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Characterization of *Colletotrichum lindemuthianum* Isolates Using Differential Cultivars of Common Bean in Santa Catarina State, Brazil

Maria Celeste Gonçalves-Vidigal[*], Claudia Thomazella, Pedro Soares Vidigal Filho, Marcus Vinícius Kvitschal and Haroldo Tavares Elias

Departamento de Agronomia; Universidade Estadual de Maringá; Av. Colombo, 5790; mcgvidigal@gmail.com; 87020-900; Maringá - PR - Brasil

ABSTRACT

In 2003 and 2004, 32 isolates of Colletotrichum lindemuthianum obtained from the infected plants of field-grown common bean (Phaseolus vulgaris L.) in Santa Catarina state, Brazil were analyzed based on the virulence to 12 differential cultivars of Phaseolus vulgaris L.. Thirteen distinct races were identified, six of which had not been reported previously in Santa Catarina. This is the first report of the occurrence of 67, 83,101,103,105, and 581 races of C. lindemuthianum. Race 65 was most common (34%). All the isolates were compatible to the cultivars Michelite and Mexico 222. Some isolates infected not only differential cultivar of Mesoamerican origin, but also the ones of Andean origin.

Key words: Anthracnose, common bean, new races, *Phaseolus vulgaris* L.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important source of food for the people in Brazil and it is a proven source of the protein and is also a good source of the carbohydrates and iron (Gonçalves-Vidigal et al., 2007b). One of the most widespread and important diseases of this crop is anthracnose, caused by the fungus *Colletotrichum lindemuthianum* (Sacc. et Magn.), which causes complete yield loss when susceptible genotypes are used in favorable conditions to the pathogen (Pastor-Corrales and Tu, 1989; Poletine et al. 2000). The first studies in Brazil to identify *C. lindemuthianum* races were done in São Paulo state (Kimati, 1966). The author identified the alpha, delta and Mexican II group races. In 1973, the isolates collected in Rio Grande do Sul and Santa Catarina states were characterized as belonging to alpha, beta, Mexican I and Brazilian I races, while the gamma race was only identified in Santa Catarina (Oliveira et al., 1973). The occurrence of alpha group I in Paraná state was first reported by Araújo (1973). In Minas Gerais, races from the alpha, Mexican II and Brazilian I and II groups were also characterized (Oliari et al., 1973). In 1988, 25 isolates from Santa Catarina state were characterized by Menezes and Dianese (1988). The authors identified 16 isolates belonging to Alpha race, three to Delta race, three to Epsilon, one to Kappa and two to Mu race. In Santa Catarina, Balardin et al. (1990), working on

[*] Author for correspondence

the identification of *C. lindemuthianum* races, observed the occurrence of alpha-Brazil, alpha, epsilon, kappa, Brazilian I races, and it was identified one isolate with characteristics of a new race. The binary nomenclature was used by Rava et al. (1994) to characterize the isolates from Santa Catarina and the authors identified the race 55. In the period from 1988 to 1992, Balardin (1997) identified seven races in the isolates collected in nine regions of Rio Grande do Sul. The studies of variability in *C. lindemuthianum* were carried out by Thomazella et al. (2002) who collected 18 isolates in six counties of Paraná state and characterized nine races: 7, 31, 65, 69, 73, 81, 87, 89, and 95, when races 7, 31, 69, 73, and 87 first occurred in that location. Since 1966, several studies have reported the presence of different races of *C. lindemuthianum* in Brazil and to date approximately 50 races have been characterized (Alzate-Marin and Sartorato, 2004; Kimati, 1966).

The 12-member differential the series (Pastor-Corrales, 1991), used to differentiate the races of *C. lindemuthianum,* has proved to be a valuable source of resistance genes for the breeders. The investigations have been sporadic, and no systematic characterization of *C. lindemuthianum* races has been carried out at the state level. The last identification of isolates from Santa Catarina has been done by Balardin et al. (1990), who characterized the following races: 7, 55, 65, 73, 75, 77, 81, 86, 87, 89, 95, 109, 111, 121, 217 and 249.Eleven independent resistance loci, *Co-1* to *Co-11*, resistance to anthracnose have been identified and mapped to different linkage groups of the bean genome, and allelic series at the *Co-1, Co-3* and *Co-4* loci (Kelly and Vallejo, 2004; Gonçalves-Vidigal et al., 2007a).

A source of resistance to anthracnose in common bean, *Co-2* gene, represents great importance in Brazil due to the fact that it had been used until the 1960's, but since 1977 it has shown lack of the resistance to races: 8, 9, 15, 31, 47, 63, 73, 75, 77, 89, 95, 109, 111, 121, 217, 249, 457, 521, 1673, 1993, and 2047 (Balardin et al., 1997). In addition, it has been observed that the resistance sources from the commercial cultivars in Santa Catarina, which possessed *Co-1, Co-2* and *Co-3* genes, were overcome by races identified by Balardin et al., 1990. The genetic variability studies of *C. lindemuthianum* in Santa Catarina have been developed for the last five years. It was possible to observe the low frequency of the reported works and data. Thus, considering the importance of the common bean for this state and the incidence of anthracnose, this study was conducted with the objective of characterizing *C. lindemuthianum* isolates by using differential cultivars.

