation. A small portion of the spore mass was tranferred using a heated scalpel to test tubes conmtaining medium PDA (potato-dextrose-agar) esterilized for 20 minutes in autoclave at 120ºC. The tubes were then incubated in a BOD type growth chamber at approximately 22^o C.

Pathogen cultures were pathogen cultures were distributed in test tubes containing pods partially immersed in an agar-agar medium, which was sterilized in an autoclave for 40 minutes at 120^{o}C. The tubes were then incubated at 22^{o}C in a BOD type chamber for 14 days to obtain spores.

After the incubation period, the pods were transferred from the test tubes to a Becker containing sterilized distilled water, and the resulting suspension was filtered through a double layer of gauze, to assure that it contained only spores. Five counts were made for each suspension using a hemocitometer (Neubauer-Preciss chamber), and the concentration was adjusted to 2.0 x 10^6 spores/ml of sterilized distilled water by successive dilutions.

Cultivars were sown on plastic trays containing a mixture of soil and organic matter sterilized with methyl bromide. Trays were kept in a greenhouse until the first trifoliolate leaf appeared. Six to ten seedlings of each differential cultivar were inoculated by passing a brush previously moistened in the spore suspension on the leaf surfaces and described for disease reaction seven days later. The inoculated seedlings were transferred to a mist chamber, where they remained for 72 hours at approximately 22^{o}C under controlled light (12 light/12 dark) and relative humidity close to 100%. Symptoms were scored on a 5-point scale: scores 1 and 2 are attributed to resistant plants and 3 to 5 to susceptible plants (Yerkes Jr. and Ortiz ,1956).

The numerical system used to characterize and distinguish different races is based on the sum of the binary values assigned to those CIAT differential cultivars on which the unknown races is pathogenic (CIAT, 1988; Pastor-Corrales, 1991; Rava et al., 1993; Kelly et al., 1994; Pastor-Corrales et al., 1995; Sicard et al., 1997; González et al., 1998; Mesquita et al., 1998; Balardin and Kelly, 1998; Thomazella et al., 2000).

This system of nomenclature facilitates the identification of races, so the differential cultivars are assigned a binary value in the same fixed order (Table 2). Denomination of races is facilitated by this system,

Table 1. Isolates of *Colletotrichum lindemuthianum* obtained in Paraná State.

Isolate	Genotype	Local	Regions
1	IAPAR 14	São João do Ivaí	Northwest
2	LP 96-72	São João do Ivaí	Northwest
3	Carioca	Paranavaí	Northwest
4	Pérola	São João do Ivaí	Northwest
5	Pérola	São João do Ivaí	Northwest
6	Pérola	Jandaia do Sul	Northwest
7	Carioca	Paranavaí	Northwest
8	Pérola	São João do Ivaí	Northwest
9	Pérola	Jandaia do Sul	Northwest
10	Carioca	Paranavaí	Northwest
11	Pérola	Jandaia do Sul	Northwest
12	Carioca	Paranavaí	Northwest
13	Pérola	Jandaia do Sul	Northwest
14	Genotype A	Capitão Leônidas Marques	Southwest
15	Pérola	São João do Ivaí	Northwest
16	FT Nobre	Lapa	South
17	Genotype B	Irati	South
18	FT Nobre	Irati	South

since only odd numbers are assigned to races pathogenic on the most susceptible differential, for example cultivar Michelite.

RESULTS AND DISCUSSION

The resistance or susceptibility reaction of the 12 differential cultivars allowed the identification of nine *Colletotrichum lindemuthianum* races in 18 tested isolates, showing the genetic variability of the pathogen of Paraná State (Table 2). Pathogen race 89 was observed at high frequency in more than one locality. It was disseminated in five of the six regions sampled, and was repeatedly found in three isolates from a single region. Race 89 had been previously found in Paraná State (Rava et al., 1994) and in Minas Gerais and Mato Grosso do Sul states.

Race 81, which was found in three different localities, is also found in the states of Bahia, Minas Gerais and Pernambuco, while race 65 (epsilon), which was observed in two isolates collected in the same region, has been reported in Bahia, Espirito Santo and Paraná states (Rava et al., 1994).