MATERIAL AND METHODS

The experiments were carried out under the greenhouse and laboratory conditions in the Núcleo de Pesquisa Aplicada à Agricultura (Nupagri) at the Universidade Estadual de Maringá, Paraná state. During the period between 2003 and 2004, several common bean commercial cultivars were infected by the pathogen in the production field of Guatambu, Ponte Serrada, Campos Novos and Ituporanga in Santa Catarina state, Brazil. In this period, a total of 32 samples of *C. lindemuthianum* were collected from leaves or pods.

Preparation of *C. lindemuthianum* isolates

The monosporic cultures of the pathogen, kept in the test tubes with PDA (potato-dextrose agar) medium, were transferred to the test tubes containing the pods partially immersed in agar-agar culture medium (Mathur et al., 1950). The test tubes were incubated at 22ºC in the BOD conditions for 14 days in order to obtain the spores from the fungus. After this, the pathogen culture was kept in a refrigerator and used as culture stock for the next experiments. After the incubation period, the pods were transferred from the test tubes to a Becker containing distilled and sterilized water, generating a suspension which was filtered in the double layer gauze to assure that it contained only the spores. Each suspension was counted five times by using a hemacytometer (Neubauer-Preciss chamber), adjusting the concentration to 1.2×10^6 spores mL^{-1} of distilled and sterilized water to the dilutions.

Genetic Plant Material

To distinguish the races derived from different *C. lindemuthianum* isolates, the differential cultivars set was used. This set consisted on 12 cultivars, each with a designated binary number as following: Michelite, 1; Michigan Dark Red Kidney, 2; Perry Marrow, 4; Cornell 49-242, 8; Widusa, 16; Kaboon, 32; Mexico 222, 64 ; PI 207262, 128; To, 256; Tu, 512; AB 136, 1024; and G 2333, 2048. The sum of the numbers assigned to each infected cultivar of the differential set determined the number or race designation. The

cultivars were sown in the plastic trays containing a mixture of the soil and sterilized organic material. These trays were kept under the greenhouse conditions until the seedlings reached the primary leaf stage. Ten seedlings of each differential cultivar were inoculated with the spore suspension by using De Vilbiss air compression.

The inoculation was done in 12 differential cultivars and the plants were moved to a mist chamber, where they remained for 72 h at 20ºC, 12 h light / 12 h dark and relative humidity nearly to 100%. Ten days after the inoculation, the plants were scored as resistant (R) or susceptible (S) as described by Pastor-Corrales (1991), where 1 to 3 = resistant and 4 to 9 = susceptible.

Table 1 - Number of isolates, cultivars of common bean, race designation of *C. lindemuthianum* and local.

Isolate	Cultivar	Race*	Local
5	População 7	65	Guatambú
6	Vagem branca	65	Guatambú
9	PSC – CH 10	65	Ponte Serrada
10	GX 9772 1169	65	Guatambú
14	Pérola	65	Campos Novos
18	Carioca	65	Ponte Serrada
21	CNFC 8063	65	Ponte Serrada
22	VI 4599	65	Ponte Serrada
26	Empasc 201	65	Campos Novos
29	Pérola	65	Campos Novos
33	FT – Bionobre	65	Ituporanga
25	Cavalo – crioulo	67	Campos Novos
35	Pérola	67	Ituporanga
11	FT 84.824	73	Guatambú
27	Comum vermelho	73	Campos Novos
28	LH 11	73	Ituporanga
30	LP 97-13	73	Ituporanga
32	Carioca – CH 06	73	Campos Novos
31	Crioulo CN 474	75	Campos Novos
12	PSC – CH 04	81	Ponte Serrada
13	Pérola	81	Campos Novos
3	Carioca Precoce	81	Guatambú
1	Carioca MG	83	Guatambú
15	Crioulo	83	Guatambú
34	CNFC – 8044	89	Campos Novos
17	FT 07-175	89	Ponte Serrada
2	Carioca 433	95	Guatambú
16	Taquara	101	Guatambú
4	Empasc 201	103	Guatambú
23	LP 96-153	105	Ponte Serrada
24	CNFC 7569	121	Ponte Serrada
19	CNFC 8045	581	Ponte Serrada

RESULTS AND DISCUSSION

The reactions of a differential cultivars set to the 32 isolates of *C. lindemuthianum* permitted the identification of the races 65, 67, 73, 75, 81, 83, 89, 95, 101, 103, 105, 121 and 581 (Table 1) from Guatambu, Ponte Serrada, Campos Novos and Ituporanga counties in Santa Catarina. This is the first report of the occurrence of 67, 83,101,103, 105 and 581 races in this state.