The occurrence of other races such as 7, 73, 69, 95 87 and 31 was limited to a single collection region. According to Balardin (1997), these results can be explained by the small number of samples from each locality and by the regional predominance of some varieties, which may favor the selection of specific pathogen races.

Races 69, 73, 81 and 89, which were found in Paraná State, occur most frequently in Minas Gerais (Paula Jr. et al., 1998). Race 73 was characterized by Balardin and Kelly (1996) as the race that is one of the most largely found ones in North, South and Central American countries.

The results showed that only six of the 12 differential cultivars were resistant to the *Colletotrichum lindemuthianum* isolates collected in Paraná. The Michelite, Mexico 222, Widusa and Cornell 49242 cultivars were the most susceptible. Considering all the cultivars originated in the Andes (Dark Red Kidney, Perry Marrow, Widusa and Kaboon), only Kaboon was resistant to all the identified races in the present study. In *Colletotrichum lindemuthianum* genetic diversity studies, Balardin et al. (1997) found that the Kaboon cultivar was highly resistant to 38 of the 40 analyzed races. Similarly, Melotto and Kelly (1999) concluded that the Kaboon cultivar carries a dominant gene that expresses resistance to *Colletotrichum lindemuthianum* races 7 and 73.

Table 2. Reaction of *Colletotrichum lindemuthianum* differential cultivars *Phaseolus vulgaris* L. to 18 isolates collected in Paraná State.

Isolate	Local	Races	Differential cultivars [1/, 2/]											
			A	B	C	D	E	F	G	H	I	J	K	L
1	São João do Ivaí	69	S	R	S	R	R	R	S	R	R	R	R	R
2	São João do Ivaí	81	S	R	R	R	S	R	S	R	R	R	R	R
3	Paranavaí	73	S	R	R	S	R	R	S	R	R	R	R	R
4	São João do Ivaí	89	S	R	R	S	S	R	S	R	R	R	R	R
5	São João do Ivaí	95	S	S	S	S	S	R	S	R	R	R	R	R
6	Jandaia do Sul	89	S	R	R	S	S	R	S	R	R	R	R	R
7	Paranavaí	89	S	R	R	S	S	R	S	R	R	R	R	R
8	São João do Ivaí	89	S	R	R	S	S	R	S	R	R	R	R	R
9	Jandaia do Sul	65	S	R	R	R	R	R	S	R	R	R	R	R
10	Paranavaí	7	S	S	S	R	R	R	R	R	R	R	R	R
11	Jandaia do Sul	81	S	R	R	R	S	R	S	R	R	R	R	R
12	Paranavaí	81	S	R	R	R	S	R	S	R	S	R	R	R
13	Jandaia do Sul	65	S	R	R	R	R	R	S	R	R	R	R	R
14	C.Leônidas Marques	31	S	S	S	S	S	R	R	R	R	R	R	R
15	São João do Ivaí	89	S	R	R	S	S	R	S	R	R	R	R	R
16	Lapa	89	S	R	R	S	S	R	S	R	R	R	R	R
17	Irati	87	S	S	S	R	S	R	S	R	R	R	R	R
18	Irati	89	S	R	R	S	S	R	S	R	R	R	R	R

[1/] Differential cultivars and respectively binary value: A, Michelite: 1; B, Michigan Dark Red Kidney: 2; C, Perry Marrow: 4; D, Cornell 49242: 8; E, Widusa: 16; F, Kaboon: 32; G, Mexico 222: 64; H, PI 207262: 128; I, TO: 256; J, TU: 512; K, AB 136: 1024 and L, G2333: 2048; [2/] S: susceptible and R: resistant.

Melotto and Kelly (2000) reported that the major dominant resistance gene present in the Kaboon cultivar is an allele of the *Co-1* gene, called *Co-1²*. According to these authors, this gene is very important for common bean breeding programs, as it gives resistance to both Andean and Central American races recently identified in Michigan. Among the cultivars of Central American origin, only Michelite, Cornell 49242 and Mexico 222 were susceptible. Backcrossing between cultivars with desirable agronomic traits and the differential cultivars AB 136, carrier of the resistance *Co-6* gene, and G 2333, carrier of the resistance genes *Co-4², Co-5* and *Co-7*, is an option for breeding programs.

Race 31 was virulent to the Michelite, Dark Red Kidney, Perry Marrow, Cornell 49242 and Widusa cultivars, while race 95 was also virulent to the Mexico 222 cultivar.

Races 65 and 73, which were isolated from cultivars belonging to the Central American genetic group, were virulent to the differential cultivars of Central American origin. These races were also isolated in hosts of Central American origin in Rio Grande do Sul (Balardin, 1997). According to this author, this led to the conclusion that there is a pathogen co-evolution with the genetic group of the *Phaseolus vulgaris* L. host.

This study identified races 7, 31, 69, 73 and 87 for the first time in Paraná State. Also in Paraná State, Rava et al. (1994) identified races 55, 64, 65, 81, 89 95 102 and 453 of *Colletotrichum lindemuthianum* using the binary nomenclature. Some of these races were observed in other Brazilian states, such as Minas Gerais, Mato Grosso do Sul, Bahia, Espírito Santo, Paraíba, Pernambuco, the Federal District and Goiás (Rava et al., 1994). According to Balardin (1997), the widespread of the new race 73 and also of the gamma race in Rio Grande do Sul, suggests that the best strategy for genetic breeding is to obtain cultivars carrying genes which provide durable resistance to *Colletotrichum lindemuthianum*. Based on the resistance reaction, the differential cultivars PI 207262, TO, TU, AB 136 and G 2333 stood out as main resistance sources to the nine *Colletotrichum lindemuthianum* races identified. The Kaboon cultivar was resistant to all the races identified in this study, but it has a susceptibility reaction to race 55 (Rava et al., 1994; Alzate-Marin et al., 1997). The AB 136 and the G 2333 cultivars, carrier of the *Co-6* and of the *Co-4², Co-5* and *Co-7* genes, respectively, were resistant to all the races identified in this study. The G 2333 cultivar can be used as donor parent in backcrosses to adapted cultivars to transfer resistance genes that have not been broken by the races tested in various parts of the world.

The results showed the existence of variability of the *Colletotrichum lindemuthianum* pathogen, highlighting the importance of constant monitoring of the races and resistant bean cultivars relationship. This is especially important in cropping regions where the damage can cause serious loss in yield.

CONCLUSIONS

The races identified were 7, 31, 65, 69, 73, 81, 87, 89 and 95.

The 89 race was disseminated in several regions of Paraná State.

The 95 race was virulent in a larger number of differential cultivars.

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RESUMO

Caracterização de raças de *Colletotrichum lindemuthianum* no Estado do Paraná, Brasil

A antracnose em *Phaseolus vulgaris* L. causada por *Colletotrichum lindemuthianum*, é uma das doenças mais importantes dessa cultura, afetando cultivares de feijoeiro suscetíveis. O objetivo deste trabalho foi obter informações sobre a ocorrência de raças de *C. lindemuhianum* no Paraná, identificando-as por meio de cultivares diferenciadores. Dos 18 isolados testados foram identificadas nove raças, quais sejam: 7, 31, 65, 69, 73, 81, 87, 89 e 95. Observou-se ainda que, Dark Red Kidney foi suscetível às raças 7, 31, 87 e 95, Cornell 49242, mostrou-se suscetível às raças 31, 73, 89 e 95, e as raças 65, 69, 73, 81, 87, 89 e 95 foram virulentas ao cultivar México 222. O cultivar AB 136 portador do gene *Co-6* e G 2333 dos genes *Co-4²*, *Co-5* e *Co-7* mostraram-se resistentes às raças identificadas. Esses resultados evidenciam a variabilidade patogênica e a importância do monitoramento das raças de *C. lindemutianum* e de cultivares de feijoeiro resistentes.

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