The race 65 showed the highest frequency, characterized in 11 isolates collected in Ponte Serrada, Campos Novos and Ituporanga counties, corresponding to 34.37% of the studied isolates. Race 73 was the second more frequent corresponding to 5 isolates, participating with 15.62% of the studied isolates collected in Campos Novos, Guatambu and Ituporanga regions. Similar results were obtained by Carneiro (1999), when studies were carried out to characterize the isolates in Paraná state. The author observed that the races 65 and 73 corresponded to 23% and 16%, respectively. However, in 2002, the race 89 was widely distributed in Paraná than any other, corresponding approximately to 39% of the identified isolates (Thomazella et al., 2002). The races 65, 73 and 81, identified in Santa Catarina, were more frequent and widely distributed in the country, as in Goiás, Paraná and Distrito Federal states (Alzate-Marin and Sartorato, 2004).

According to Table 2, all the races identified in this study were compatible with Michelite (A) and Mexico 222 (G) cultivars, both of Mesoamerican origin. Also, only four of all the races overcame the resistance of Kaboon (F) and Perry Marrow (C), while five races infected Michigan Dark Red Kidney (B). Cornell 49-242 (D) cultivar was compatible with six of the characterized races. This behavior is characteristic of the isolates with the typical Mesoamerican reaction, different of Andean isolates, which demonstrated wide virulence on the genes of a differential group (Pastor-Corrales, 1996). In addition, only four of all the races overcame the resistance of Kaboon (F) and Perry Marrow (C), and five races infected Michigan Dark Red Kidney. Only three of the 32 isolates were also virulent in more than one differential Andean group that were isolates 2, 4 and 16, all from the locality of Guatambú.

Table 2 - Reaction of differential cultivars to *C. lindemuthianum* isolates from Santa Catarina state.

Isolate	Differential cultivars*												Race
	A	B	C	D	E	F	G	H	I	J	K	L	
5	S	R	R	R	R	R	S	R	R	R	R	R	65
6	S	R	R	R	R	R	S	R	R	R	R	R	65
9	S	R	R	R	R	R	S	R	R	R	R	R	65
10	S	R	R	R	R	R	S	R	R	R	R	R	65
14	S	R	R	R	R	R	S	R	R	R	R	R	65
18	S	R	R	R	R	R	S	R	R	R	R	R	65
21	S	R	R	R	R	R	S	R	R	R	R	R	65
22	S	R	R	R	R	R	S	R	R	R	R	R	65
26	S	R	R	R	R	R	S	R	R	R	R	R	65
29	S	R	R	R	R	R	S	R	R	R	R	R	65
33	S	R	R	R	R	R	S	R	R	R	R	R	65
25	S	S	R	R	R	R	S	R	R	R	R	R	67
35	S	S	R	R	R	R	S	R	R	R	R	R	67
11	S	R	R	S	R	R	S	R	R	R	R	R	73
27	S	R	R	S	R	R	S	R	R	R	R	R	73
28	S	R	R	S	R	R	S	R	R	R	R	R	73
30	S	R	R	S	R	R	S	R	R	R	R	R	73

(Cont. ...)

(Cont. Table 2)

32	S	R	R	S	R	R	S	R	R	R	R	R	73
31	S	S	R	S	R	R	S	R	R	R	R	R	75
12	S	R	R	R	S	R	S	R	R	R	R	R	81
13	S	R	R	R	S	R	S	R	R	R	R	R	81
3	S	R	R	R	S	R	S	R	R	R	R	R	81
1	S	S	R	R	S	R	S	R	R	R	R	R	83
15	S	S	R	R	S	R	S	R	R	R	R	R	83
34	S	R	R	S	S	R	S	R	R	R	R	R	89
17	S	R	R	S	S	R	S	R	R	R	R	R	89
2	S	S	S	S	S	R	S	R	R	R	R	R	95
16	S	R	S	R	R	S	S	R	R	R	R	R	101
4	S	S	S	R	R	S	S	R	R	R	R	R	103
23	S	R	R	S	R	S	S	R	R	R	R	R	105
24	S	R	R	S	S	S	S	R	R	R	R	R	121
19	S	R	S	R	R	R	S	R	R	S	R	R	581

* Common bean differential cultivars used to characterize races of *C. lindemuthianum* followed by there respective binary value (Pastor-Corrales, 1991): A- Michelite (1); B- Michigan Dark Red Kidney (2); C- Perry Marrow (4); D- Cornell 49-242 (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); L- G 2333 (2048). S = Susceptible, R = Resistant.

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RESUMO

Em 2003 e 2004, isolados de *Colletotrichum lindemuthianum* obtidas de plantas infectadas de feijoeiro comum (*Phaseolus vulgaris* L.) no Estado de Santa Catarina, Brasil foram analisadas baseando-se na virulência em 12 cultivares diferenciadoras de *Phaseolus vulgaris* L.. Treze raças distintas foram identificadas, sendo que seis delas não haviam sido reportadas anteriormente em Santa Catarina. Este é o primeiro registro de ocorrência das raças 67, 83, 101,103,105, e 581 do *C. lindemuthianum*. A raça mais comum foi a 65 (34%). Todos os isolados foram compatíveis com as cultivares Michelite e México 222. Alguns dos isolados infectaram tanto cultivares diferenciadoras de origem Mesoamericana como de origem Andina.

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